How nitrogen availability constrains insect herbivores



William Benedict Vigar Langdon

Pembroke College

University of Oxford

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Declaration

I declare that this thesis was composed by myself and that the work contained herein is my own except where explicitly stated in the text. The work has not been submitted for any degree or professional qualification except as specified.

William Langdon, Trinity Term 2024

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Abstract

It is now clear that nitrogen pollution is radically re-structuring plant communities, impacts that appear to be cascading up the food web to insect herbivores. Understanding of the mechanisms driving the impacts of nitrogen pollution on insects, and how these affect different insect species remains sparse however. Fundamentally, the impacts of nitrogen pollution on insect herbivores depend on how nitrogen levels constrain the availability of their foodplants, and the nutritionally and thermally-suitable subsets of these. In this thesis I aim to improve understanding of how insect herbivores are affected by nitrogen pollution by exploring the nature of these constraints for lepidoptera, a group expected to be particularly sensitive to the effects of nitrogen pollution. I examine how these constraints are structured, and how they interact to govern the overall response of different insect species to nitrogen availability, and in turn nitrogen pollution.

I find that the effect of nitrogen availability on the nutritional quality of host plants for the larvae of three butterfly species is unimodal. Using a novel analysis of the nutrients remaining in frass, I am able to detect the nutrient deficiencies that may be driving this. These results imply strong nutritional constraints for these butterfly species, but in a field study of one of these butterfly species I find that a requirement for high temperatures around host plants is more important than host nutritional quality in shaping habitat use. This work underlines the importance of considering how nitrogen levels constrain the availability of all aspects of insect niches when attempting to predict their responses to nitrogen pollution. I therefore attempt to model the relationship between occurrence and nitrogen availability for a variety of lepidoptera species in Great Britain. This relationship should integrate all of these constraints, and this approach provides a promising method for better understanding the sensitivity of insect species to nitrogen pollution in the future.

Overall, this thesis provides new insights into how nitrogen availability constraints insects, and new tools for exploring this in the future to better understand species' sensitivity to nitrogen pollution.

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Chapter 1: General Introduction

Of all the biogeochemical cycles altered by human activity, the nitrogen cycle has been perhaps the most radically and rapidly changed. In spite of the abundance of nitrogen gas in the earth's atmosphere, forms of nitrogen that living organisms can use (so-called 'reactive nitrogen') are scarce in most terrestrial ecosystems (LeBauer & Treseder, 2008; Vitousek & Howarth, 1991). Prior to the Industrial Revolution, inputs of these forms were largely provided by nitrogen-fixing bacteria, at a rate that was insufficient to release these systems from the nitrogen-limitation that pervades on the earth's surface (Fowler et al., 2013). The advent of fossil fuel combustion (producing oxidised nitrogen) and the Haber-Bosch process (producing reduced nitrogen), however, mean that total input of reactive nitrogen into terrestrial ecosystems has more than doubled (Fowler et al., 2013), and the nitrogen handbrake on biological productivity has been released.

The likely impacts on plant communities are now well-characterised across ecosystems (Bobbink et al., 2010). As plant productivity and biomass increase, and competition for light replaces competition for nutrients as the primary structuring force, there is a shift from low-growing, stress-tolerant plant species, to faster-growing, taller, light-demanding species. This usually results in an overall loss of botanical species richness and diversity at the community level, alongside an increase in the nitrogen content of individual plants. In some systems on weakly-buffered soils, deposition can also lead to soil acidification and a shift towards more acid-tolerant plant species.

In recent years, there has been an increasing appreciation that these effects will be propagated up to the insects living on and among plant communities (Nijssen et al., 2017), as large-scale trends in insect abundance and diversity in Europe have been related to levels of nitrogen pollution, or traits thought to underpin their sensitivity to it (Carvalheiro et al., 2020; Roth et al., 2021; WallisDeVries & van Swaay, 2017). Such observations are deeply worrying in the context of reported declines in insect abundance and increasing appreciation of the importance of insects to human society (Losey & Vaughan, 2006;

van Klink et al., 2020), but at such large scales, do not provide much insight into the mechanisms driving insect responses to nitrogen pollution or the sensitivity of different insect species to nitrogen pollution. Fundamentally, this depends on how the availability of reactive nitrogen in the environment affects the availability of habitat for different insect species, via a variety of mechanisms. Smaller-scale experimental and field studies assessing the responses of insect species to changes in habitat structure and host plants that are likely to arise under nitrogen pollution have provided some of this insight. However, most of this work has not been done in the context of nitrogen pollution, nor compared the responses of multiple species, or tested the effects of different mechanisms side-by-side to understand how they interact. The result is a fragmented picture of how insects are likely to vary in their sensitivity to nitrogen pollution. In light of this, this thesis explores the separate mechanisms via which nitrogen levels constrain habitat availability for insects, and how they are ultimately likely to combine to affect species' sensitivity to pollution.

The Nitrogen Cycle

Life on earth is carbon-based and carbon-powered, but underpinned by nitrogen in the proteins that catalyse reactions to build and run living organisms, and the nucleic acids that encode these proteins (Novoa & Loomis, 1981). Surprisingly, given the abundance of nitrogen gas on earth (making up 78% of its atmosphere), the scarcity of nitrogen relative to its demand by living organisms means that its availability is often the primary constraint on plant productivity in terrestrial ecosystems (LeBauer & Treseder, 2008; Vitousek & Howarth, 1991). This is because nitrogen gas is diatomic (so-called 'dinitrogen'), formed from two nitrogen atoms joined by a triple bond with an extremely high activation energy of 945 kJ mol^{-1} that requires a great deal of energy to break before the nitrogen atoms can be incorporated into living organisms (Galloway et al., 2004; Geng et al., 2019).

The only organisms that have really succeeded in doing this are nitrogen-fixing bacteria, that use the nitrogenase enzyme to break the strong triple bond in dinitrogen, and reduce nitrogen to various

ammonium compounds (Elser, 2011; Galloway et al., 2004). Until recently, this 'biological nitrogen fixation' (BNF) was the principal input of useable nitrogen compounds to terrestrial ecosystems (Fowler et al., 2013; Galloway et al., 2004). This useable nitrogen is often referred to as 'reactive nitrogen' (N_r) and includes inorganic oxidised (NO_x, N_2O, HNO_3) and reduced forms (NH_x), and more complex organic forms (such as proteins, urea, amines) (Erisman et al., 2011). Far smaller quantities of reactive nitrogen are also produced by lightning strikes and volcanic eruptions, in oxidised and reduced forms respectively (Fowler et al., 2013). While some nitrogen-fixing bacteria in terrestrial ecosystems live as mutualists in nodules in the roots of plants (particularly the Fabaceae) and make their nitrogen available to their hosts in exchange for carbohydrates and amino acids, most live freely in the soil (Fowler et al., 2013). Reactive nitrogen fixed by them is made available to living organisms after the death of a nitrogen-fixing bacterium or its host, and the mineralisation by microbes of the nitrogen contained within it into reduced forms that can then enter the nitrogen cycle. Nitrogen atoms can then cascade through different 'pools' of reactive nitrogen via a variety of processes. They can be dissolved in precipitation, washed out of the soil surface into surface water ('run-off') or groundwater ('leaching'), and ultimately into the sea (Fowler et al., 2013). This is common for the more soluble oxidised forms of nitrogen, which can be produced from reduced nitrogen by nitrifying bacteria (Fowler et al., 2013). From the oceans or the soil, reactive nitrogen can then cycle through living organisms, being taken up by producers and later consumers, and released on their decomposition or in their excreta, or it can enter atmospheric pools, via reduction (producing ammonia from nitrate), denitrification (producing dinitrogen gas and N_2O) and, in the case of solid forms in the soil, volatilisation to gas (Fowler et al., 2013; Holmes et al., 2019). Having entered atmospheric gaseous pools, reactive nitrogen can be returned to the land or ocean via deposition. This can be 'dry' – direct deposition at the surface via sedimentation, interception and diffusion, or 'wet', dissolved in precipitation (Fowler et al., 2013).

Nitrogen limitation of terrestrial ecosystems

In spite of the existence of biological nitrogen fixation, the productivity of most terrestrial ecosystems appears to be limited by the availability of reactive nitrogen, and increases when it is added. In a meta-analysis of 126 nitrogen-addition experiments, LeBauer & Treseder (2008) found that above-ground plant growth responded positively to nitrogen additions in most terrestrial ecosystems: temperate forests, temperate grasslands, tropical grasslands, wetlands and tundra. Only deserts and tropical forests did not show consistent increases. The former are water-limited, but the latter showed a mixture of responses with nitrogen limitation on young soils, but not on older soils.

This observation provides a clue as to why most terrestrial ecosystems may be limited by the availability of nitrogen and not other chemical elements, such as phosphorus which is also important for the formation of nucleic acids and membranes in living organisms (Elser et al., 2007). While reactive nitrogen is derived from the atmosphere, phosphorus comes mostly from weathering of phosphorus-containing rocks. This means that phosphorus is at its most abundant in young soils, declining to a terminal steady state over time as it is leached, while nitrogen is initially scarce (Vitousek & Howarth, 1991). Thus, in temperate and boreal systems, where glaciation has constantly renewed soils, nitrogen limitation dominates, whereas very old, deeply-leached soils in the tropics have arrived at phosphorus limitation (LeBauer & Treseder, 2008; Vitousek & Howarth, 1991). The differing mobility of phosphorus and nitrogen may also contribute to maintaining this limitation. Phosphorus can be retained in the soil by living organisms, and also adsorbed and precipitated with metals while nitrogen is only retained by living organisms, and more easily lost by volatilisation or leaching (Vitousek & Howarth, 1991).

Nevertheless, pervasive nitrogen limitation of organisms seems surprising given the existence of biological nitrogen fixation. This may be a result of the higher demand of nitrogen-fixers for energy and other nutrients, such as phosphorus (Vitousek et al., 2002; Vitousek & Howarth, 1991). This means

that nitrogen-fixation only conveys an advantage when nitrogen is scarce but these other resources are abundant. Its advantage declines as nitrogen accumulates in soils, competition for other resources increases and nitrogen-fixation demands relatively more of a limited energy and nutrient budget (Vitousek et al., 2002; Vitousek & Howarth, 1991). This is a common trajectory during ecological succession (eg. Vitousek et al., 1993), perhaps explaining why nitrogen-fixing plant species are often common in early-successional habitats and scarcer at later seral stages and on more fertile sites where competition for light between plants increases (limiting the rate of photosynthesis and energy supply) (Gutschick, 1981). Thus, ecosystem nitrogen-limitation really represents a steady-state of nitrogen limitation for non-nitrogen-fixing plants, and limitation by phosphorus or light for nitrogen-fixing plant species (Vitousek & Howarth, 1991).

Human impacts on the availability of reactive nitrogen

Human activity has however been releasing the nitrogen-limitation brake on the productivity of terrestrial ecosystems via three main processes (Erisman et al., 2011).

1. The planting of nitrogen-fixing crops either for food production, or to add nitrogen to the soil to facilitate it. This can enter terrestrial ecosystems on the decomposition of the nitrogen-fixing crops, and the mineralisation of the nitrogen they contain in the soil.
2. The production of nitrous oxides (mainly NO and NO_2.) in fossil fuel combustion. This oxidises both atmospheric nitrogen and organic nitrogen in the fuels themselves, thus both creating new reactive nitrogen and mobilising sequestered reactive nitrogen (Galloway et al., 2004). This releases oxidised nitrogen directly into an atmospheric pool.
3. The production of reduced nitrogen as Ammonia via the Haber-Bosch process, primarily to be used as a fertiliser in agriculture. This nitrogen can enter terrestrial ecosystems via inaccurate application, volatilisation to a gas after application, or on the decomposition of the organisms that take it up after application.

Although considerable uncertainty exists around the exact scale of different nitrogen fluxes (Galloway et al., 2013; Van Vuuren et al., 2011), it is clear that the anthropogenic increase in the inputs of nitrogen to terrestrial ecosystems has been considerable, with most reviews agreeing that it represents at least a doubling of annual inputs since the Industrial Revolution (Fowler et al., 2013; Galloway et al., 2004, 2008; Kanakidou et al., 2016). Fowler et al. (2013) for example, estimated current anthropogenic Nr creation as 210 TG N yr^{-1}, exceeding natural fixation at 203 TG N yr^{-1}. This represents an extraordinary increase from the 15 Tg N yr^{-1} estimated to have been produced by human activity (mainly cultivation of nitrogen-fixing crops) in 1860 by Galloway et al. (2004). The majority of this increase is reduced nitrogen produced via the Haber-Bosch process, approximately 120 TG N yr^{-1}, with 30 TG N yr^{-1} coming from oxidised nitrogen produced during fossil fuel combustion, and 60 TG N yr^{-1} from cultivation-induced biological nitrogen fixation (Fowler et al., 2013).

The majority of reactive nitrogen emissions are deposited close to where they are emitted. At the continental scale for example, only between 12 and 24% of oxidised nitrogen emissions are estimated to be exported to other regions (Sanderson et al., 2008). Reduced nitrogen is usually transported smaller distances owing to its shorter atmospheric lifetime (Erisman et al., 2011) meaning that the distribution of reactive nitrogen deposition closely matches its production, with hotspots in Europe, the eastern United States and south and east Asia (Galloway et al., 2004; Lamarque et al., 2010).

Nevertheless, emitted reactive nitrogen can still travel long distances before being deposited, meaning that no ecoystem on earth is entirely untouched by it – 11% of the world's natural vegetation is estimated to receive more than 10 kg N $ha\ yr^{-1}$, and signals of nitrogen deposition can be detected in some of the most remote regions on earth. Holtgrieve et al. (2011) found, for example, that a rise in oxidised nitrogen deposition following the Industrial Revolution was detectable in lake sediments in remote watersheds across the northern hemisphere, congruent with shifts in the isotopic signature of nitrate in Greenland ice cores at the same time (Hastings et al., 2009).

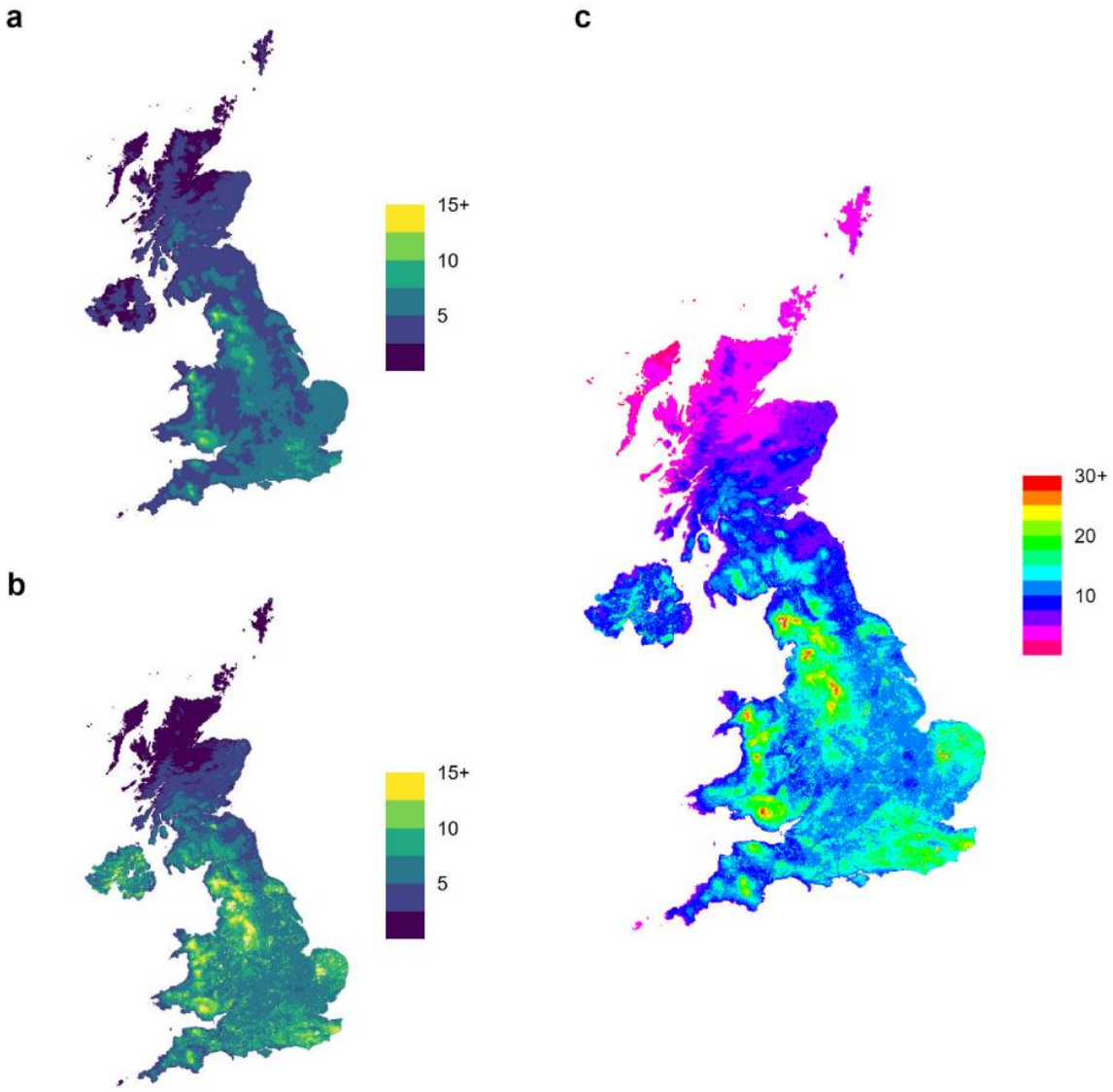


Figure 1: *Levels of a) oxidised, b) reduced and c) total atmospheric nitrogen deposition (kg ha^{-1} yr^{-1}) in the United Kingdom in 2017 plotted at 1km resolution. Higher concentrations of reduced nitrogen are visible in areas with more arable production (in the south and east) and higher rainfall (in the west), while oxidised nitrogen is driven more by population centres and rainfall. Total calculated by summing wet and dry oxidised and reduced deposition for 2017 in Tomlinson et al. (2020).*

At smaller spatial scales, the different properties of wet and dry deposition shape deposition patterns. Wet deposition is nearly always longer-range than dry, due to transport of reactive nitrogen in more elevated atmospheric layers, meaning that dry nitrogen deposition is very heterogeneous and strongly reflects the distribution of sources, while wet deposition has a smoother-grained distribution, mapping onto rainfall patterns. The result is that overall, deposition patterns reflect a combination of weather patterns and reactive nitrogen production patterns, with the highest levels in the UK in the wetter uplands in the west (where wet deposition is highest) and agricultural areas in the south-east (where dry deposition of reduced nitrogen is highest) (Tomlinson et al., 2021) (Figure 1).

Much uncertainty exists about future trends in reactive nitrogen production, largely dependent on the extent to which proposed climate change policies (to reduce carbon emission) are implemented. These are already reducing emissions of oxidised nitrogen in developed nations, but may be insufficient to offset rises in emissions in industrialising countries (Van Vuuren et al., 2011), even though many of these are also implementing their own control measures (Cofala et al., 2007). Kanakidou et al. (2016) suggest that even if policy is sufficient to reduce oxidised nitrogen emissions, this will be offset by a rise in reduced nitrogen production to feed a growing population, meaning that while the rate of increase in emissions may fall, levels are unlikely to drop below current levels before the end of the 21st century, except under the most optimistic scenarios (Kanakidou et al., 2016; Van Vuuren et al., 2011). Thus levels of reactive nitrogen emissions will remain high, but their composition and distribution may change, with higher levels of reduced nitrogen, and rising emission levels in industrialising countries, particularly in Asia (Stevens et al., 2018).

Impacts of nitrogen pollution on plant communities

Given the widespread nitrogen limitation of plant productivity on land, the most immediate impact of this enormous increase in inputs of reactive nitrogen to terrestrial ecosystems is an increase in plant productivity. This leads to a shift from competition for nutrients to competition for light being the

primary force structuring plant communities in open habitats, leading to community change - so-called 'soil eutrophication' (Bouwman et al., 2002). In some contexts, additional reactive nitrogen can also increase the acidity of soil, when gaseous nitrous oxides dissolve in precipitation to produce nitric acid which is deposited in rainfall, or when reduced nitrogen is deposited into the soil and nitrified, releasing hydrogen ions (Goulding, 2016; van Breemen et al., 1982). Uptake of the nitrate ions produced during nitrification can prevent acidification, as nitrate is taken up via ion channels in roots in a process that simultaneously requires hydrogen ions (Goulding, 2016), but if nitrate is leached before it can be taken up , hydrogen ions will accumulate, meaning that acidification is particularly likely on free-draining soils in areas of high rainfall.

Goulding (2016) lists four main processes that buffer soils against acidification, each operating at different pHs : the dissolution of carbonates and other basic rocks (at pH 7-8), the replacement of exchangeable base cations (Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}) by hydrogen (H^{+}) and aluminium (Al^{3+}) (the latter being more soluble at lower pHs) through the cation exchange buffer (at pH 5-6), the dissolution of aluminium and manganese-bearing minerals (at pH 4) and the dissolution of iron-bearing minerals (at pH 3). Thus, the extent of acidification by nitrogen pollution will depend on the chemistry of the soil receiving the pollution, and its concentration of surface sites able to hold H^{+} ions. As a result, acidification is primarily a problem on more weakly-buffered, acid soil types, such as those found on heathlands in temperate regions (Dise et al., 2011) , or more widely in tropical climates (Kuylenstierna et al., 1995). In these weakly-buffered soils it causes a gradual loss of essential nutrient cations that are liberated and then leached (Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}), and an accumulation of aluminium, iron and manganese that eventually inhibits plant growth (Bartakova et al., 2001; Bojórquez-Quintal et al., 2017; Millaleo et al., 2010).

While most changes to plant communities as a result of deposition are mediated by the soil, in rare instances plants are directly impacted by the toxicity of gaseous reactive nitrogen (Pearson & Stewart,

1993). Such instances are mainly reported close to point sources of reduced deposition (Bobbink et al., 2010; Dise et al., 2011).

The impacts of eutrophication and acidification on terrestrial plant communities caused by reactive nitrogen deposition have been widely-studied and frequently synthesised in reviews at global (eg. Bobbink et al., 2010; Stevens et al., 2018), regional (eg. Bobbink et al., 1998; Dise et al., 2011) and ecosystem levels (eg. Stevens et al., 2022). A relatively consistent picture emerges from this work, described by Bobbink et al. (2010). Enhanced levels of reactive nitrogen in the soil initially increase plant productivity, biomass and litter production. This leads to taller, denser vegetation, and reduced penetration of light to ground level (eg. Bobbink, 1991), creating enhanced competition for light and leading to the competitive exclusion of slower and lower growing plant species (DeMalach et al., 2017; Hautier et al., 2009). Eventually, with increasing inputs of nitrogen, the system becomes saturated, and productivity limited by some other nutrient, often phosphorus. Similar changes occur when nitrogen inputs lead to acidification, as this is accompanied by eutrophication, but as pH declines, there is a shift towards acid-tolerant plant species.

Increases in plant nitrogen content resulting from the uptake of the additional reactive nitrogen are a widely-reported consequence of these changes (eg. Hicks et al., 2000), and this can continue even after productivity stops increasing (Bobbink et al., 2010). This leads to a fall in the C:N ratio of plant tissues, and an increase in their N:P ratios (Sardans et al., 2012). In environments where acidification is also occurring, the latter may be increased further by the rise in dissolved aluminium and iron in the soil, which can bind phosphorus (Vogels et al., 2017), as well as reduced mycorrhizal infection of plant roots (Pearson & Stewart, 1993), both of which reduce phosphorus uptake by plants.

These results primarily concern field-layer vegetation, and there appears to be little evidence to suggest that nitrogen inputs are leading to changes in the composition or structure of tree and shrub communities (Bobbink et al., 2010), perhaps owing to their much slower rates of turnover. Variable

changes in the chemistry of tree tissues, and reductions in vigour and growth rate (mostly due to acidification) have been reported however (Schmitz et al., 2019). In field-layer communities, increased inputs of reactive nitrogen therefore cause vegetation to become less open, and the loss of plant species requiring these conditions – bare ground and shorter vegetation is gradually lost, followed by lichens and mosses, and eventually forbs of small stature and dwarf shrubs, which are replaced by graminoids (Bobbink et al., 2010; Dise et al., 2011). This leads to an overall loss of heterogeneity, both in terms of vegetation structure, and plant diversity (Midolo et al., 2019; Payne et al., 2017), with a few nitrophilic plant species replacing a larger variety of nitrophobic species (Staude et al., 2020) – the 'nitrogen homogeneity hypothesis' advanced by Gilliam (2006).

Studies of these changes across a wide variety of plant communities have enabled the development of 'critical loads' for acidification and eutrophication for different ecosystems. These were first proposed by Nilsson (1988) as 'the highest load [of reactive nitrogen] that will not cause chemical changes leading to long-term harmful effects on most sensitive ecological systems [via acidification or eutrophication]'. Analyses overlaying critical loads onto land-cover maps and estimated levels of deposition can provide an insight into the scale of changes to plant communities by nitrogen deposition. Bouwman et al. (2002) for example, estimated that globally, the critical load for acidification is exceeded in 7-17% of ecosystems, and for eutrophication, 7-18%. These impacts are not evenly-distributed however, and are much higher in industrialised nations with high levels of reactive nitrogen production and deposition, In the United Kingdom, for example, where levels of atmospheric nitrogen deposition average 10.7 kg N ha^{-1} yr^{-1} but can get as high as 70 kg N ha^{-1} yr^{-1} (Tomlinson et al., 2021), many sites exceed their critical loads (Table 1). Rowe et al. (2022) found that in 2010, 63.4% of acid-sensitive habitats in the UK and 74.4% of nitrogen-sensitive habitats exceeded their critical loads for acidification and eutrophication respectively, although both proportions have fallen somewhat since (to 52.9% and 67.7% in 2019).

Habitat	Eutrophication Critical Load (kg N ha^{-1} yr^{-1})
Dune Grasslands	9-12
Raised and Blanket Bogs	8-10
Calcareous Grassland	15
Dry Acid Grassland	10
Wet Heaths	10
Dry Heaths	10
Woodland	10-20

Table 1: *Critical loads for eutrophication for a sample of UK habitats. Values are the 'UK Mapping Value' for each habitat from Hall et al. (2015). Ranges are given in cases where multiple values are given for sub-habitats (eg. 9 kg N ha^{-1} yr^{-1} for acid dunes, and 12 kg N ha^{-1} yr^{-1} for non-acid dunes).*

Understanding of the likely nature and extent of the impacts of nitrogen pollution on plant communities comes from three main sources – experiments adding different forms of reactive nitrogen to natural vegetation; space for time studies, linking variation in plant community structure to variation in nitrogen pollution levels, usually at large scales; and longitudinal studies, linking changes in plant communities over time to levels of nitrogen pollution (Dise et al., 2011). Experiments provide clear evidence of the potential effects of reactive nitrogen on plant communities (Bobbink et al., 2010), but it is often questioned whether these are realised under nitrogen pollution in the field. Many experiments use large doses of nitrogen applied over short periods and may not realistically represent chronic deposition (Clark & Tilman, 2008). For this reason, field studies relying on natural variation in deposition levels provide a useful complement to experimental results. Small-scale gradients of deposition next to point sources for example, provide a natural experiment (Pitcairn et al., 1998, 2003),

436 although such gradients are rare in the most sensitive habitats, and like true experiments, can only
437 provide evidence of the potential large-scale effects of deposition. Larger-scale space-for-time studies,
438 examining variation in plant communities along gradients of deposition across countries or regions
439 have been a popular approach for this reason, reporting declines in plant richness and loss of habitat
440 specialists across a variety of ecosystems, but it is a challenge to resolve weak signals of deposition
441 from other differences between sites (Damgaard et al., 2017; Field et al., 2014; Maskell et al., 2010;
442 Pescott & Jitlal, 2020; Roth et al., 2013; Stevens et al., 2004, 2009). Longitudinal data from re-surveys
443 of vegetation plots and national plant monitoring schemes can suffer from this challenge too, but at
444 least provides clear signals of changes in plant communities over time (Smart et al., 2003, 2005).

445 Collectively, these sources of evidence provide a relatively consistent picture of the impacts of
446 anthropogenic inputs of reactive nitrogen on plant communities, even if individually their results need
447 to be interpreted with caution. One problem that is common to all approaches is that much of the work
448 takes place in regions where deposition levels are very high, providing the motive for inquiry. Thus,
449 many of the sites used will already have been heavily impacted by reactive nitrogen, reducing the
450 extent to which they will change under further inputs in experiments or differ across gradients of
451 deposition if all sites are already at or close to saturation in space for time studies (Phoenix et al.,
452 2012). Longitudinal studies might provide a solution to this problem if there were sufficient historical
453 data, but even in well-studied northern European ecosystems, almost no analyses have been performed
454 with historical data prior to the 1970s, when emissions of reactive nitrogen had been high for some
455 time. In consequence, although studies of plant communities provide a relatively consistent picture of
456 the impacts of nitrogen pollution, it seems likely that many studies will underestimate impacts on plant
457 communities, and over-estimate thresholds at which sites may exhibit significant changes.

Impacts of nitrogen pollution on insect communities

It has long been appreciated that plant communities can exert significant bottom-up control on the structure of insect communities (de Sassi et al., 2012; Jerrentrup et al., 2014; Murdoch et al., 1972). It therefore seems inevitable that the reported impacts of reactive nitrogen inputs on plant communities will be propagated up to the insects that live on and among them. Indeed, nitrogen-addition experiments of the kind that provide much of the knowledge of the impacts on plant communities usually also report strong impacts on invertebrate community structure when this is assessed. A recent meta-analysis of these experiments by Nessel et al. (2021), for example, reported declines in invertebrate abundance with nitrogen addition across terrestrial ecosystems. Gallego-Zamorano et al. (2023) performed a similar analysis and found that responses varied between arthropods with incomplete metamorphosis and complete or no metamorphosis, the former increasing, the latter decreasing. The latter trend was particularly strong in warmer environments, and Gallego-Zamorano et al. (2023) suggest that this heterogeneity is why neither they nor Nessel et al. (2021) were able to detect an overall effect on species richness. Like plants then, insect responses to nitrogen additions are diverse and context-dependent. This likely reflects the way in which several simultaneous changes to vegetation structure and composition under nitrogen addition interact with diverse insect requirements to affect insect communities by a number of different mechanisms.

These findings are striking in the context of increasing reports of declines in insect abundance, particularly in Europe and North America where levels of reactive nitrogen inputs are highest. Large declines in abundance have been noted in a variety of taxa in recent years (Conrad et al., 2006; Forister et al., 2021; van Klink et al., 2020; van Strien et al., 2019; Wagner, 2020), even on protected areas (Cooke et al., 2023; Hallmann et al., 2017), implying that rather than just habitat loss, diffuse anthropogenic impacts like nitrogen pollution and climate change could be driving changes in insect abundance (Wagner et al., 2021). In spite of this, studies exploring the impact of nitrogen pollution on

insect populations and communities are relatively scarce, particularly when compared to those considering other diffuse drivers of change. As a result, there is currently weak evidence of large-scale impacts on insect communities and understanding of the mechanisms that might drive these impacts is mostly derived from theory rather than experimental tests or direct observation.

Mechanisms for impacts of nitrogen pollution on insect communities

Nijssen et al. (2017) reviewed the main mechanisms via which consumers are expected to be affected by nitrogen pollution, in the context of atmospheric nitrogen deposition. They identified six main pathways, which are grouped into three by Vogels et al. (2023), and roughly follow the three main changes observed in plant communities – increases in vegetation growth lead to changes in the structure of habitat, changes in soil and plant chemistry lead to chemical changes to habitat (changes in host plant nitrogen content, or chemical stress in the soil), and changes in plant community composition lead to quantitative changes to habitat (reduction or increase in host plants). Of these, chemical stress is unlikely to be relevant to most terrestrial insects as it is restricted to aquatic environments, and so it is not discussed further here.

Changes in habitat structure and microclimate

It has long been appreciated that many insect species of grassland and heathland habitats are strongly thermally-constrained and require high temperatures in order to successfully complete their development (Van Noordwijk et al., 2012; van Wingerden et al., 1991). For species at the northern edges of their ranges, such conditions can be rare, constraining them to only use a small subset of the warmest habitats , and often just the warmest micro-habitats within these (Roy & Thomas, 2003). In open habitats such as grasslands and heathlands, such warm microhabitats are often provided by bare ground and litter. These warm up in the sun and radiate heat on sunny days. In contrast, taller green vegetation has less sunlight penetration and air circulation, reducing warming and buffering

temperature changes, meaning that these often remain close to ambient temperature (Curtis & Isaac, 2015; WallisDeVries, 2006).

Van Wingerden et al. (1991) were the first to observe that the increase in plant productivity, vegetation density and height observed under eutrophication (eg. Bobbink, 1991) would lead to a loss of these warm micro-habitats and might negatively impact species dependent on them. The idea was formalised into a hypothesis of 'microclimatic cooling' under nitrogen deposition by WallisDeVries & van Swaay (2006). They suggest that these effects are most likely for species do most of their feeding in early spring when temperatures are low and warm microclimates are therefore most important, and add that earlier plant growth in spring as a result of climate change may exacerbate this problem if insects do not also advance their development.

Yang et al. (2017) carried out an experimental test of the link between inputs of reactive nitrogen, plant growth, microclimate and insect performance by adding nitrogen to plots of alpine grasslands on the Tibetan plateau where the caterpillars of the moth *Gynaephora alpherakjj* were feeding on *Carex* spp. This was applied as Ammonium Nitrate at levels similar to ambient deposition in the region. They found that this treatment increased plant nitrogen content by 10%, improving the performance of larvae in the lab, but not in the field where plant height increased by 25% and temperatures at the ground surface were reduced (by approximately 5.5°C in the mornings), decreasing the time caterpillars could spend feeding on a given day by 27%. Larval growth rates declined, and the duration of the larval period increased, although there was no overall impact on survival. This work strongly supports the possibility of microclimatic cooling as described by van Wingerden et al. (1991), although it did not seek to test the interaction with climate change suggested by WallisDeVries & van Swaay (2006). While the Yang et al. (2017) study does not show an effect of microclimatic cooling scaling up to a population-level impact, there is ample evidence that the availability of suitably warm microclimates can dictate the occurrence and abundance of specialist insect species (Bourn & Thomas, 2002; J. A. Thomas et al., 2001, 2011), and the work of Yang et al. (2017) strongly supports the ability

of nitrogen inputs to shape this. The potential for impacts on insects by microclimatic cooling therefore seems well-supported, at least in contexts where management does not remove biomass and prevent the build-up of green vegetation.

The strongest evidence of microclimatic cooling scaling up to impact insect populations comes from Roth et al. (2021) who examined how spatial variation in nitrogen deposition in Switzerland impacted the structure of plant and in turn butterfly communities. They found that at low altitudes, nitrogen deposition mainly led to denser, cooler and more humid vegetation, leading to a decline in butterfly richness, which they interpret as evidence of microclimatic cooling. Other work relates spatial variation in nitrogen deposition to trends in the abundance and richness of insect taxa with different traits, interpreting stronger impacts on thermophilous species as consistent with cooling. Hendriks et al. (2013) for example related spatial variation in Orthoptera richness trends at a variety of spatial scales in the Netherlands to spatial variation in nitrogen deposition. They found the strongest negative effects of deposition on the richness of species with low dispersal and high habitat specificity, laying eggs in the soil. Because the latter are strongly thermally constrained (van Wingerden et al., 1991), they conclude that this also represents evidence of microclimatic cooling induced by nitrogen deposition.

Similarly, at larger scales, WallisDeVries & van Swaay (2006) tested for signals of microclimatic cooling in an analysis of the population trends of butterflies across north-west Europe. They found that a greater proportion of species that overwinter as eggs or larvae were in decline than species overwintering as pupae or adults, and that this proportion was higher in countries with warmer springs and higher levels of atmospheric nitrogen deposition, arguing that this is indicative of microclimatic cooling driven by climate change and nitrogen deposition. McDermott Long et al. (2017) also found that UK butterflies overwintering as larvae and eggs showed significant declines in abundance after warmer winters, and more negative population trends for lepidoptera overwintering in less advanced stages is a finding repeated in similar analyses of lepidoptera trends to Wallisdevries & van Swaay

(2006) (albeit not in a nitrogen pollution context) across north-west Europe (reviewed by Tordoff et al. (2022)). Various explanations for these correlations are offered by the authors of these papers, including phenological mismatches between lepidoptera and their hosts, and increased vulnerability of egg and larval stages to attack by pathogens during warmer, wetter winters. Thus, the results of Wallisdevries & van Swaay (2006) though consistent with the impacts of widespread loss of warm microclimates due to nitrogen deposition, do not confirm them.

Changes in food quality

Like microclimate, the impacts of plant nitrogen content on insect performance have a long history of study, much of it prior to an appreciation of its possible relevance to the impacts of nitrogen pollution. This body of work clearly shows that variation in plant nitrogen content can have strong effects on insect performance (Throop & Lerdau, 2004), and that this can lead to impacts on population size under experimental manipulations of plant nitrogen content (Kyto et al., 1996), natural variation in plant nitrogen content (Cease et al., 2012; Clissold et al., 2006; Zhu et al., 2019), and along gradients of nitrogen pollution (Jones et al., 2008, 2011).

Historically, these impacts were mainly expected to be positive, owing to the far higher nitrogen content of most insects than their host plant (Boersma & Elser, 2006; Elser et al., 2000), reflecting the fact that the main structural component in insects is the nitrogen-containing polysaccharide chitin, and in plants structural carbohydrates cellulose and lignin. The result is that to obtain sufficient quantities of nitrogen, insects are expected to have to over-eat and excrete carbon at a cost of time and energy, and therefore experience benefits from increasing plant nitrogen content - the 'nitrogen limitation hypothesis' (Mattson, 1980; White, 1993). However, contrary to this hypothesis, some nitrogen addition experiments report negative responses of insects to increased plant nitrogen content, sometimes for species that also show positive responses in other experiments (eg. Kurze et al., 2018; Mevi-Schütz et al., 2003; Soontiens & Bink, 1997).

In some cases this may be caused by accumulations of nitrogen-based plant defensive compounds, but work in nutritional ecology suggests that more fundamental constraints underlie these differences, and that they can be reconciled as part of an overall unimodal response to plant nitrogen content. This may come about when insects do not attempt to maximise their intake of nitrogen, but carefully regulate it alongside a variety of other nutrients, aiming to ingest each in a specific quantity (Simpson et al., 2004; Simpson & Raubenheimer, 1993; Sperfeld et al., 2017; Waldbauer & Friedman, 1991). As a result, while insects might normally experience a deficit of nitrogen, high levels may exceed their requirements and create a deficit of other essential nutrients, requiring them to over-eat and excrete nitrogen to compensate (Behmer, 2008; Simpson & Raubenheimer, 1993), with a reduction in performance as a result (Boersma & Elser, 2006; Raubenheimer et al., 2005; Simpson et al., 2004). Thus, all else being equal, insect responses to increasing levels of plant nitrogen (and any nutrient) should be unimodal. From a position of deficit, increasing levels of nitrogen will reduce the need for costly over-eating of other nutrients (mainly carbon), until levels rise so that the insect's requirements are met, and performance peaks. Performance then begins to decline with further increases, as the food becomes increasingly deficient in other nutrients relative to nitrogen, again requiring insects to over-eat.

This means that the positive and negative responses of insects to increasing plant nitrogen content probably represent different sides of an overall unimodal response (Kurze et al., 2018; Vogels et al., 2023). Experiments raising insect herbivores on agar-based diets varying in their protein and carbohydrate content have repeatedly shown the generality of these unimodal patterns (Raubenheimer et al., 2005; Simpson et al., 2004), as has work in aquatic systems with consumers fed algal food varying in its carbon and phosphorus content (Benstead et al., 2014; Elser et al., 2016; Laspoumaderes et al., 2015). However, perhaps owing to the historic expectation of positive, linear responses under the nitrogen limitation hypothesis, application of this theory to insect responses to increasing plant nitrogen content have been rare, with few experiments using more than one or two levels of nitrogen

addition, and even fewer attempting to understand which nutrients may become limiting when nitrogen is excessive (Vogels et al., 2023).

Such work is vital to understanding the likely impacts of nitrogen pollution on host quality in practice. Experiments with small numbers of fertilisation treatments will be unable to detect a unimodal response and precisely determine insect nutrient requirements that shape the direction of species' responses, while a failure to investigate which nutrients are likely to constrain performance when nitrogen is excessive limits understanding of the environmental contexts in which negative impacts are most likely. Phosphorus, for example, has been widely suggested to become limiting under high levels of nitrogen, based on evidence of positive responses to phosphorus additions for a number of insect species and its clear importance in aquatic systems (Perkins et al., 2004; Vogels et al., 2023). This suggests that negative effects of nitrogen are most likely in contexts where phosphorus is already scarce, for example on weakly-buffered heathland soils (Vogels et al., 2017).

Even where experiments do attempt to address these questions or parts of them, they do not compare the responses of multiple species, precluding a more detailed understanding of the variation in nutrient requirements among even closely-related species that work in nutritional ecology suggests exists (Audusseau et al., 2015; Behmer & Joern, 2008; Joern & Behmer, 1998; Raubenheimer & Simpson, 2004). This variation appears to arise from the complex interplay of species' food sources and life-history strategies. Species' habitats and host plants govern the nutrients available to them, thereby shaping their body stoichiometry and life-history strategy (Behmer, 2008; Frost et al., 2006; Ibanez et al., 2017), and in turn their requirements. Ultimately though, more experiments comparing species' responses to rising nitrogen content and relating their requirements to variation in life-history traits are required to fully understanding this complexity and the likely re-structuring of insect communities by changes to host quality under nitrogen pollution (Audusseau et al., 2015; Vogels et al., 2023).

Thus, while it is clear that rising plant nitrogen content under nitrogen pollution is likely to affect insect populations and community structure, the lag between the development of theory of insect nutrition and its application in this context means that there remains great uncertainty about the likely impacts – in which contexts and for which species they will be positive or negative.

Changes in food abundance

Many insects have strong associations with just one or a few host plant species and in such cases, the abundance of host plants or favoured floral resources may limit population size (Fourcade & Öckinger, 2017; WallisDeVries, 2004). This, combined with the consistent changes in plant community composition caused by nitrogen pollution suggests that it may affect insect populations via changes in the abundance and distribution of their host plants.

At large scales, there is some support for the role of declines in food or forage abundance in driving insect trends. Scheper et al. (2014) for example, found that the population trends of Dutch bee species in the 20th century were positively correlated with those of their primary pollen sources. Interestingly, they found the strongest declines in bees that depend on nitrogen-fixing Fabaceae (a finding replicated in the UK, e.g. Goulson et al., 2005), plants that are often reported to decline under rising levels of soil nitrogen (e.g. Helsen et al., 2014). More explicit attempts to link insect trends to the impacts of nitrogen pollution on their host plants have been made by comparing the population trends of insects feeding on hosts with different Ellenberg nutrient scores. These scores were derived for most central European plant species by Ellenberg (1979) and score plant species based on their preferred nutrient levels from 1 (low nutrients) to 8 (high nutrients). Fox et al. (2014), Betzholtz et al. (2012), Öckinger et al. (2006) and Pöyry et al. (2017) found relationships between host plant Ellenberg scores and UK moth occupancy trends, range expansion of Swedish lepidoptera, local extinctions of Swedish lepidoptera and trends in frequency of Swedish lepidoptera, respectively. Species using plants with higher Ellenberg nutrient scores fared better in each case, although other work has failed to find

correlations between species' trends and the Ellenberg scores of their foodplants, suggesting this is not a universal pattern (Hallmann et al., 2020; Tordoff et al., 2022). Carvalheiro et al. (2020) also compared richness trends of 'nitrophobic', 'nitrophilous' and 'nitrogen generalist' Dutch butterfly and bee species (based on the Ellenberg N scores of their food and forage plants) and found that historically, the richness of nitrophobic taxa had declined more than nitrophilic taxa, but both had shown recent recoveries (either increases, or a reduction in the rate of decline). Unlike other studies, they tested the effect of spatial variation in nitrogen pollution on recent trends and found that these recoveries were constrained by atmospheric nitrogen deposition, with high levels reducing the rate of recovery.

The analysis of Pöyry et al. (2017), however, showed that the Ellenberg score of the host plants of Swedish lepidoptera was positively correlated with their voltinism (species with more broods had higher scores) and body size, the latter being correlated in turn with dietary breadth and dispersal ability. Shifts in insect communities along these trait axes have been found in response to a variety of stressors other than nitrogen pollution (eg. Macgregor et al., 2019; Merckx, Kaiser, et al., 2018; Merckx, Souffreau, et al., 2018). Thus, like the results of WallisDeVries & van Swaay (2006) for microclimatic cooling, results from these correlative studies are consistent with the hypothesis that changes in the abundance of species' host plants due to nitrogen pollution are driving their trends, but cannot be taken as unambiguous evidence of it.

Studies clearly linking changes in nitrogen pollution, plant abundance and insect abundance, either experimentally or observationally would therefore be very valuable, but are largely lacking. A report by Weiss (1999) is perhaps the closest, chronicling the local extinction of the butterfly *Euphydryas editha* at a number of sites in the San Fransico bay area of California. He suggests that this was driven by a decline in the abundance of its host plant *Plantago erecta* due to invasion of its habitat by non-native annual grasses when grazing management ceased. Crucially, this shift in vegetation structure only happened at sites with higher levels of atmospheric nitrogen deposition.

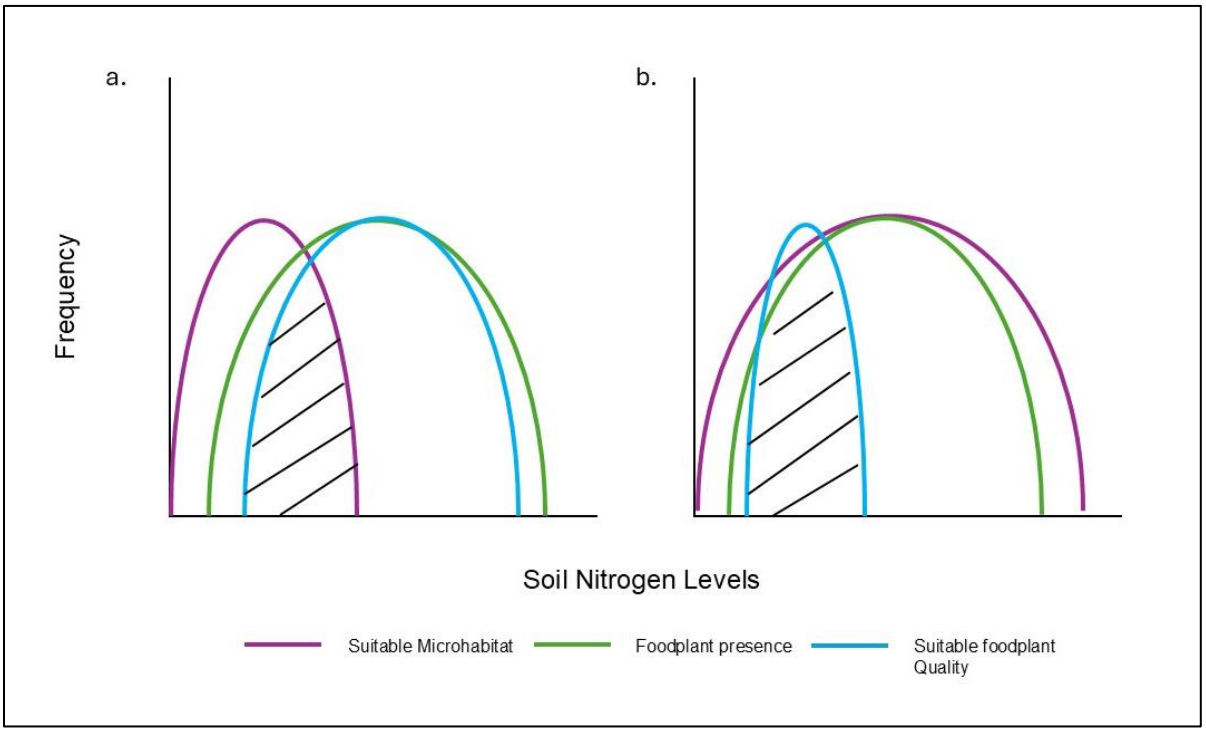
Shortcomings of the current evidence for impacts of nitrogen pollution on insect communities

In summary, there are three main aspects of habitat quality for insects that nitrogen levels shape the availability of – the abundance of their host plants, the nutritional quality of their host plants, and the availability of suitably warm microhabitats, that give rise to the three main changes via which nitrogen pollution is expected to impact insects: changes in vegetation structure, increases in plant nitrogen content altering food quality, and changes in plant community composition altering the abundance of food and forage plants. All of these mechanisms are supported by theory or a combination of insect responses to plant communities, and plant community responses to nitrogen pollution, but experimental tests of these mechanisms are scarce. This hinders understanding of how these mechanisms will impact insects in practice, when the effects of increased reactive nitrogen inputs are moderated by soil, plants and other stressors, and means that a clear idea of how the sensitivity of insect species to each mechanism varies, and why, is lacking, particularly for changes in plant quality. As a result, to some extent, these mechanisms remain theoretical, rather than robustly demonstrated at small or large scales.

Furthermore, perhaps because the theory supporting these mechanisms has been developed in separate contexts, their impacts on insect species or communities are usually considered in isolation. In practice, all three will act simultaneously, and how species are impacted by nitrogen pollution, and why, will depend on their thermal, nutritional and foraging requirements, and how the availability of all of these is shaped by soil nitrogen levels. Thus, focusing on one aspect of habitat quality that is affected by nitrogen levels might detect effects or predict sensitivity of species that are most strongly constrained in this dimension, but is likely to underestimate the impacts of nitrogen pollution for others (Figure 2). Work that integrates all of these mechanisms is therefore required to develop a more

699 rounded understanding of how insects are constrained by nitrogen levels, and their sensitivity to
700 nitrogen pollution.

701



702

703 **Figure 2:** *Hypothetical figures showing how soil nitrogen levels could shape the availability of*
704 *suitable microhabitat, suitable host plant quality, and the presence of host plants for two insect species*
705 *'a' and 'b'. Each species can only occur where their requirements in all of these dimensions are met*
706 *(black hashed area). Here species a and b are constrained by soil nitrogen and affected by nitrogen*
707 *pollution for different reasons, a mostly by the availability of suitable microhabitat (since other aspects*
708 *of its niche mostly overlap in the nitrogen levels at which they are available), and b mostly by the*
709 *availability of its host plant. Thus, using host plant Ellenberg score as a proxy for species' sensitivity*
710 *to nitrogen pollution might work well for species b but not a, while using traits associated with*
711 *microhabitat preference might work well for species a, but not b. A view that integrates all the ways in*
712 *which species are constrained by nitrogen pollution is therefore required to predict their sensitivity.*

Thus, although experiments indicate that insect communities will be impacted by nitrogen pollution, and large-scale analyses of insect population trends suggest that this is happening, a combination of a lack of detailed experimental work and integration of mechanisms means that an overall understanding of how different insect species are constrained by nitrogen levels and will be affected by nitrogen pollution is lacking. This thesis aims to investigate these different mechanisms via which nitrogen levels affect habitat availability for insects, alone and in combination, to better understand these constraints and insect sensitivity to nitrogen pollution.

Throughout, I focus on lepidoptera as a model group for this investigation. These are among the best-studied of insect herbivores, but they are also likely to be more strongly constrained than other taxa by nitrogen levels – many have highly-specialised foodplant associations (Wagner, Fox, et al., 2021), they contain a higher proportion of thermophilous species relying on warm microhabitats than other insect taxa (Hambler & Speight, 2004), and their tissues are among the most nitrogen-poor and phosphorus-rich of insect orders (implying low requirements for nitrogen in their foodplants, and the strong possibility of nitrogen-induced phosphorus limitation) (Fagan et al., 2002; Woods et al., 2004). Thus, nitrogen pollution is likely to be an important driver of changes in lepidoptera communities (WallisDeVries & van Swaay, 2017), and understanding how they are constrained by nitrogen levels is important, while previous work provides an abundance of insight and data to support the development and testing of hypotheses about these constraints.

Thesis Data Chapters

1. ***The effects of nitrogen levels on host nutritional quality for insect herbivores***

While theory suggests that the effect of plant nitrogen content on insect herbivore performance should be unimodal, this has rarely been tested and it is unclear which other nutrients become limiting at high levels of nitrogen, and how the nutrient requirements driving this unimodal response vary among species.

My first data chapter therefore tests the response of three butterfly species to a range of host plant nitrogen levels. Three species (*Lycaena phlaeas*, *Coenonympha pamphilus* and *Polyommatus icarus*) are tested, to contribute to understanding of how nutritional requirements vary among lepidoptera, and one species (*L. phlaeas*) is subject to more focused experiments to understand whether an induced phosphorus deficit is the reason for declining performance at high nitrogen levels.

2. The role of host nitrogen levels in structuring habitat use by insect herbivores

My first data chapter shows that insect nutritional requirements mean that plant nitrogen content can have strong effects on insect performance, but outside the lab, other aspects of habitat quality vary alongside host nutritional quality, and may not overlap with host plants of high nutritional quality, creating trade-offs for insect herbivores. My second chapter therefore examines the importance of these nutritional requirements relative to thermal requirements in dictating habitat use by a grassland butterfly, *Lycaena phlaeas*.

This interaction has rarely been studied, limiting understanding of the relative importance of these constraints for insects, and how they are likely to be affected by nitrogen pollution in practice, when it simultaneously affects the availability of their thermal and nutritional requirements.

3. Modelling the effect of nitrogen levels on insect distributions

My second data chapter shows the importance of taking a multi-dimensional view of insect requirements – habitat use is not just dictated by species' nutritional requirements and host-plant requirements, but also their micro-habitat requirements. Together, these dictate how nitrogen levels constrain habitat availability for a given insect herbivore.

In this chapter I therefore attempt to model the relationship between insect occurrence and nitrogen levels that arises from these different constraints to provide a better method for predicting species' sensitivity to nitrogen pollution and detecting the effects of nitrogen pollution on insect communities.

Chapter 2: The effects of nitrogen levels on host nutritional quality for insect herbivores

Contributors: William Langdon, Joost Vogels, Richard Fox, Owen Lewis

Nitrogen pollution can cause increases in plant nitrogen content. Experimental work shows that this is a change which insect herbivores respond strongly but heterogeneously to, with the same species often responding positively and negatively in different studies. Theory suggests that this heterogeneity can be reconciled in an overall unimodal response to rising levels of host-plant nitrogen, driven by species-specific optima. Performance should rise as nitrogen levels approach these optima and decline when host-plants are further from them and nitrogen is either deficient or excessive relative to other nutrients. We test for this pattern in three butterfly species, rearing larvae on a wide range of host-plant nitrogen levels. We find unimodal effects on performance for all three species, and indications that high nitrogen levels cause phosphorus deficiency for one species, though we were not able to link this to changes in performance. Mechanisms behind the unimodal responses may also have differed across the three species. These results suggest that the impacts of nitrogen pollution on host nutritional quality for insects will be dose, context and species dependent.

Introduction

Human activity has more than doubled inputs of reactive nitrogen into terrestrial ecosystems since the industrial revolution (Galloway et al., 2008; Vitousek et al., 1997). The impact of this change on plant communities has been profound (Bobbink et al., 2010), and appears likely to be propagated up the food web to herbivorous insects dependent on these plants (Throop & Lerdau, 2004). While it is increasingly clear that this is the case, with reports of insect community structure shifting in response to atmospheric nitrogen deposition (Carvalheiro, Biesmeijer, Franzén, Aguirre-Gutiérrez, et al., 2020;

782 T. Roth et al., 2021; WallisDeVries & van Swaay, 2017), understanding of the mechanisms
783 underpinning these responses remains poor (Nijssen et al. 2017). This hampers understanding of the
784 likely nature and extent of these shifts, and prevents interventions to mitigate negative biodiversity
785 impacts.

786 Nijssen et al. (2017) reviewed mechanisms via which nitrogen pollution can impact fauna and drive
787 these changes. For insects, these can be divided into two categories: changes in the abundance of key
788 resources via effects on vegetation structure and composition, and changes in their quality, via effects
789 on plant chemistry. In general terms, the primary change in plant chemistry under increasing inputs of
790 nitrogen is an increase in the nitrogen content of plant tissues (Throop & Lerdau, 2004), reflecting the
791 fact that plant nitrogen content is usually correlated with soil nitrogen levels (Fujita et al., 2013). As a
792 result, increases in plant nitrogen content are commonly reported in nitrogen-addition experiments
793 (Sardans, Rivas-Ubach, & Penuelas, 2012; Throop & Lerdau, 2004), and along both small (Pitcairn et
794 al., 1998) and large-scale (Hicks et al., 2000; Pitcairn et al., 1995) gradients of atmospheric nitrogen
795 deposition.

796 Plant nitrogen content has often been regarded as a key determinant of food quality for insect
797 herbivores, owing to the far higher nitrogen content of insects than their host plants, which implies
798 widespread nitrogen limitation (Mattson, 1980; White, 1993). Many studies have demonstrated
799 changes in measures of individual insect performance (including survival, growth rate, and mass of
800 adults or pupae) in response to nitrogen fertilisation of hostplants (Throop & Lerdau, 2004), and some
801 have gone further, showing population-level responses to natural variation in plant nitrogen levels
802 (Clissold et al., 2006), experimental nitrogen additions (Kyto et al., 1996), agricultural activity (Cease
803 et al., 2012) and small-scale gradients of atmospheric nitrogen deposition (Port & Thompson, 1980).

804 This work paints a complex picture of insects' responses however, and while there are many positive
805 responses to increasing plant nitrogen content (Mattson, 1980; White, 1993), some species also show

negative responses (Fischer & Fiedler, 2000), or even a mix of the two (eg. Kurze et al., 2018; Mevi-Schütz et al., 2003; Soontiens & Bink, 1997). Research in nutritional ecology suggests that these varied responses to plant nitrogen content reflect the complexity of insect nutrient requirements, and their interaction with experimental design – how much nitrogen is added, and in what way. This work has shown that while insects are often limited by nitrogen and have to regulate their consumption and processing of food to make best use of what is available, they must also do the same for a wide variety of other nutrients, with each required in a specific amount (Simpson et al., 2004; Simpson & Raubenheimer, 1993; Sperfeld et al., 2017; Waldbauer & Friedman, 1991). Food selection and processing for insects is therefore not simply about maximising the intake of one nutrient like nitrogen, but working towards a global optimum of a large number of different nutrient species (Raubenheimer et al., 2009).

Attaining such an optimum is a substantial challenge for herbivorous insects when the stoichiometry of the plants on which they feed varies greatly, and often fails to meet their requirements (Boersma & Elser, 2006; Persson et al., 2010). In part, this is achieved by feeding selectively on foods that closely match requirements (so-called 'pre-ingestive regulation'), but if such foods are not available, insects have to over-eat and excrete nutrients that are in excess on unbalanced foods in order to obtain sufficient quantities of the deficient nutrients (Behmer, 2008; Simpson & Raubenheimer, 1993). Both processes come at a cost of time, energy, and ultimately fitness (Boersma & Elser, 2006; Raubenheimer et al., 2005; Simpson et al., 2004). Thus, the effect of a change in the availability of a given nutrient on insect behaviour and performance cannot be understood without reference to other nutrients that are required by insects.

For this reason, insect nutrient requirements and food quality are often expressed in terms of ratios. Performance is maximised when the availability of the focal nutrient relative to others (i.e. the ratio of nutrients in food) meets insect requirements, and declines when it is deficient relative to other nutrients and insect performance is limited by it, or when it is in excess relative to other nutrients, and it creates

a limitation of these (Vogels et al., 2023). This implies that if levels of other nutrients are held constant, the relationship between nitrogen availability and insect performance will overall be unimodal (Vogels et al., 2023) (Figure 1). Under such a relationship, nitrogen additions, e.g. from atmospheric deposition, can reduce nitrogen limitation and improve food quality for herbivores, improving performance, or induce limitations of other nutrients, leading to a decline in performance, perhaps explaining some of the variability of the results of nitrogen addition experiments.

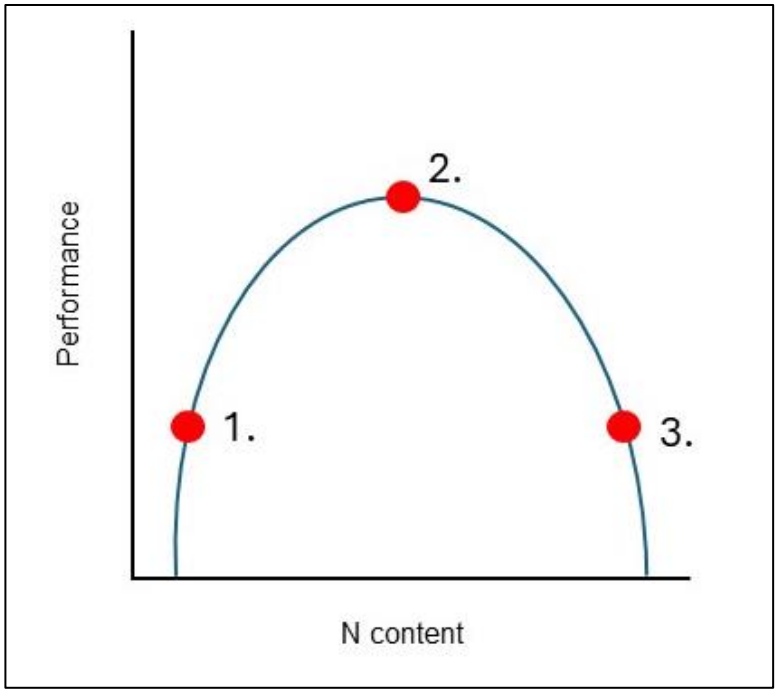


Figure 1: *The expected relationship between insect performance and plant nitrogen content when the availability of other nutrients is held constant. At low levels (1) nitrogen is deficient relative to insect requirements, and insects are nitrogen-limited. Increasing nitrogen levels therefore improve performance until (2), when nitrogen levels relative to other nutrients match insect requirements and performance is optimised. Further increases will result in a decline in performance (3), as nitrogen is in excess relative to insect requirements for other nutrients. Insects therefore have to over-eat and excrete nitrogen to maintain their intake of these other nutrients, at an increasing cost of time, energy and performance.*

While experiments raising insect herbivores on artificial, agar-based diets support this hypothesis and have validated the existence of unimodal responses to the availability of a variety of nutrients (Clancy & King, 1993; Raubenheimer et al., 2005; Simpson et al., 2004), studies showing such responses to nitrogen availability using insects' natural hostplants are scarce. In part, this probably reflects the challenge of predictably manipulating plant nitrogen content and keeping other aspects of host quality constant (eg. Tao et al., 2014), but more simply, most experiments have too few or too small a range of treatments to detect non-linear responses, perhaps owing to the historic expectation of positive, linear responses (Vogels et al., 2023). The same problem has been noted in studies of the impact of phosphorus additions in aquatic systems (Boersma & Elser, 2006). Where unimodal responses are detected, experiments still tend to use a small number of treatments, preventing a full characterisation of the shape of the response (Boersma & Elser, 2006; Vogels et al., 2023).

Accurate characterisation of species' responses to nitrogen availability is important because work on artificial diets in nutritional ecology demonstrates that even closely-related insect species vary in their requirements (Audusseau et al., 2015; Behmer, 2008; Behmer & Joern, 2008; Ibanez et al., 2017; Joern & Behmer, 1998), occupying specific 'nutritional niches' (González et al., 2017; Vogels et al., 2023). This implies that responses to nitrogen availability will also be species-specific, and the same nitrogen additions under the same conditions could move plant nitrogen content closer to the requirements of one species, but further from another, thus re-structuring insect communities. Understanding the nature and extent of this re-structuring depends on accurately characterising species' requirements, and understanding what drives variation in these, but with few experiments precisely defining species' requirements, and even fewer comparing responses among species, this remains difficult. (González et al., 2017; Vogels et al., 2023).

Here therefore we test for predicted unimodal, species-specific responses to nitrogen availability by assessing the performance of larvae of three widespread butterfly species (*Lycaena phlaeas*, *Coenonympha pamphiulus* and *Polyommatus icarus*) across a broad range of host plant nitrogen levels.

We focus on lepidoptera as they are one of the most nitrogen-poor lineages of insect herbivores (Fagan et al., 2002), implying a relatively low demand for nitrogen, a decline in performance at relatively low levels of plant nitrogen availability, and potentially negative responses to increasing plant nitrogen content under nitrogen pollution. We also test for the role of other nutrients in shaping these responses in a separate factorial experiment with one species (*L. phlaeas*) where both nitrogen and phosphorus levels are varied factorially. We focus on phosphorus as lepidoptera are one of the most phosphorus-rich insect orders (Woods et al., 2004), implying a relatively high demand for this nutrient which, like nitrogen, is scarce in their host plants. Indeed, there is evidence of a number of lepidoptera species responding positively to increases in the availability of phosphorus (Clancy & King, 1993; Goverde et al., 2000, 2004), suggesting that some species are already limited by it and that phosphorus is likely to be the commonest limitation suffered by lepidoptera under rising nitrogen levels (Tao & Hunter, 2012).

Methods

Study Species

All three of our study species occur in similar oligotrophic or mesotrophic habitats, often side by side, yet have host plants with large differences in nitrogen content. The nitrogen-fixing Fabaceae used by *P. icarus* are the most nitrogen-rich, followed by the *Rumex* spp. on which *L. phlaeas* is oligophagous (primarily using *Rumex acetosa* and *Rumex acetosella* in the UK) and the grasses used by *C. pamphilus* (primarily fine-leaved species) (J. A. Thomas & Lewington, 2014) (Figure S1). We expected this difference in host nitrogen content to shape species' tolerances, and *C. pamphilus* performance to peak at the lowest levels of nitrogen, and *P. icarus* the highest. This notion is partially supported by previous work by Kurze et al. (2018) who found negative effects of nitrogen fertilisation on *C. pamphilus* and *L. phlaeas* survival, which were strongest for *C. pamphilus*. The fact that Mevi-Schütz et al. (2003) also showed positive effects of a single level of nitrogen fertilisation on *C. pamphilus* pupal mass and Soontiens & Bink (1997) demonstrated that growth rate and pupal mass

peaked either at intermediate levels, or at the high levels of fertilisation, supports the suggestion that the overall response for this species will be unimodal. There is no previous work exposing *P. icarus* to nitrogen fertilisation, but two previous studies have shown strong positive effects of increased host phosphorus content on growth rate (Goverde et al., 2004) and survival (Goverde et al., 2000) suggesting that on its nitrogen-rich hosts, it may be more phosphorus limited than *C. pamphilus* or *L. phlaeas*.

Plant cultivation

In our experiment, *P. icarus* was fed on *Lotus corniculatus*, *L. phlaeas* on *Rumex acetosa*, and *C. pamphilus* on *Poa pratensis*. Plug plants of *L. corniculatus* and *R. acetosa* were purchased in compost in mid-April 2023 (Cumbria Wildflowers, Carlisle, United Kingdom and Naturescape, Nottingham, England). On receipt, plants were evenly divided into plastic trays (Garland Titan Tray, Garland Products Limited, Kingswinford, England) of dimensions 100 x 55 x 15cm, 10 for *L. corniculatus*, one for each of the treatments in the main experiment, and 14 for *R. acetosa* (with 4 extra for the factorial experiment). These contained a 5:2 mix (by volume) of horticultural sharp sand (Oxford Garden Centre, Yarnton, England) and vermiculite (Sinclairs Medium Grade, Dungannon, Northern Ireland) to provide a low-nutrient medium that would retain some water and fertiliser. These were kept in a greenhouse (Wytham Field Station, University of Oxford, England) with an 18:7 light:dark cycle, and a temperature maintained at 21°C. *P. pratensis* plants were grown from seed (Emorsgate Seeds, Wisbech, England) planted in sharp sand in the same greenhouse, germinating in early May 2023. After approximately 5 weeks they were also evenly divided into 10 plastic trays (Garland Deep Garden Tray, Garland Products Limited, Kingswinford, England) of dimensions 53 x 40 x 9.5cm, containing the same growing medium as used for the other plants.

Nitrogen and Phosphorus Addition

The trays of each plant species were randomly allocated to one of ten levels of nitrogen addition for the 'main' experiment with an additional four factorial nitrogen and phosphorus addition treatments for *R. acetosa* in the 'factorial' experiment. Fertiliser was applied weekly, crushed to powder and dissolved in 6.5l of water in the large trays, and 2.85l of water in the smaller trays. Weekly doses were calculated based on the proposed annual dose for each treatment divided by 52, multiplied by the tray area in hectares, and weighed out using a microbalance (OHAUS Pioneer PX224, OHAUS, Nänikon, Switzerland). We chose to apply fertiliser weekly in this manner, so as to avoid large spikes in nitrogen content and to more closely mimic chronic nitrogen pollution. Fertiliser was first applied to *R. acetosa* and *L. corniculatus* on 2nd August 2023, and *P. pratensis* on 17th September 2023, reflecting a later start date for *C. pamphilus* larvae.

Nitrogen was added in the form of ammonium nitrate (Scientific Laboratory Supplies, Wilford, England). In the main experiment, treatments were 0, 20, 40, 70, 110, 170, 230, 290 and 350kg N ha^{-1} $year^{-1}$. In the factorial experiment with *R. acetosa*, phosphorus was added in the form of triple superphosphate ($Ca(H_2PO_4)_2$) (Elixir Gardening Supplies, Morecambe, England). This was applied to two trays in a dose of 25.83 kg P ha^{-1} $year^{-1}$, alone in one and in combination with a dose of 350kg N ha^{-1} $year^{-1}$ in another. The latter was also applied in isolation to another tray, to give a separate nitrogen treatment, while the fourth was kept as a control.

The nitrogen treatments in the main experiment were chosen to cover the full range of nitrogen addition levels used in experiments with our study species previously. Both positive and negative responses have been observed over this range, implying that species' optima should fall within it. We anticipated that this would be towards the lower end of our range of nitrogen levels, and that differences in plant nitrogen content would be smaller between treatments with higher levels of nitrogen addition as saturation occurred (Gebauer et al., 1984; Kurze et al., 2018), hence the larger

increments between the highest treatments. We assumed that nutrient limitation induced by excess nitrogen would be strongest in the highest nitrogen addition treatment and therefore chose to use this for the factorial experiment. The phosphorus dose was designed to give a molar N:P ratio of 30 in the fertiliser added, and offset this limitation, as the optimum food molar N:P ratio for most insects is thought to be between 22 and 38 (Vogels et al., 2023).

Larval Rearing

Larvae for the experiment were sourced from females captured in the wild which were kept in netted cages indoors under heat lamps. These contained two live, unfertilised host plants (elevated on upturned pots to be near the roof of the cage where butterflies spent most of their time), as well as nectar sources kept in water and changed every two days. They were sprayed regularly and lined with moistened tissue paper to maintain humidity. Eggs were removed every two to three days and kept in a fridge in the laboratory to delay hatching until the start of the experiment.

Larvae were introduced into the experiment on the day they hatched, using a fine paint brush to move them individually into plastic pots where they were kept for the duration of the experiment (Taylor Davis, Wiltshire, England: 120ml volume, 69mm diameter, 52mm height). They were randomly allocated to a treatment and provided with fresh leaves from host plants in that treatment every two to three days. When larvae were fed, all the larvae of that species were fed, to minimise differences in the freshness of food among larvae. Upon feeding, pots were wiped to remove condensation, and old host plant material was removed. For a sample of the *L. phlaeas* larvae in the factorial experiment, all frass was then removed using a fine paint brush and frozen in a microtube for the corresponding larva. For all other larvae in the factorial and main experiment, pots were wiped to remove frass which was not retained.

Pots were kept in an incubator (PHCBI MIR-154 Cooled Incubator, PHC Europe, Breda, Netherlands) with a light:dark cycle of 18:6, temperature maintained at 25°C, and humidity mode set to ‘normal’

(corresponding to summer conditions in the United Kingdom). Pupation occurred in the rearing pots, from which all host plant material was then removed, leaving the pupa, which was checked daily until emergence when adults were frozen.

Whether larvae fed or not, larval and pupal survival, the mass of the pupa after hardening, the date that larvae were introduced to the experiment, pupated and emerged as adults were all noted for each larva, and adults were sexed based on their external morphology.

Chemical Analysis

To understand how our nitrogen treatments affected plant nitrogen content, and whether this led to nitrogen and phosphorus deficiencies in *L. phlaeas* larvae, we analysed the carbon, nitrogen and phosphorus content of samples of plant material from each experimental treatment, and the samples of *L. phlaeas* frass from the factorial experiment.

In both the main and the factorial experiment, one sample of plant material was taken for each treatment at the start of the experiment (the date on which the first larvae started feeding on that host plant) and the end of the experiment (the date on which the last larva finished feeding) (i.e. two samples per treatment). To make these samples as representative as possible, we removed material equally from each plant in each treatment (as we did when feeding larvae). Wet masses of samples averaged 10.8g (sd = 4.1) for *L. corniculatus*, 13.7g (sd = 5.1) for *R. acetosa* and 2.76g (sd = 1.4) for *P. pratensis*.

In the main experiment, *R. acetosa* and *P. pratensis* samples from all treatments at the end of the experiment were analysed, along with three samples from the start (in the 0, 110 and 350 kg N ha^{-1} $year^{-1}$ treatments) to ensure that differences between the treatments were relatively consistent throughout the experiment. For *L. corniculatus*, all samples from the start and end of the experiment were analysed, as by the end of the experiment *L. corniculatus* plants were suffering from an attack of mildew that may have affected the results of the analysis. In the factorial experiment, samples from

both the start and the end of the experiment were analysed. All frass samples from the factorial experiment were analysed.

Samples of frass were dried at 70°C for 48 hours, moved into microtubes and ground to a fine powder by bead beating (MP Bio FastPrep-24 5G, MP Biomedicals, Irvine, USA). Samples were beaten for two minutes with or two ceramic beads of diameter 2.3mm (Zirconia/Silica, Thistle Scientific, Warwickshire, England). Plant samples were dried at 70°C for 48 hours and ground to a fine powder using a Cyclotec laboratory mill (FOSS, Runcorn, United Kingdom).

Chemical analysis of plant samples was undertaken by Forest Research (Alice Holt, England). Approximately 10mg of each sample was analysed for carbon and nitrogen content and 100mg for phosphorus. Carbon and nitrogen analysis was done via combustion using a Thermo Flash Carlo Erba CN analyser (EA 1112 series) (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Phosphorus analysis was carried out using sulphuric acid digestion. Samples were kept in borosilicate tubes to which 1 ml of concentrated sulphuric acid is added and then two aliquots (0.4ml) of hydrogen peroxide (30%). Tubes were then heated for 30 minutes at 335 °C before further addition of hydrogen peroxide and further heating for 10 minutes, repeating this process of heating and hydrogen peroxide addition until the digests were clear. The samples were then made up to 15 ml with distilled water and analysed for phosphorus content using a Thermo Scientific ICP-OES CID spectrometer (iCap 6500, Thermo Fisher Scientific, Waltham Massachusetts, USA).

Chemical analysis of frass was carried out in the Department of Animal Ecology and Ecophysiology at Radboud University (Nijmegen, Netherlands). Analysis of phosphorus content was carried out using 10mg of material. This was digested using 4 ml nitric acid (65%) and 1 ml hydrogen peroxide (35%) in Teflon™ vessels using an Ethos D microwave (1200 MLS, Milestone, Sorisole, Italy). Digests were then diluted with ultrapure water up to 50ml and analysed using an inductively coupled plasma emission spectrophotometer (ICP-OES; model IRIS Intrepid II XDL, Thermo Fisher Scientific,

Franklin, USA). Analysis for carbon and nitrogen contents used 3 mg of material and an elemental CNS analyser (model NA 1500, Carlo Erba; Thermo Fisher Scientific, Franklin, USA).

Statistical Analysis

Plant Chemistry

In the main experiment we compared the chemistry of our host plants between species and treatments using separate linear models with treatment, plant species and their interaction as covariates for plant nitrogen content, carbon content, phosphorus content, nitrogen:carbon ratio and nitrogen:phosphorus ratio. All measurements for each element were in micromoles per gram. As we were unable to analyse large numbers of plant samples to account for temporal variation in plant nitrogen content we felt that individual measurements were not necessarily reliable and prone to sampling effects, but that the broad trend across all treatments should be robust. As a result, we used the predicted nitrogen content of each treatment from these linear models as our measure of plant chemistry for all further analyses of data from the main experiment. To do this we used the single sample from each treatment at the end of the experiment for *R. acetosa* and *P. pratensis*, and the start of the experiment for *L. corniculatus* (owing to the mildew infection on these plants at the end of the experiment). We faced a similar problem in the factorial experiment, with differences in several measures between the start and the end of the experiment. We therefore used the mean of each measurement for all further analyses involving plant chemical data from this experiment.

Larval Performance

We analysed the effect of plant chemistry on larval performance as measured by survival (to adult emergence), mass of the pupa, and duration of the larval period (in days, from hatching to pupation). We used separate models for each measurement and butterfly species and only included larvae that were noted to have fed in the experiment in the analysis, not those that refused food and died in their first instar. As our measure of plant chemistry we used predicted nitrogen content of each treatment

from our linear models, incorporated as a second-order orthogonal polynomial to test for the anticipated unimodal effect. As covariates, we then included the difference in days between the hatch date of each larva and the hatch date of the first larva of that species (hereafter ‘days since start’), to account for changes in conditions over the course of the experiment. Models of pupal mass and larval duration also included the sex of the adult. This did mean excluding larvae that did not survive the pupal stage from these analyses, but as pupal survival was high and we expected sex to explain a large amount of variation in our measures, we felt this was a reasonable trade-off. Sex was not included in survival models, as it was based on the morphology of the reared adult. Models of survival used binomial errors with a logit link, and models of pupal mass and duration used gaussian errors with an identity link. Model fit was checked with plots of residuals and fitted values.

Orthogonal polynomials were used because in ordinary polynomial regression first and second order terms are confounded with each other, preventing a clear identification of their separate contributions to the response. Orthogonal polynomial regression decomposes the relationship that arises from the first and second order terms into independent components encoding the importance of a constant rate of change (first order term) and a varying rate of change (second order term), enabling an unbiased test of their significance, and in the case of a second order term, whether the response is unimodal, as opposed to linear (Cisneros et al., 2014). However, because of this, the coefficients generated by orthogonal polynomials cannot be used to derive the equation of the resultant polynomial function.

In order to generate ordinary coefficients with which to calculate the turning point of our curves and the optimal nitrogen levels for each species we therefore re-ran the models using raw polynomial regression for each species that had significant second order orthogonal terms. From the optimal nitrogen levels derived in this way we were able to derive the optimal nitrogen addition level for each species (using our linear model of plant nitrogen content as a function of nitrogen addition) and the optimal carbon content and phosphorus content and C:N and N:P ratios for each species (using this

nitrogen addition level and our linear models of plant C:N and N:P ratios and carbon and phosphorus content as a function of nitrogen addition).

Nutrient Limitation

Our initial intention was to analyse performance of *L. phlaeas* larvae from the factorial experiment using similar model structures to the main experiment, replacing the polynomial term for nitrogen addition with nitrogen addition and phosphorus addition encoded as binary categorical predictors, with their interaction. This would enable us to understand whether phosphorus limitation induced declines in performance at high nitrogen levels in the main experiment by testing for a positive interactive effect of nitrogen and phosphorus (against neutral or negative effects in isolation), as additional phosphorus offset the negative effects of high nitrogen. We intended to further assess whether phosphorus limitation underpinned this change by assessing the extent of nitrogen and phosphorus limitation experienced by larvae in each treatment using a measure of 'relative frass enrichment' with nitrogen and phosphorus whose derivation is explained in Box 1.

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Box 1: Understanding nutrient limitation using frass chemistry

To understand the level of nitrogen and phosphorus limitation in each treatment of the factorial experiment we used a measure derived from a comparison of the chemistry of frass and host plant samples.

$$relative\ enrichment = \frac{frass\ elemental\ ratio}{food\ elemental\ ratio}$$

This metric is based on the assumption that if one element is excessive relative to another, the uptake of the deficient element or the excretion of the excessive element will be increased in proportion to the degree of deficiency or excess, to ensure intake of the two elements in the required ratio.

Either process will have the effect of increasing the concentration of the excessive element in frass relative to the deficient element. Thus, our measure of relative enrichment will be increased when the 'major' element (the element used as the numerator in the ratio) is in excess, and decreased when the 'minor' element (the element used as the denominator) is in excess. Importantly, this increase will be proportional to the degree of excess.

It therefore provides an intuitive way of understanding how balanced food is with respect to herbivore requirements, and the degree of different kinds of nutrient limitation on different food sources. Some simple rules of thumb are as follows:

Relative enrichment > 1 : The major element is in excess (eg. for C:N, there is a higher C:N ratio in frass than food, because the C:N ratio of food is greater than the C:N ratio required, and uptake of N or excretion of C has been increased to provide more N relative to C, leaving less N in frass relative to C than in food).

Relative enrichment = 1 : Food ratio matches required ratio (eg. for C:N, both C and N have been taken up or excreted with equal efficiency, resulting in the same ratio in frass as food.

Relative enrichment < 1 : The minor element is in excess (eg. for C:N, there is a lower C:N ratio in frass than food, because the C:N ratio of food is less than the C:N ratio required, and uptake of C or excretion of N have been increased to provide more C relative to N, leaving more N in frass relative to C than in food).

Thus, the optimal value for a given elemental ratio can be derived as the food ratio at which relative enrichment of frass is one, and food increasingly matches herbivore requirements and becomes more efficient as values of relative enrichment approach one for each elemental ratio, as smaller quantities of excessive nutrients have to be over-eaten and excreted. Note however that this only captures one aspect of food efficiency (the need to over-eat or not). Consider two food sources which both have a set of required elements in the right ratios but their concentrations are half as high in one. Both would have the same relative enrichment for each elemental ratio (1) but one would be half as efficient as the other.

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Like the performance measures, we intended to analyse relative N:P enrichment of frass (to see if there was a switch from nitrogen to phosphorus limitation in the high nitrogen treatment, and that N:P requirements were balanced in the nitrogen plus phosphorus treatment) as a function of nitrogen and phosphorus addition and their interaction.

However, initial analysis of the plant chemistry data from this experiment showed that our experimental manipulations had not had the expected impacts on nitrogen and phosphorus content in the hostplants (Figure 4) – nitrogen additions led to only small increases in nitrogen content, far smaller than those in the equivalent treatment in the main experiment. Phosphorus additions also had little effect, with phosphorus levels instead showing substantial variation independent of our treatments and the highest levels in the nitrogen addition treatment. This meant that the factorial experiment could not be used to examine the effect of phosphorus limitation induced by nitrogen addition on performance (although we provide plots of the performance measures in each treatment in Figure S4).

We were, however, still able to use variation in plant chemistry across the treatments to assess how nitrogen and phosphorus limitation varied on different food sources. To do this we calculated our measures of relative enrichment for C:P, C:N and N:P ratios, and analysed them as a function of their ratios in food, to understand which of these pairs of elements were in excess at different ratios in food, and enable us to estimate the optimal C:P, C:N and N:P ratios for *L. phlaeas* as the values of each ratio where relative enrichment was predicted to be one.

Previous work has not examined the relationship between frass and food elemental ratios in this fashion to understand nutrient limitation, and therefore understanding of how these should scale with one another is not well-developed. Intuitively, the relationship between frass and food ratios that underlies relative enrichment should not be linear – ratios must after all be greater than zero. Therefore, lacking a prior hypothesis about the shape of this relationship, but wanting to accommodate non-linearity, we used generalised additive models for this analysis, restricting the number of knots to

4 to accommodate potential turning points without overfitting. All other parameters were kept at their defaults. Posterior simulation was used to calculate the optimal values of each ratio for *L. phlaeas*. One thousand plausible sets of predictions (over the range of ratios in our data) were derived for each model, and from each set of predictions the value of the ratio where relative enrichment was predicted to equal one was taken as the optimum. For each ratio, the mean, 2.5^{th} and 97.5^{th} quantiles of the distribution of these optima across prediction sets were taken as the optimum, its lower confidence interval and upper confidence interval respectively.

All statistical analyses were carried out in R (R Core Team, 2024) using the readr, tidyverse, Hmisc, lubridate, forcats and broom packages for data manipulation (Grolemund & Wickham, 2011; Harrel, 2024; D. Robinson et al., 2024; Wickham, 2023; Wickham et al., 2019, 2024) and GGplot2, ggh4x and cowplot for data visualisation (van den Brand, 2024; Wickham, 2016; Wilke, 2024) and mgcv for generalised additive models (Wood, 2023).

Results

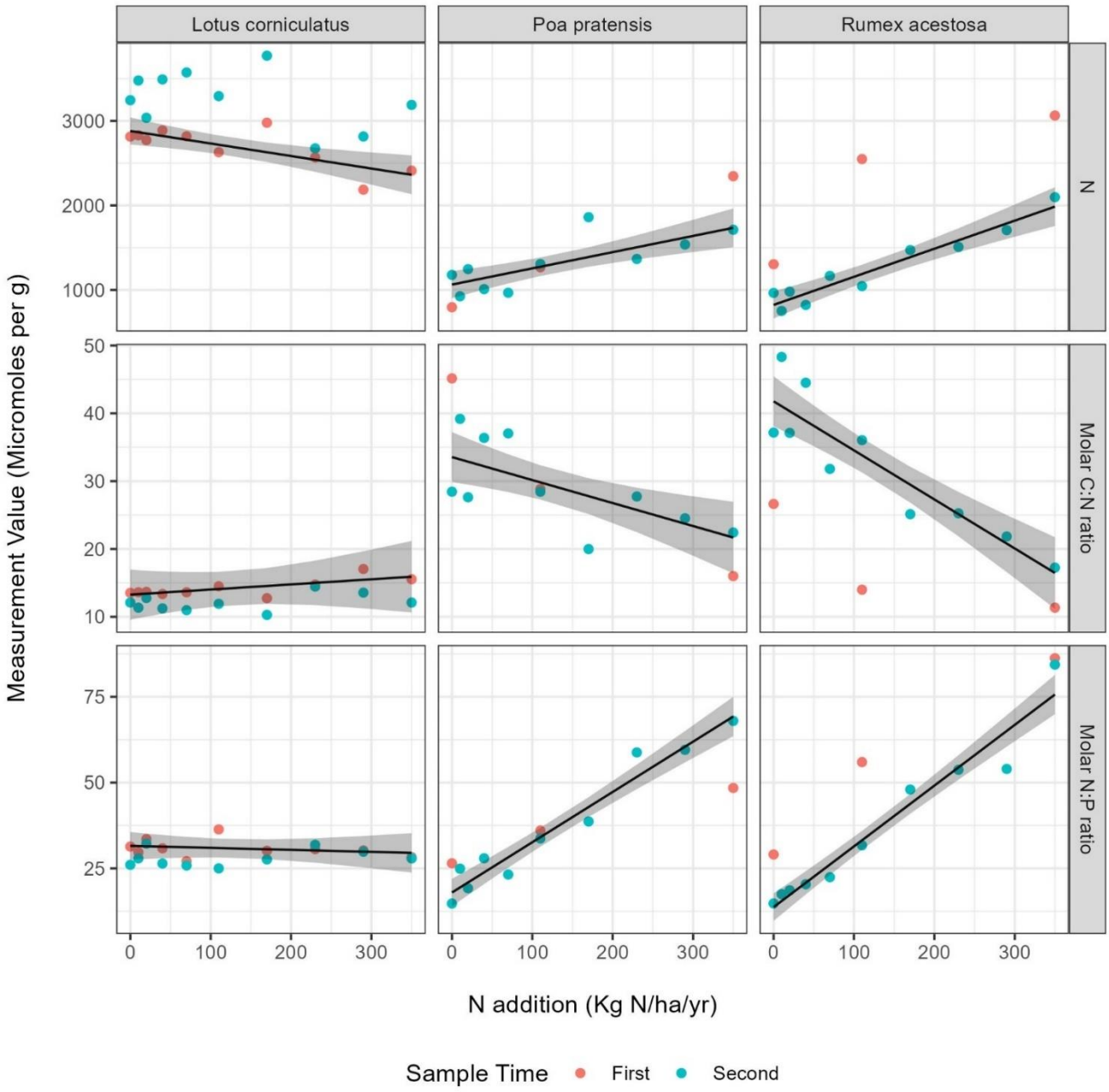
Main Experiment

Plant Chemistry

Nitrogen content increased significantly with nitrogen additions for both *R. acetosa* and *P. pratensis*, with a slightly steeper increase for *P. pratensis*. This in turn led to significant declines in C:N ratio (marginally less steep for *P. pratensis*) and increases in N:P ratio for both plant species. The response to N fertilisation in *L. corniculatus* differed significantly from *R. acetosa*, with a decline in N content, leading to significant differences in the trend in C:N ratio and N:P ratio across treatments, the former increasing slightly and the latter declining slightly.

In the control treatments, both *P. pratensis* and *L. corniculatus* had higher nitrogen contents than *R. acetosa*, although the magnitude of this difference between *R. acetosa* and *P. pratensis* was much smaller than *R. acetosa* and *L. corniculatus*. This meant that both plants also had significantly lower C:N ratios than *R. acetosa*, and higher N:P ratios, although the latter difference was only significant for *L. corniculatus*.

Model statistics for these results are displayed in table 1 and plotted in figure 2. Plots and model results for carbon and phosphorus content are also available in Figure S2 and Table S1.



1149 **Figure 2**: *Trends in N content, C:N ratio and N:P ratio across experimental treatments for L.*
1150 *corniculatus, P. pratensis and R. acetosa in the main experiment. Data from samples taken at the start*
1151 *(first) and end (second) of the experiment are plotted with predicted effects and their standard errors*
1152 *from models (Table 1). These were only fitted to data from the start of the experiment for L.*
1153 *corniculatus, and the end of the experiment for R. acetosa and P. pratensis.*

Table 1: *Results of models assessing differences in N content, C:N ratio and N:P ratio between experimental treatments and host plant species in the main experiment. R. acetosa was used as the reference group in these models. Models were only fitted to data from the start of the experiment for L. corniculatus, and the end of the experiment for R. acetosa and P. pratensis and therefore each are based on ten plant samples. All numbers are shown to 2 decimal places, apart from p values, which are shown to 3 significant figures.*

Measurement	Term	β	Standard Error	t	p
N Content	(Intercept)	823.08	80.91	10.17	< 0.001
	N	3.32	0.46	7.2	< 0.001
	L. corniculatus	2057.55	114.43	17.98	< 0.001
	P. pratensis	240.44	114.43	2.1	0.0463
	N:*L. corniculatus*	-4.8	0.65	-7.36	< 0.001
	N:*P. pratensis*	-1.4	0.65	-2.15	0.0417
C:N ratio	(Intercept)	41.77	1.86	22.45	< 0.001
	N	-0.07	0.01	-6.81	< 0.001
	L. corniculatus	-28.51	2.63	-10.83	< 0.001
	P. pratensis	-8.22	2.63	-3.12	0.00461
	N:*L. corniculatus*	0.08	0.02	5.32	< 0.001
	N:*P. pratensis*	0.04	0.02	2.56	0.0172
N:P ratio	(Intercept)	13.72	2.02	6.78	< 0.001
	N	0.18	0.01	15.33	< 0.001
	L. corniculatus	17.85	2.86	6.24	< 0.001
	P. pratensis	4.25	2.86	1.48	0.151
	N:*L. corniculatus*	-0.18	0.02	-11.2	< 0.001
	N:*P. pratensis*	-0.03	0.02	-1.87	0.0744

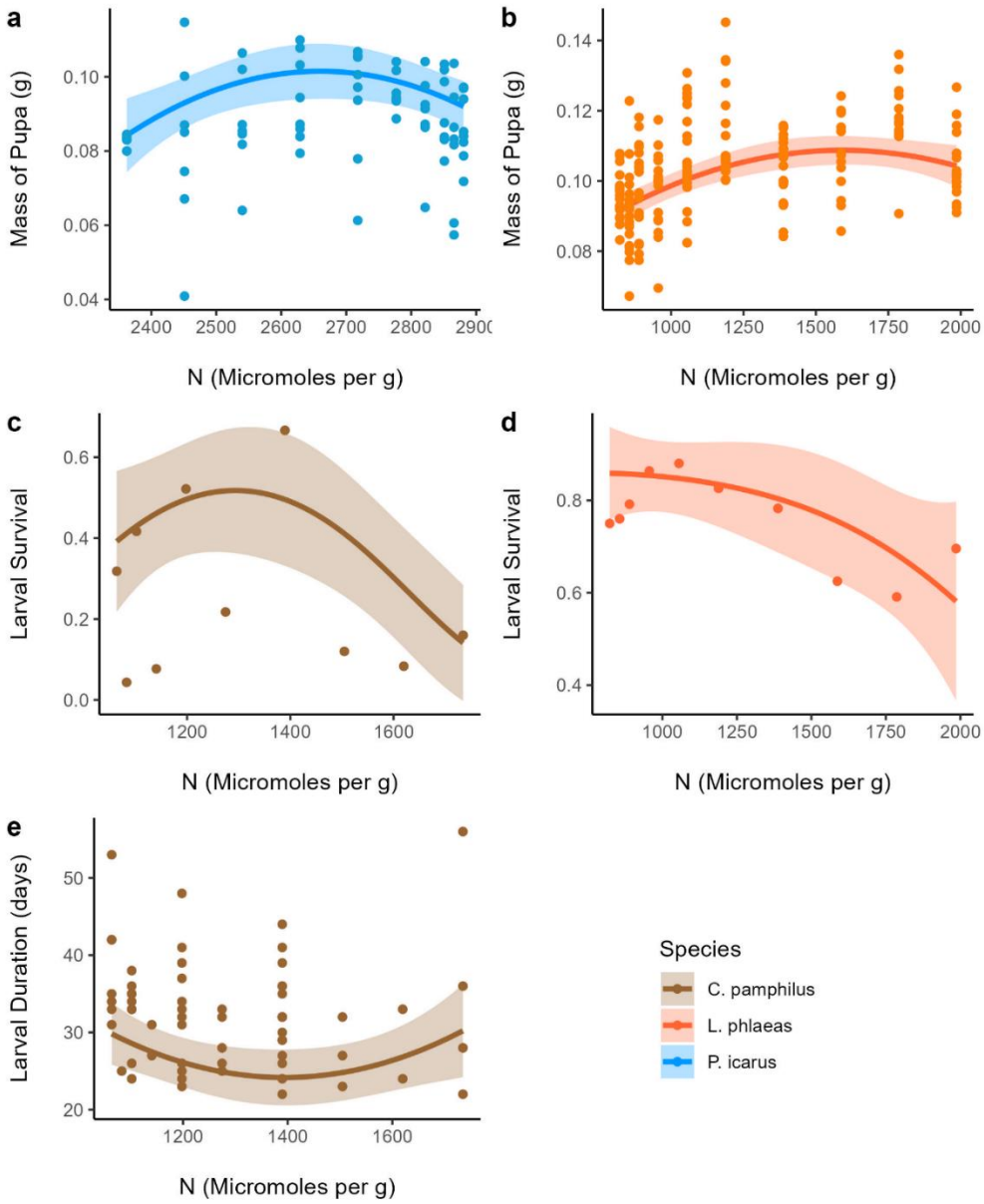
Larval Performance

All species showed a significant unimodal response to plant nitrogen content in at least one performance measure with performance peaking in intermediate treatments (Table 2, Figure 3). For *C. pamphilus* these were for larval survival and larval duration, and for *L. phlaeas* and *P. icarus* pupal mass. There was also a significant negative linear effect on survival in *L. phlaeas* (Table 2, Figure 3).

The highest-performing treatment for *P. icarus* was estimated to be more N rich than for *L. phlaeas* and *C. pamphilus*, and had both a lower C:N ratio and N:P ratio (Table 3). N content and C:N ratio in the optimal treatments for *L. phlaeas* and *C. pamphilus* were relatively similar, although the N:P ratio for *L. phlaeas* was higher (Table 3).

299 larvae of *L. phlaeas* were used in the main performance experiment, of these 277 accepted food (and were used in the survival models), and 226 survived to adulthood (and were used in pupal mass models and larval duration models). For *C. pamphilus*, 239 larvae were used, 192 accepting food and 51 surviving to adulthood, and for *P. icarus* the equivalent statistics were 87, 82 and 66 respectively.

Start date did not have a significant effect on any performance measure, except larval duration in *C. pamphilus*, which was increased with later start dates (Table 2). Sex had significant effects on pupal mass for all species (Table 2). Males weighed more than females in *P. icarus*, but less in *C. pamphilus* and *L. phlaeas*. For all species, males had shorter larval periods than females, but this difference was not significant in *P. icarus* (Table 2).



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1185 **Figure 3**: *Relationships between larval performance and N content for P. icarus, L. phlaeas and C.*

1186 *pamphilus. Only significant responses are plotted, non-significant responses are available in Figure*

1187 *S3. Lines show model predictions on the first day of the experiment for males (in the pupal mass and*

1188 *duration models, sex was not included in the survival models) and ribbons their 95% confidence*

1189 *intervals.*

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Table 2: *Results of models using second order orthogonal polynomials to describe the effect of plant nitrogen content on larval performance for P. icarus, L. phlaeas and C. pamphilus. Significant second order terms for N content (indicating a unimodal response) are highlighted with bold text. Test statistics for pupal pass and larval survival are t values, for larval survival z values. The reference sex is female.*

Species	Metric	Term	Estimate	Standard Error	Statistic	p
P. icarus	**Pupal Mass (g)**	Intercept	0.09	0	32.81	<0.001
		N (Micromoles per g, 1st order)	0.02	0.02	1.05	0.297
		N (Micromoles per g, 2nd order)	**-0.04**	**0.01**	**-2.94**	**0.00472**
		Days Since Start	0	0	-1.14	0.26
		Sex (male)	0.01	0	2.72	0.00857
	Larval Survival	Intercept	3.13	0.8	3.89	< 0.001
		N (Micromoles per g, 1st order)	3.91	2.83	1.38	0.167
		N (Micromoles per g, 2nd order)	-3.89	3.02	-1.29	0.198
		Days Since Start	-0.03	0.01	-1.84	0.0664
	Larval Duration (days)	Intercept	18.56	0.37	50.05	< 0.001
		N (Micromoles per g, 1st order)	1.77	2.1	0.85	0.401
		N (Micromoles per g, 2nd order)	0.62	2.02	0.31	0.759
		Days Since Start	0	0.01	-0.52	0.605
		Sex (male)	-0.27	0.42	-0.63	0.53
L. phlaeas	**Pupal Mass (g)**	Intercept	0.11	0	69.19	< 0.001
		N (Micromoles per g, 1st order)	0.07	0.01	5.31	< 0.001
		N (Micromoles per g, 2nd order)	**-0.05**	**0.01**	**-3.71**	**< 0.001**
		Days Since Start	0	0	0.31	0.756
		Sex (male)	-0.01	0	-2.83	0.00519
	Larval Survival	Intercept	1.41	0.27	5.31	< 0.001
		N (Micromoles per g, 1st order)	-7.01	2.57	-2.73	0.00637
		N (Micromoles per g, 2nd order)	-1.65	2.57	-0.64	0.52
		Days Since Start	0.02	0.02	1.04	0.296
	Larval Duration (days)	Intercept	18.64	0.25	73.55	< 0.001
		N (Micromoles per g, 1st order)	-0.37	2.3	-0.16	0.873
		N (Micromoles per g, 2nd order)	1.67	2.22	0.75	0.452
		Days Since Start	-0.02	0.01	-1.85	0.0664
		Sex (male)	-0.89	0.3	-2.96	0.00357
C. pamphilus	Pupal Mass (g)	Intercept	0.07	0	28.89	< 0.001
		N (Micromoles per g, 1st order)	0.01	0.02	0.64	0.523
		N (Micromoles per g, 2nd order)	0.01	0.01	1.02	0.314
		Days Since Start	0	0	1.48	0.145
		Sex (male)	-0.01	0	-4.61	< 0.001
	Larval Survival	Intercept	-0.44	0.26	-1.71	0.0874
		N (Micromoles per g, 1st order)	-5.63	2.59	-2.17	0.0297
		N (Micromoles per g, 2nd order)	**-5.33**	**2.43**	**-2.19**	**0.0286**
		Days Since Start	-0.03	0.01	-1.71	0.0864
	Larval Duration (days)	Intercept	31.79	1.9	16.77	< 0.001
		N (Micromoles per g, 1st order)	-2.47	14.04	-0.18	0.861

Species	Metric	Term	Estimate	Standard Error	Statistic	p
		N (Micromoles per g, 2nd order)	**28.66**	**11.44**	**2.5**	**0.016**
		Days Since Start	0.31	0.09	3.59	< 0.001
		Sex (male)	-4.73	1.82	-2.6	0.0126

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1198 **Table 3:** *Estimated levels of N addition, N content, C:N ratio, N:P ratio, P content and C content for*
1199 *the optimal treatment for each combination of species and performance measure with a significant*
1200 *second order effect of N content. Optimal N contents for each species x performance measure*
1201 *combination were calculated using raw performance models (Table S2), the optimal level of N addition*
1202 *from the N content ~ N addition linear model, and then other measures from models relating these to N*
1203 *addition.*

1204

Species	Metric	N Addition (kg N ha-1 yr-1)	N	C:N Ratio	N:P Ratio	P	C
C. pamphilus	Larval Duration	172.07	1393.55	27.72	43.19	37.75	36941.58
	Larval Survival	119.92	1293.53	29.48	35.54	42.98	36412.82
L. phlaeas	Pupal Mass	231.38	1591.73	25.04	54.67	31.52	37056.09
P. icarus	Pupal Mass	149.01	2659.93	14.39	30.70	87.11	37951.17

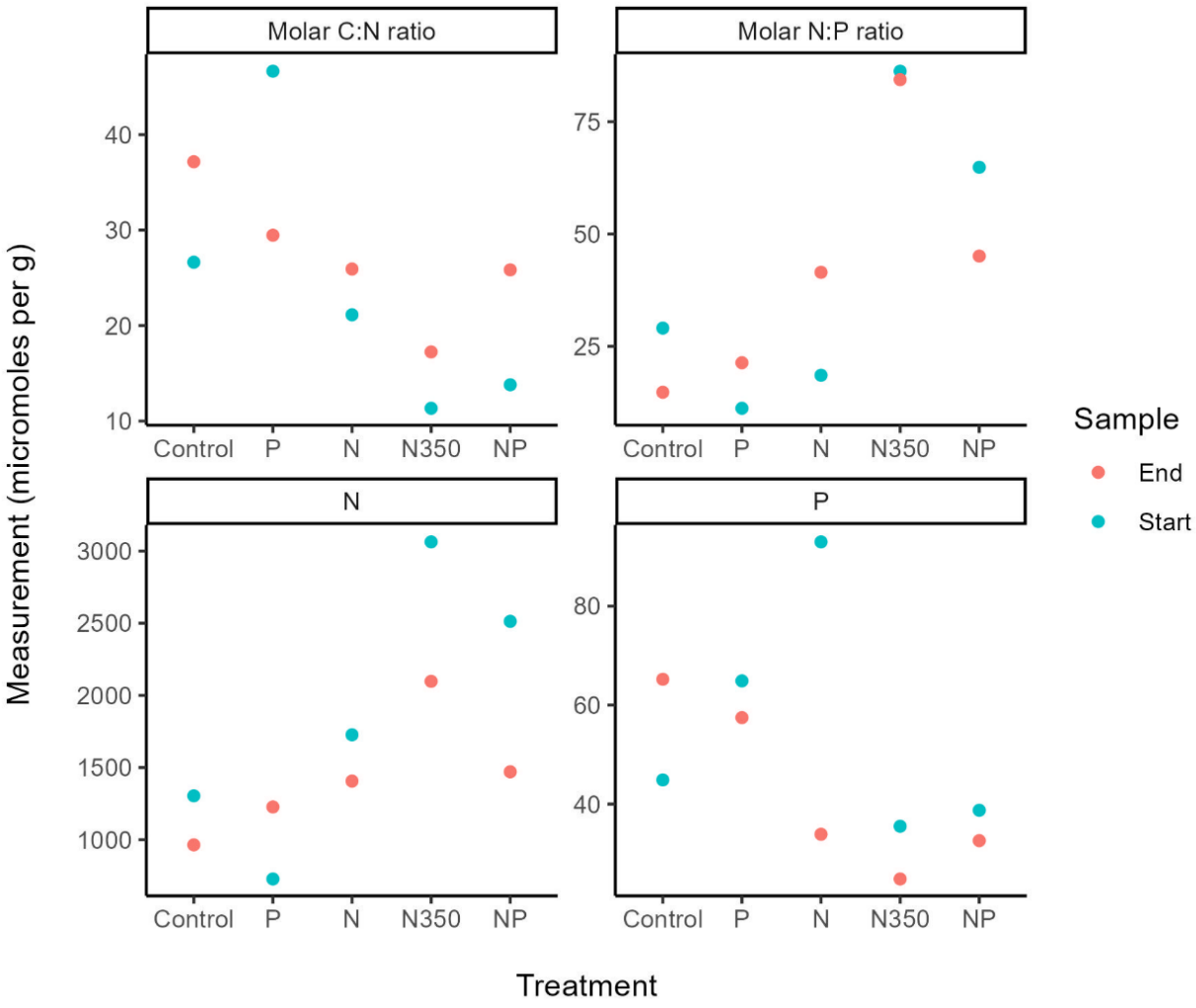
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Factorial Experiment

Plant Chemistry

In isolation, the nitrogen addition treatment in the factorial experiment only led to a small increase in *R. acetosa* nitrogen content, far lower than was achieved with the same N addition level in the main experiment, and rather than causing a drop in P content and N:P ratio, plants had a high P content and a low N:P ratio (Figure 4). Phosphorus addition led to a small increase in *R. acetosa* P content and fall in N content in isolation (Figure 4).The combined N and P addition treatment also had a lower N content than the N addition treatment in the main experiment, but a similar P content and N:P ratio, i.e. even with only a small increase in N content relative to the control, still showing little offsetting of the effect of increased N content on P content and N:P ratio.



1228

1229 **Figure 4**: *Differences in the chemistry of R. acetosa samples between treatments in the factorial*

1230 *experiment, compared with the highest N addition treatment in the main experiment (N350) which had*

1231 *the same dose of N as was used in the N addition and factorial N and P addition in this experiment.*

1232 *Samples are coloured by whether they were taken at the start of the experiment (when the first larva*

1233 *started feeding) or the end (when the last finished).*

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Relative Enrichment

Relative enrichment of *L. phlaeas* frass for C:P, N:P and C:N in the factorial experiment were all positively related to frass C:P, N:P and C:N ratios respectively. These relationships were significant for C:P ratio ($F = 5.49$, $p = 0.013$) and N:P ratio ($F = 20.5$, $p = 0.00040$) but not for C:N (though only marginally, $F = 4.24$, $p = 0.057$). No non-linearity was predicted for C:N and N:P (edf = 1), but some for C:P ratio (edf = 1.56).

The predicted optimal values for C:N ratio was 18.1 ($Q_{2.5} = 13.06$, $Q_{97.5} = 27.74$), for C:P ratio 746.08 ($Q_{2.5} = 537.39$, $Q_{97.5} = 904.59$), and for N:P ratio 38.27 ($Q_{2.5} = 31.54$, $Q_{97.5} = 48.56$).

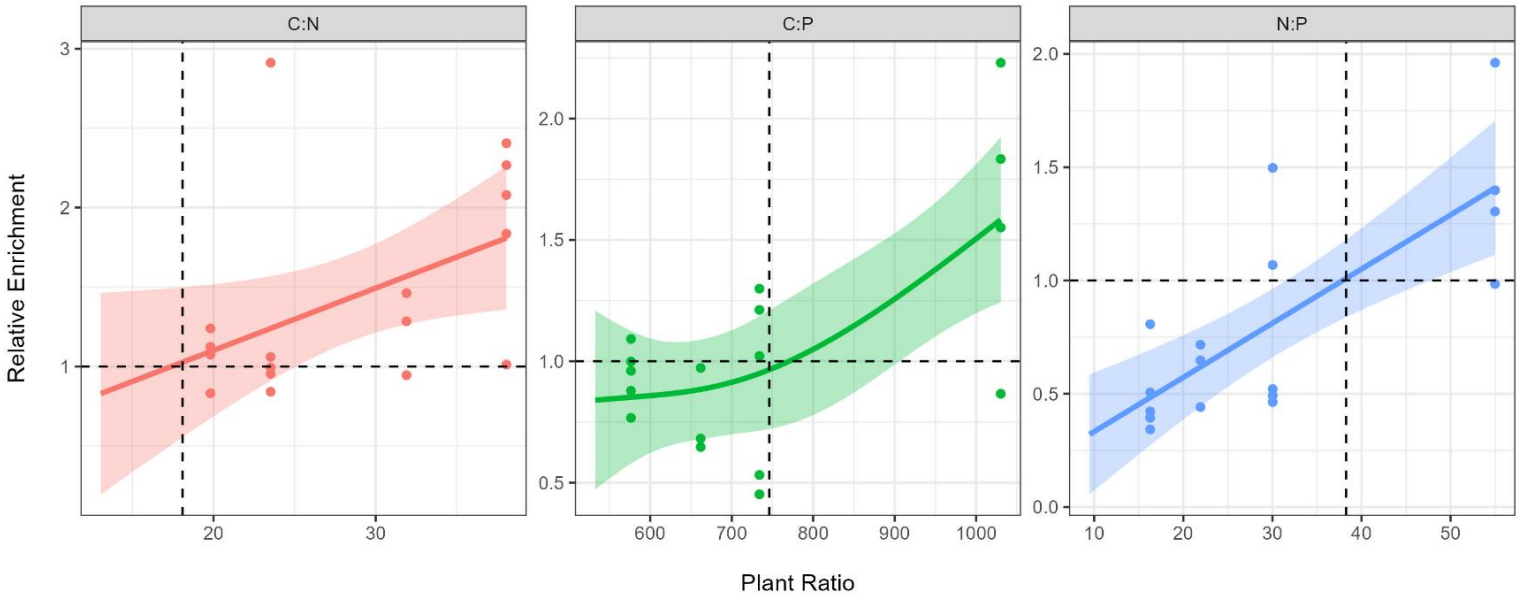


Figure 5: *Relative enrichment of frass for C:N, C:P and N:P as a function of C:N, C:P and N:P ratios in food. Solid lines show model predictions, and ribbons their 95% confidence intervals. The point at which relative enrichment = 1 (i.e. food is balanced with respect to L. phlaeas requirements) is highlighted for each ratio with dashed lines.*

Discussion

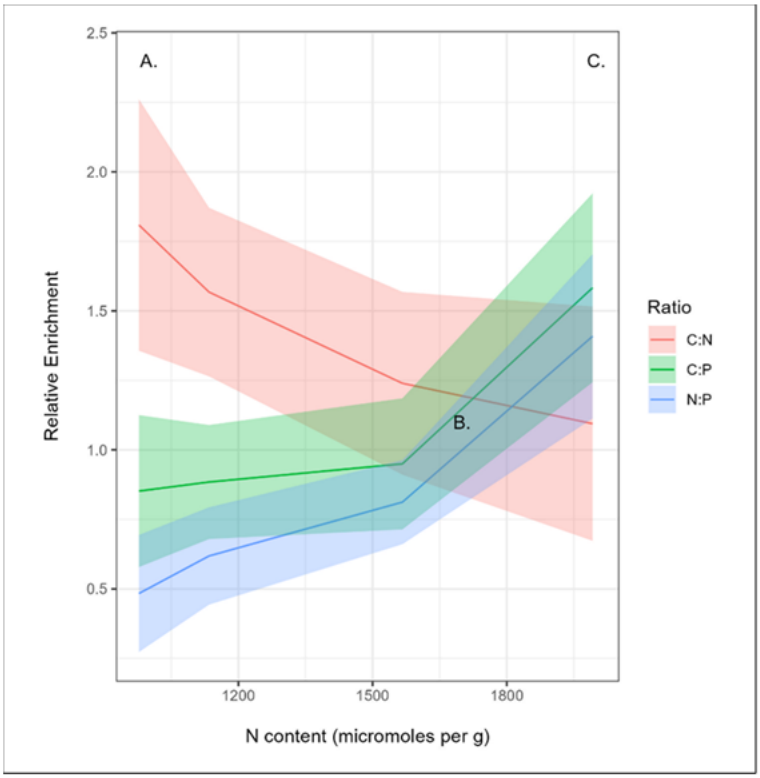
Interpretation

Rising levels of plant nitrogen content are a commonly reported consequence of real and simulated nitrogen pollution and are expected to strongly impact food quality for insect herbivores. Theory suggests that the direction and strength of these impacts will depend on species and context-specific unimodal responses of herbivore performance to plant nitrogen content, which have rarely been reported in nitrogen addition experiments. Our results show responses of the performance of three butterfly species to nitrogen addition that, in all bar one case, are unimodal, with performance improved relative to the control treatment by a small increase in plant nitrogen content, peaking at intermediate levels, and declining at high levels. Such unimodal responses were found across all performance measures examined (larval survival, pupal mass and duration of the larval period).

These results are consistent with the hypothesis that nitrogen additions initially relieve the nitrogen limitation on performance that is pervasive in insects, before causing a switch to limitation by another element, which is exacerbated by further additions, reducing the efficiency of feeding and reducing performance. This may reconcile the variety of positive and negative responses to rises in plant nitrogen content reported for insect herbivores in work with fewer treatments.

Our analysis of the C:P, C:N and N:P enrichment of frass relative to hostplants in the factorial experiment suggests that this may occur for *L. phlaeas* on *R. acetosa*. When enrichment for each ratio is plotted as a function of the nitrogen level in the host plants (rather than the respective ratio, as in figure 5), there is a strong indication that rising nitrogen levels initially improve the efficiency of food, as nitrogen limitation is relieved, before decreasing it again, as larvae switch to phosphorus limitation and further nitrogen additions exacerbate this (see Box 2).

Box 2: A switch from nitrogen to phosphorus limitation in the factorial experiment?



Predicted C:P, C:N and N:P enrichment of L. phlaeas frass relative to host plants in each treatment in the factorial experiment, plotted with nitrogen content of these treatments on the x axis to provide a common axis.

At low nitrogen levels (point A.) food is highly deficient in nitrogen relative to larval requirements (high C:N enrichment while C:P and N:P enrichment are less than one), but adequate, if not slightly enriched in phosphorus (frass is enriched in phosphorus relative to carbon, and to nitrogen). Larvae will thus have to over-eat and excrete phosphorus and carbon to maintain their intake of nitrogen, and nitrogen availability is the primary determinant of the efficiency of food (how much has to be eaten to meet insect nutrient requirements).

Increasing nitrogen availability reduces the nitrogen deficit (falling C:N enrichment) and improves the efficiency of food (less has to be eaten to meet insect nutrient requirements). The resultant decline in phosphorus content however leads to a deficit of phosphorus relative to carbon (rising C:P enrichment). This deficiency is initially smaller than the deficiency of nitrogen relative to carbon (C:P enrichment is less than C:N enrichment) and over-eating of food for nitrogen meets and exceeds *L. phlaeas* phosphorus requirements. Eventually however, these deficiencies become equal (point B) - C:P enrichment and C:N enrichment of frass is equal, meaning that N:P enrichment is one – phosphorus and nitrogen are taken up equally because they are equally deficient and present in the correct ratio.

At this point, any further increase in nitrogen content (B to C) will mean that phosphorus is more deficient relative to carbon than nitrogen is (C:P enrichment exceeds C:N enrichment), and therefore nitrogen is excessive relative to phosphorus (N:P enrichment exceeds one). Thus, the amount of food that *L. phlaeas* larvae have to eat to meet their nitrogen requirement is less than they have to eat to meet their phosphorus requirement, and phosphorus availability becomes the primary determinant of the efficiency of food. Further nitrogen additions reduce phosphorus levels, decreasing the efficiency of food.

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Predictions of frass C:N, C:P and N:P enrichment in the main experiment based on the chemistry of the plants in each treatment and our models suggest that *L. phlaeas* larvae would have experienced a similar shift from nitrogen to phosphorus limitation at around 140 kg N ha^{-1} yr^{-1} of nitrogen addition (Figure S5). This supports the notion that a switch from nitrogen to phosphorus limitation could occur under nitrogen addition and that this may be a driver of the unimodal responses observed in the main experiment, at least for *L. phlaeas* and *C. pamphilus* whose host plants show increasing C:N and N:P ratios with nitrogen addition. Interestingly, the reverse trends in C:N and N:P ratios were observed in the *L. corniculatus* fed to *P. icarus*. This decline in N:P ratio is consistent with previous work by Morecroft et al., (1994) who observed a similar trend in nitrogen fertilised *L. corniculatus* that also contrasted with other plant species in their experiment. As they did not find a strong trend in nitrogen content across treatments, they suggest that this may be driven by phosphorus limitation. In our case however, phosphorus levels were relatively stable for *L. corniculatus* (Figure S2), and this shift appears instead to be driven by a reduction in nitrogen content (Figure 2). Nitrogen fertilisation usually suppresses nitrogen fixation by Fabaceae (Reinprecht et al., 2020; Salvagiotti et al., 2008; Smith, 1992), and it may be that in our system this was not compensated for by uptake of nitrogen fertiliser, leading to an overall decline in plant nitrogen content. This decrease in N:P ratio and C:N ratio suggests that the opposite mechanism may have acted on *P. icarus* to *L. phlaeas* and *C. pamphilus*, and it experienced a shift from phosphorus limitation to nitrogen limitation. This is supported by the strong positive responses of this species to increases in plant phosphorus content in previous experimental work, implying that it may experience strong phosphorus limitation under normal conditions (Goverde et al., 2000, 2004).

The nitrogen addition level at which our relative enrichment models predicted *L. phlaeas* larvae in the main experiment would have switched from nitrogen to phosphorus limitation (140kg N ha^{-1} yr^{-1}, Figure S5) was lower than that at which their performance (in terms of pupal mass) peaked (231.38 kg N ha^{-1} yr^{-1}, Table 3). This lack of overlap may be biological in origin, perhaps reflecting the fact that

nitrogen and phosphorus deficiency do not have equal impacts on performance, with a relatively lower impact for phosphorus. Such differing fitness impacts have been reported before for protein and carbohydrate deficiency in Orthoptera (Simpson et al., 2004), and although phosphorus deficiency is predicted to be far greater than nitrogen deficiency at the point at which performance peaks, there is still nitrogen deficiency. Alternatively, it may simply be statistical in origin; *L. phlaeas* showed only a rather weak unimodal performance response, with little differentiation between intermediate treatments, and some uncertainty about the position of the peak as a result. *P. icarus* and *C. pamphilus* showed stronger responses, and the N:P ratios at which their performance peaked (27.72 and 29.48 for *C. pamphilus* larval duration and survival, and 30.7 for *P. icarus* pupal mass) are much lower than for *L. phlaeas* (54.67), and closer to the optimum derived for *L. phlaeas* from its frass enrichment plot (38.27). Furthermore, these lower values also closely match estimates of optimal N:P ratios for insect herbivores from previous work by Vogels et al. (2023) who analysed 13 experiments manipulating N:P ratios in the food of terrestrial consumers and suggested that phosphorus limitation occurs at N:P ratios of greater than 38, and detrimental effects of increasing phosphorus at N:P ratios of less than 22.

The difference between the optimum N:P ratio for *L. phlaeas* derived from its performance curve and from its frass enrichment plot therefore suggest that our performance curves may struggle to accurately resolve species' optimal food chemistry. Thus, while differences in the nitrogen levels at which each species' performance peaked are broadly consistent with our initial hypotheses – highest for *P. icarus*, and lowest for *C. pamphilus*, it is uncertain whether interspecific differences in our calculated optima (Table 3) really reflect species-specific responses and provide much insight into what drives interspecific variation in nutrient requirements. Nevertheless, the contrasting trend in N:P and C:N ratios in *L. corniculatus* demonstrate the likelihood of interspecific variation in how and why insect herbivores respond to nitrogen pollution, and our results thus emphasise the species-dependent nature of species' responses to increases in nitrogen content of their host plants.

It is important to note that our results, although consistent with phosphorus limitation, do not robustly demonstrate it, as our factorial nitrogen and phosphorus additions did not succeed in creating sufficiently high nitrogen or phosphorus levels to allow us to test whether phosphorus addition offset the decline in *L. phlaeas* performance observed at high nitrogen levels. The importance for food quality of other elements beyond nitrogen, phosphorus and carbon, so-called 'micronutrients', is increasingly appreciated for insect herbivores (Kaspari, 2021; Vogels et al., 2023), and there is evidence of insects suffering from limitations of these under normal conditions (Borer et al., 2019; Welti et al., 2019), and their role in structuring insect herbivore communities and interaction with nitrogen additions (Joern et al., 2012; Prather et al., 2018). Given that nitrogen was the only nutrient added to the plants in the main experiment, these micronutrients would probably have shown similar trajectories in C:nutrient and N:nutrient ratios to phosphorus, and perhaps be deficient relative to nitrogen and carbon in high nitrogen treatments as well. We focused on phosphorus in part because we were not able to accurately determine the concentration of most of these micronutrients in the small frass samples available from the factorial experiment, but mainly because of the widely-acknowledged importance of phosphorus to consumers (Boersma & Elser, 2006; Elser et al., 1996; Ibanez et al., 2017; Tao & Hunter, 2012) . It is the most common nutrient limitation in systems with adequate levels of nitrogen (Elser et al., 2007; Vitousek et al., 2010), and where phosphorus limitation has been tested for in terrestrial insect herbivores it has often been found (Goverde et al., 2004; Perkins et al., 2004).

If shifts from nitrogen to phosphorus limitation (or the reverse for *P. icarus*) do indeed drive the structure of the unimodal performance curves we observed, then responses are driven as much by the availability of phosphorus as nitrogen and the point at which herbivore performance peaks (assuming equal costs of nitrogen and phosphorus deficiency) will be that at which the N:P ratio of their host plants matches their requirements. This means that measuring plant carbon, nitrogen and phosphorus content is essential to understanding insect responses to nitrogen addition, and we emphasise the

importance of reporting phosphorus levels alongside nitrogen and carbon in future work attempting to understand insect nutritional requirements.

Methodological Insights

More convincing evidence of nitrogen additions inducing phosphorus limitation would come from experiments manipulating levels of both nutrients simultaneously, either in separate factorial experiments like we attempted, or in more of a response-surface design. Leaving aside the possibility of incorporating phosphorus addition alongside nitrogen addition, response-surface designs with the same number of larvae spread over more treatments may in general be a more powerful way to detect insect responses to changing nitrogen levels (eg. Clancy & King, 1993). Kreyling et al. (2018) compared such designs to more replicated approaches like the one we used (with more sampling units per treatment, but the same number of sampling units overall) and found them to be much more powerful for detecting responses to ecological gradients, a situation analogous to our gradient of nitrogen levels. We did not detect the expected unimodal impacts on performance in all combinations of species and metric, which could reflect differences in the responses of different performance measures and species, but this seems unlikely given that each species and performance measure did show at least one unimodal response. Rather, relatively weak effects combined with a lack of power, partly reflecting small sample sizes for *C. pamphilus* and *P. icarus*, but perhaps also our experimental design may be responsible, and a shift to response surface designs may be an improvement on our approach.

Our frass data provided a simple and intuitive way to explore the relative deficiencies and overall efficiency of different hostplants for *L. phlaeas*. The interpretation of these analyses would benefit from a better understanding of the relationship between food and frass elemental ratios, and how this maps onto the disparity between food and required elemental ratios for different herbivores. Nevertheless, the simple rules of thumb (Box 1) that underlie our analysis and interpretation seem

reasonable. These enabled us to explore possible nutrient limitation in our performance experiment, and derive the optimum food C:N, N:P and C:P ratios for *L. phlaeas*, producing estimates and dynamics that were a close fit with existing theory, all from a relatively small sample of frass data. The latter could, in theory, be done for any nutrient for which levels can reliably be measured in frass. Pairing these analyses with performance data could thus provide a powerful way of developing a fuller understanding of insect nutrient requirements and their contribution to insect responses to rising levels of plant nitrogen content.

We treated nutrient levels in our hostplants as static over the course of both experiments. This is the typical approach used in such experiments, perhaps because much of the relevant theory was developed using artificial, agar-based diets where this assumption holds, and also because of the expense of sample analysis. Analysis of nutrient levels in host plants with samples from both the start and the end of the experiment however show that this assumption is not necessarily met in our experiment (Figure S6, Table S3). In *R. acetosa*, levels of non-carbon nutrients declined over the course of the experiment, while in *L. corniculatus*, they increased. This is not necessarily problematic if it is assumed that although nutrient levels might decline or increase over time within treatments, the relationship between treatments remains relatively consistent, and the interest is solely in determining the impacts of relative N content on herbivore performance. However, it may be more problematic for analyses attempting to estimate optimal nutrient levels from the relationship between performance and host plant chemistry, or frass and host plant chemistry, as we have done.

For our frass analyses, we used the mean of the host plant nutrient levels at the start and end of the experiment, the two time points at which nutrient levels were likely to be most different, assuming a linear trend over time. In this instance, the mean therefore seems a reasonable approximation of the nutrient levels experienced by most larvae during the experiment. However, in the main experiment, we used only samples from the end of the experiment for *R. acetosa* and *P. pratensis*, and samples from the start for *L. corniculatus*. It is noticeable that *L. corniculatus* samples were far more variable

in their nutrient levels at the end of the experiment than the start. This may reflect the impact of a mildew outbreak on this particular hostplant, but the differential harvesting of material to feed larvae may also have had an impact. Nitrogen additions caused an increase in plant biomass in some treatments, and this combined with variation in the number of surviving larvae in each treatment meant that relatively more plant material was harvested from some treatments, potentially leading to an extra source of variability in nutrient levels alongside our experimental treatments, given the known response of plant chemistry to herbivory or harvesting (Jaramillo & Detling, 1988; Ruess, 1984).

In general, realistically capturing the nutrient levels experienced by larvae that feed selectively on hostplants with complex chemistry that varies between plant parts, and over time, is a challenge when few samples are available. Approaches that combine the stable nutrient levels of artificial diets with the realistic chemistry of real host plants would therefore be desirable for a more detailed understanding of insect responses to variation in host nitrogen content. Such a compromise could be provided by pellets of food derived from homogenised material from fertilised plants which have successfully been used in recent work examining the response of Field Crickets (*Gryllus campestris*) to fertilisation of hostplants with nitrogen, phosphorus and lime (Vogels et al., in press; 2021).

Conclusions

Overall, our experiments are the first to show unimodal responses of the performance of multiple herbivore species to the same gradient of nitrogen addition levels on their natural host plants. This is consistent with the hypothesis that under normal conditions many insects are nitrogen-limited and will benefit from increases in plant nitrogen content up to the point that nitrogen deficiency is equalled by deficiency of other nutrients, which is exacerbated by further nitrogen additions, reducing the efficiency of food and causing a decrease in performance. Previous work suggests that the most likely deficiency induced by nitrogen addition is phosphorus, and analysis of frass data for one species (*L. phlaeas*) suggests that this does indeed occur in our study system and may be responsible for these

unimodal responses, although we were not able to manipulate phosphorus levels to test this. On the other hand, the host plant of another species, *P. icarus*, showed declining nitrogen content and N:P ratios with increasing nitrogen addition, and a switch from phosphorus to nitrogen limitation may drive its response. Our results thus suggest that changes to plant nitrogen content under nitrogen pollution will be dose-dependent (depending on whether they move host plant N:P ratios closer to herbivore requirements, or further from them), context-dependent (reflecting the levels of phosphorus in hostplants as much as nitrogen) and species-dependent.

Supplementary Material

Host plant chemistry from the TRY plant database

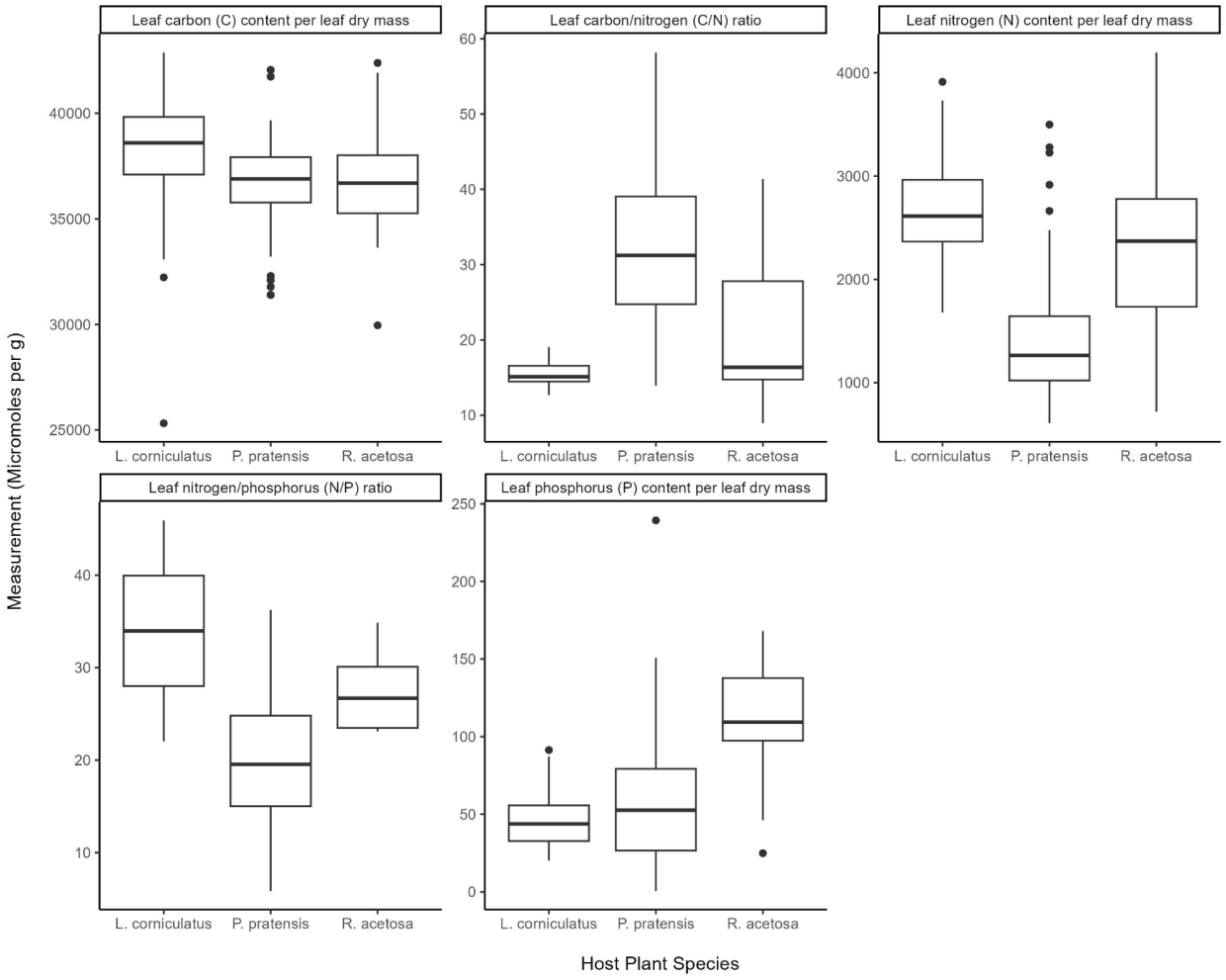


Figure S1: *The chemistry of the three host plants in our experiment from the TRY plants database (measured in micromoles g^{-1}) (Kattge et al., 2020). Our hypotheses about the preferred nitrogen levels of each butterfly species were based on the strong differences between plants, and these provide useful context for the range of measurements of host plants in our experiment. Boxes show the first, second (median) and third quartiles for each measurement, whiskers 1.5 x IQR above the third quartile, and below the first quartile.*

Other Measures of Plant Chemistry in the main experiment

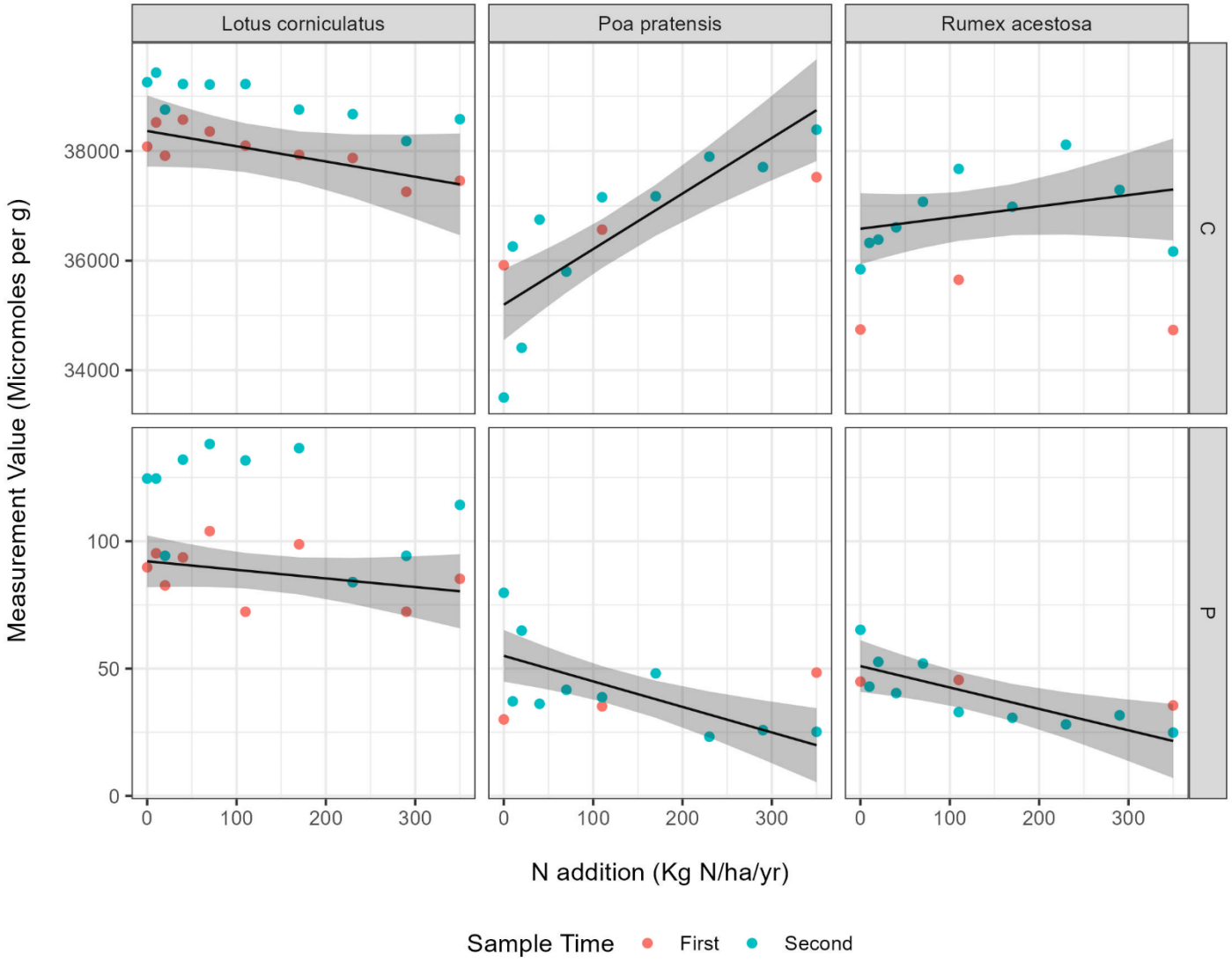


Figure S2: *Trends in C and P content across experimental treatments for L. corniculatus, P. pratensis and R. acetosa in the main experiment. Data from samples taken at the start and end of the experiment are plotted with predicted effects from models in table S1 and with their standard errors. These were only fitted to data from the start of the experiment for L. corniculatus, and the end of the experiment for R. acetosa and P. pratensis.*

Table S1: *Results of models assessing differences in plant chemistry between experimental treatments and host plant species in the main experiment. R. acetosa was used as the reference group in these models. Models were only fitted to data from the start of the experiment for L. corniculatus, and the end of the experiment for R. acetosa and P. pratensis*

Measurement	Term	Estimate	Standard Error	t	p
C Content	(Intercept)	36581.8	327.84	111.58	< 0.001
	N	2.05	1.87	1.1	0.284
	L. corniculatus	1783.87	463.64	3.85	< 0.001
	P. pratensis	-1385	463.64	-2.99	< 0.01
	N:L. corniculatus	-4.83	2.64	-1.83	0.0801
	N:P. pratensis	8.09	2.64	3.06	0.00538
P Content	(Intercept)	50.98	5.13	9.93	< 0.001
	N	-0.08	0.03	-2.87	< 0.01
	L. corniculatus	41.15	7.26	5.67	< 0.001
	P. pratensis	4.03	7.26	0.55	0.584
	N:L. corniculatus	0.05	0.04	1.22	0.236
	N:P. pratensis	-0.02	0.04	-0.39	0.699

Performance results in the main experiment

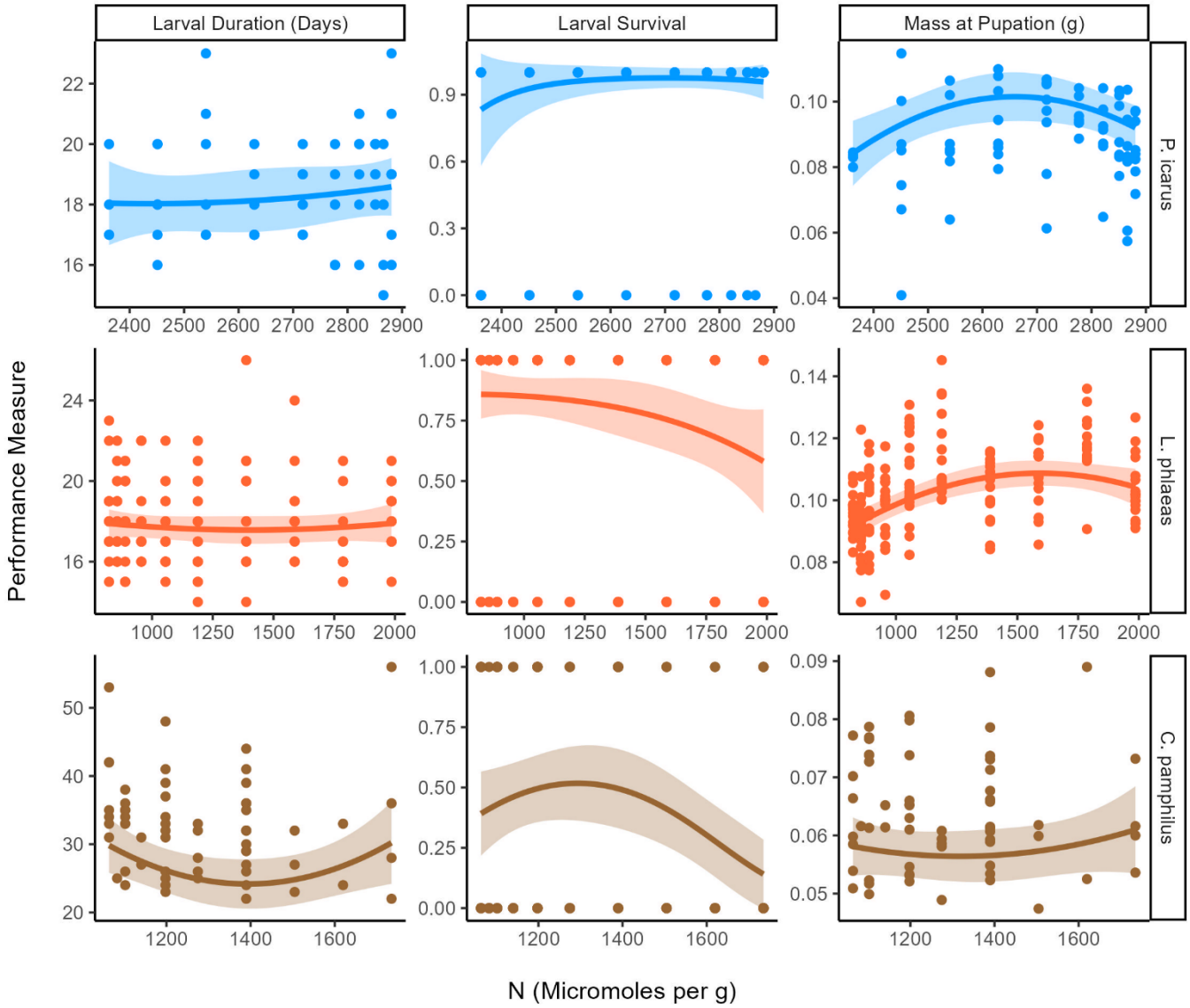


Figure S3: *Relationships between larval performance and N content for P. icarus, L. phlaeas and C. pamphilus. All responses are plotted (only results where there were significant second order N content terms are shown in the main text). Lines show model predictions and ribbons their 95% confidence intervals.*

Raw polynomial performance model outputs

Table S2: *Results of models using second order raw polynomials to describe the effect of plant nitrogen content on larval performance for P. icarus, L. phlaeas and C. pamphilus. Significant terms for N content are highlighted with bold text. Test statistics for pupal mass and larval survival are t values, for larval survival z values. The reference sex is female. Co-efficients for N in these models were used to calculate the optimal treatments in table 3.*

Species	Metric	Term	Estimate	Standard Error	Statistic	p
P. icarus	**Pupal Mass (g)**	Intercept	-1.29	0.47	-2.76	0.0077
		N (Micromoles per g, 1st order)	**0**	**0**	**2.94**	**0.00464**
		N (Micromoles per g, 2nd order)	**0**	**0**	**-2.94**	**0.00472**
		Days Since Start	0	0	-1.14	0.26
		Sex (male)	0.01	0	2.72	0.00857
	Larval Survival	Intercept	-126.66	94.61	-1.34	0.181
		N (Micromoles per g, 1st order)	0.1	0.07	1.33	0.184
		N (Micromoles per g, 2nd order)	0	0	-1.29	0.198
		Days Since Start	-0.03	0.01	-1.84	0.0664
	Larval Duration (days)	Intercept	35.3	65.15	0.54	0.59
		N (Micromoles per g, 1st order)	-0.01	0.05	-0.28	0.778
		N (Micromoles per g, 2nd order)	0	0	0.31	0.759
		Days Since Start	0	0.01	-0.52	0.605
		Sex (male)	-0.27	0.42	-0.63	0.53
L. phlaeas	**Pupal Mass (g)**	Intercept	0.04	0.01	2.97	0.00339
		N (Micromoles per g, 1st order)	**0**	**0**	**4.3**	**< 0.001**
		N (Micromoles per g, 2nd order)	**0**	**0**	**-3.71**	**< 0.001**
		Days Since Start	0	0	0.31	0.756
		Sex (male)	-0.01	0	-2.83	0.00519
	Larval Survival	Intercept	1.3	2.71	0.48	0.633
		N (Micromoles per g, 1st order)	0	0	0.34	0.736
		N (Micromoles per g, 2nd order)	0	0	-0.64	0.52
		Days Since Start	0.02	0.02	1.04	0.296
	Larval Duration (days)	Intercept	20.36	2.25	9.04	< 0.001
		N (Micromoles per g, 1st order)	0	0	-0.77	0.442
		N (Micromoles per g, 2nd order)	0	0	0.75	0.452
		Days Since Start	-0.02	0.01	-1.85	0.0664
		Sex (male)	-0.89	0.3	-2.96	0.00357
C. pamphilus	Pupal Mass (g)	Intercept	0.11	0.05	2.35	0.023
		N (Micromoles per g, 1st order)	0	0	-0.98	0.333
		N (Micromoles per g, 2nd order)	0	0	1.02	0.314
		Days Since Start	0	0	1.48	0.145
		Sex (male)	-0.01	0	-4.61	< 0.001
	Larval Survival	Intercept	-16.07	8.03	-2	0.0455
		N (Micromoles per g, 1st order)	**0.02**	**0.01**	**2.08**	**0.0379**

Species	Metric	Term	Estimate	Standard Error	Statistic	p
		N (Micromoles per g, 2nd order)	**0**	**0**	**-2.19**	**0.0286**
		Days Since Start	-0.03	0.01	-1.71	0.0864 .
		Intercept	129.66	38.36	3.38	0.0015
		N (Micromoles per g, 1st order)	-0.14	0.06	-2.55	0.0143
		N (Micromoles per g, 2nd order)	**0**	**0**	**2.5**	**0.016**
		Days Since Start	0.31	0.09	3.59	< 0.001
	Larval Duration (days)	Sex (male)	-4.73	1.82	-2.6	0.0126

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Performance measurements from the factorial experiment

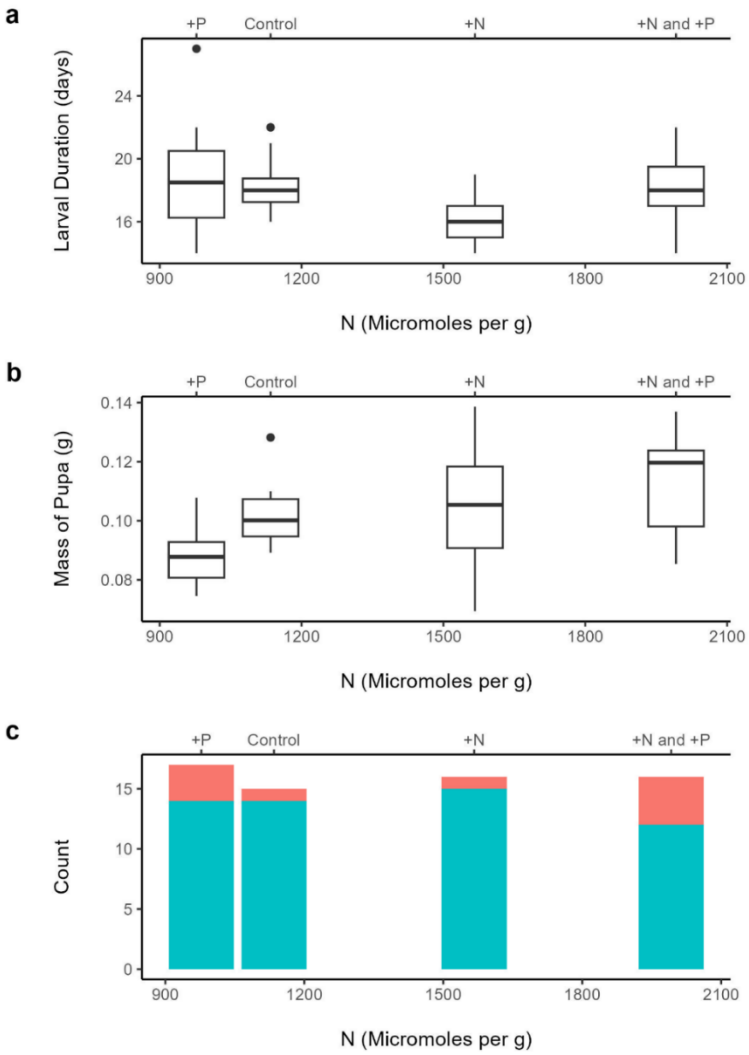


Figure S4: *Performance measures of L. phlaeas larvae in the factorial experiment: a) larval duration b) mass of pupa c) number of larvae surviving (blue) and dying (red). These are plotted for each treatment with the mean nitrogen content of the plant material sampled at the start and the end of the experiment on the lower x axis, and its initial treatment on the upper x axis. Bars are stacked not overlapping, and boxes in boxplots show the first, second (median) and third quartiles for each measurement, whiskers 1.5 x IQR above the third quartile, and below the first quartile.*

Potential phosphorus limitation in the main experiment

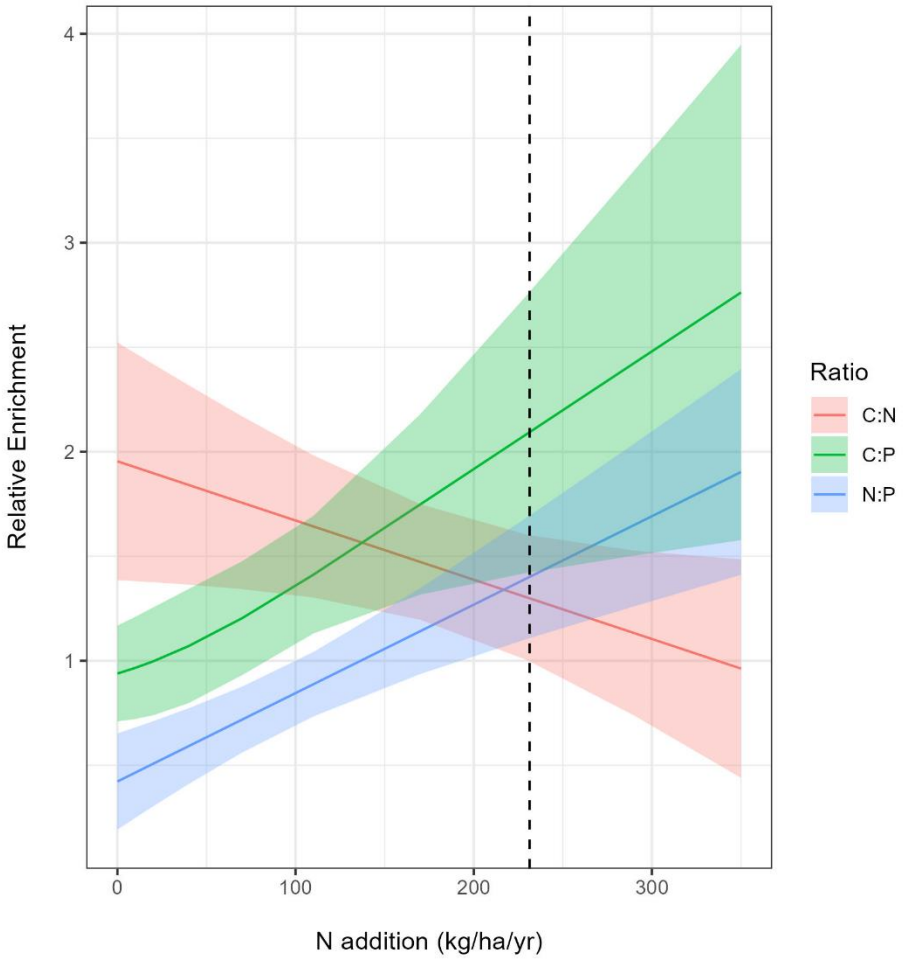


Figure S5: *Predicted relative frass C:N, C:P and N:P enrichment for L. phlaeas larvae in the main performance experiment, shown alongside the level of N addition at which L. phlaeas pupal mass peaked (vertical dashed line). Predictions use the C:N, C:P and C:N content predicted for plants at each nitrogen addition level in the main experiment (from models in table 1) as inputs to the GAMs of relative enrichment ~ elemental ratio from the factorial experiment. The predictions are plotted with solid lines, and their 95% confidence intervals with ribbons, against N addition level to give a common axis.*

Changes in plant chemistry over time

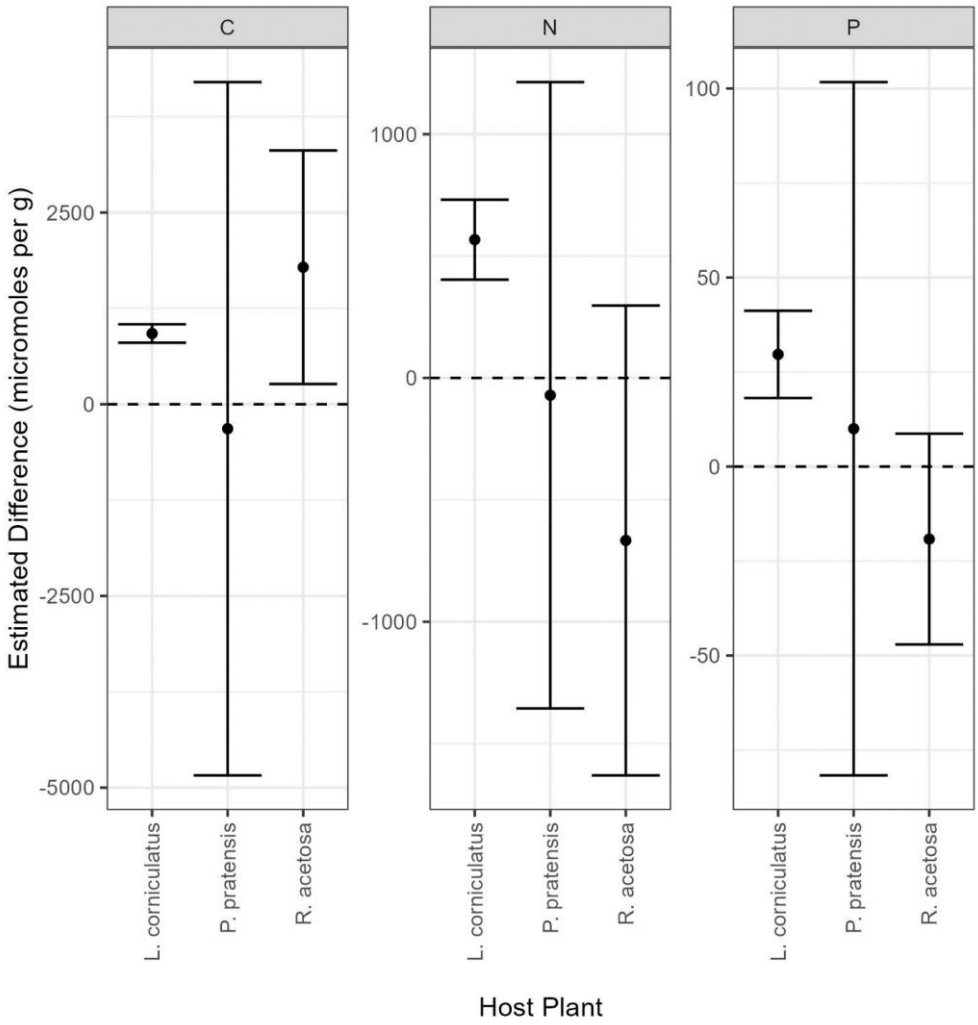


Figure S6: *Results of paired T-tests of measurements of plant carbon, nitrogen and phosphorus content at the end of the experiment vs the start of the experiment for plants in the same treatment. Positive values therefore represent increasing nutrient content over time, and negative values decreasing nutrient content. The mean differences are shown with their 95% confidence intervals. There are 10 paired measurements for L. corniculatus, 5 paired measurements for R. acetosa (both factorial and main experiment are used), and 3 for P. pratensis.*

Table S3: *Results of paired T-tests of measurements of plant carbon, nitrogen and phosphorus content at the end of the experiment vs the start of the experiment for plants in the same treatment. Positive estimates therefore represent increasing nutrient content over time, and negative values decreasing nutrient content. There are 10 paired measurements for L. corniculatus, 5 paired measurements for R. acetosa (both factorial and main experiment are used), and 3 for P. pratensis.*

Host	Element	Estimate	Statistic	DF	Lower Confidence Interval	Upper Confidence Interval	p
R. acetosa	P	-19.18	-1.91	4	-47.06	8.7	0.129
R. acetosa	N	-666.54	-1.92	4	-1630.05	296.97	0.127
R. acetosa	C	1786.7	3.26	4	264.26	3309.13	< 0.01
P. pratensis	P	10.01	0.47	2	-81.68	101.69	0.685
P. pratensis	N	-70.92	-0.24	2	-1355.45	1213.61	0.834
P. pratensis	C	-319.15	-0.3	2	-4839.73	4201.42	0.79
L. corniculatus	P	29.67	5.82	9	18.14	41.21	< 0.001
L. corniculatus	N	567.23	7.82	9	403.18	731.27	< 0.001
L. corniculatus	C	922.49	17.33	9	802.09	1042.88	< 0.001

Chapter 3: The role of host nitrogen levels in structuring habitat use by insect herbivores

Contributors: William Langdon, Richard Fox, Owen Lewis

The abundance of insect herbivores is often dictated by the availability of suitable host plants on which their most specialised immature stages can survive. Their survival can be strongly influenced by the microclimate in which their host plants grow, and the nutritional quality of the host plants themselves, depending on the extent of their thermal and nutritional constraints. We assessed the role of microclimate around host plants and host plant quality in determining habitat use by the butterfly *Lycaena phlaeas*, to understand the relative importance of thermal and nutritional constraints for this species. We found that this was mostly dictated by the need for high temperatures, and that the preferred warmer plants tended to be of lower nutritional quality. We suggest that this reflects the common influence of soil nitrogen on plant nitrogen content (increasing it) and temperature (decreasing it via enhanced plant productivity and denser, cooler vegetation) and therefore that strongly thermophilous insect herbivores like *L. phlaeas* may often face a trade-off between the temperature around host plants and their nutritional quality.

Introduction

The quality of habitat for insects is primarily dictated by the availability of sites in which the least mobile and most specialised early stages can survive (Bourn & Thomas, 2002; J. A. Thomas et al., 2011). For many herbivorous insects of open habitats, this is the availability of suitably warm micro-habitats, which are particularly important for species at their cool range margins (Oliver et al., 2009; J. A. Thomas et al., 1999). Here ambient temperatures are often lower than those required for these insects to complete their development (van Wingerden et al., 1991), meaning that with limited ability

to thermoregulate behaviourally (Ashe-Jepson et al., 2023), and little ability to move between feeding and thermoregulating sites, larvae can often only survive on hostplants growing in particularly warm microhabitats (Curtis & Isaac, 2015; WallisDeVries, 2006). As a result, the availability of host plants in such microhabitats often dictates the abundance of thermophilous insect species (Curtis & Isaac, 2015; J. A. Thomas et al., 2001). Microhabitat aside, however, not all host plants are of equal nutritional quality for insects, thanks to their varying chemistry (Persson et al., 2010). Insects require a wide variety of nutrients, each in specific amounts (Raubenheimer et al., 2009; Raubenheimer & Simpson, 1993, 2004). When the chemistry of food does not match these requirements, insects are forced to over-eat and excrete excessive nutrients to make up for deficiencies of others, at a cost of time and energy (Chapter 2, this thesis; Raubenheimer et al., 2005; Simpson et al., 2004). Thus, host nutritional quality is defined as the match between insect requirements and host chemistry (Sterner & Elser, 2002; Vogels et al., 2023), and large mis-matches can have strong effects on insect fitness that can scale up to population-level impacts (Cease et al., 2012; Clissold et al., 2006).

Insects are therefore strongly constrained by their thermal and nutritional requirements, and their fitness will be highest on hosts where both of these are met. Such conditions may be rare however, if thermally and nutritionally suitable conditions do not overlap. For example, the host plants of many herbivorous insects have a far higher carbon content and a far lower nitrogen content than the insects themselves (Elser et al., 2000). Thus, to attain sufficient quantities of nitrogen to complete their development, insects generally have to over-eat large quantities of carbon, and plant nitrogen content is therefore often regarded as a key indicator of host nutritional quality for insects, with performance generally improved on higher-nitrogen hostplants (Mattson, 1980; Throop & Lerdau, 2004; White, 1993). Plant nitrogen content typically increases where soil nitrogen levels are higher (Fujita et al., 2013; Sardans, Rivas-Ubach, & Peñuelas, 2012; Throop & Lerdau, 2004), but in open habitats, higher soil nitrogen also enhances vegetation productivity, leading to taller, less open vegetation, with reduced penetration of sunlight to ground level (Bobbink, 1991; DeMalach et al., 2017; Hautier et al., 2009).

Microhabitat temperatures in such situations are lower than in more open situations where bare ground and dead plant material exposed to sunlight warm up rapidly and radiate heat (Curtis & Isaac, 2015; WallisDeVries, 2006). Therefore, in general, host plants with higher nutritional quality (nitrogen content) should not overlap with warm micro-habitats, creating a trade-off for thermophilous insects in open habitats.

The roles of thermal and nutritional requirements in dictating insect microhabitat use are rarely studied simultaneously however (eg. Bourn & Thomas, 1993; Ellis, 2003). This may reflect the fact that most work is interested in testing a specific nutritional or thermal hypothesis, either for theoretical reasons, or because previous work or observations have identified them as an important population constraint in advance. As a result, it is difficult to understand the relative strength of thermal and nutritional constraints for many insect herbivores, and whether our hypothesised trade-off exists. Such an understanding is important when both climate change and nitrogen pollution are altering the thermal and nutritional conditions experienced by insects simultaneously. Rising ambient temperatures under climate change may lessen thermal constraints on some insect species, as microhabitats that were previously too cool become sufficiently warm (Roy & Thomas, 2003; Thomas et al., 2001). On the other hand, warming extends the growing season (Menzel et al., 2006), enhancing plant productivity and potentially leading to cooler microhabitats overall (WallisDeVries & van Swaay, 2006). Rising levels of soil nitrogen under nitrogen pollution are expected to enhanced plant productivity and alter vegetation structure alongside warming (WallisDeVries & van Swaay, 2006), while also leading to increases in plant nitrogen content and changes in host nutritional quality for insect herbivores (Vogels et al., 2023).

Thus, the impacts of two major anthropogenic stressors on insect populations depend to a great extent on the interplay of changing host nutritional quality, vegetation structure and macroclimate with insect thermal and nutritional requirements. If there is a trade-off between host plants with high nutritional quality and warm microhabitats, species requiring warmer conditions may be unable to access the

potential benefits of higher host nutritional quality driven by nitrogen pollution, unless higher macroclimatic temperatures enable them to use cooler microhabitats. Here, we therefore investigate how host plant nutritional quality (nitrogen content) and microclimate shape habitat use by a grassland butterfly, *Lycaena phlaeas* (Linnaeus, 1761), and the relationship between these two aspects of habitat quality in this system. Specifically, we assess the evidence for our hypothesised trade-off between high nutritional quality host plants and high temperatures around host plants for *L. phlaeas*, and how differences in vegetation structure bring this about.

We do this by studying the distribution of *L. phlaeas* eggs and larvae on their host plants and comparing the microclimatic temperature and nutritional quality of host plants occupied by *L. phlaeas* immature stages with unoccupied plants. Female butterflies generally select host plants that maximise larval survival (Gripenberg et al., 2010; Jaenike, 1978), and the availability of hosts that meet larval requirements has consistently been shown to be the main factor constraining butterfly populations in systems like ours (Bourn & Thomas, 2002; J. A. Thomas et al., 2011). Thus, we expect female oviposition choice, measured by the distribution of eggs, to indicate how *L. phlaeas* populations are constrained by thermal and nutritional requirements. By replicating this work across seasons we attempt to understand how variation in macroclimate might alter thermal constraints, and we then examine our data on micro-climate and host nutritional quality to understand whether and how these variables are related in our system.

Specifically, we test the hypotheses:

1. **In autumn, *L. phlaeas* egg-laying is determined more by microclimate around host-plants than their nutritional quality.**
2. **In spring when macroclimatic temperatures are higher than in autumn, microclimate is less important in dictating egg-laying patterns, and there is a shift to host plants with higher nutritional quality.**

3. **Nutritional quality of host-plants is negatively correlated with the temperature under them.**

Methods

Study System

Lycaena phlaeas is a widespread Lycaenid butterfly in open habitats across Eurasia and North America. It is oligophagous on a variety of *Rumex* spp. but in north-west Europe, primarily uses *Rumex acetosa* and *Rumex acetosella* (J. A. Thomas & Lewington, 2014). *Lycaena phlaeas* is multivoltine, with the ability to breed continuously where conditions allow, but in the United Kingdom it undergoes a winter diapause in the larval stage, and usually has between one and three generations per year depending on latitude (J. A. Thomas & Lewington, 2014). Like many butterfly species, *L. phlaeas* requires warm microhabitats (Streitberger et al., 2014), but will also respond strongly to changes in host nutritional quality in laboratory experiments. In oligotrophic or mesotrophic environments *L. phlaeas* larvae appear to be nitrogen-limited, and to benefit from moderate increases in plant nitrogen content, before performance declines at high nitrogen levels (Chapter 2, this thesis; Kurze et al., 2018). Thus, they have high potential to be impacted by changes in vegetation structure but also by changes in plant nitrogen content and predicting the likely outcome of these changes requires an understanding of the relative strength of their thermal and nutritional constraints.

Data Collection

Our work took place at Shotover Country Park (Oxfordshire, United Kingdom, 51°44′42″N , 001°11′01″W). This is a typical habitat for *L. phlaeas* in lowland Britain, a mesotrophic grassland where it uses *R. acetosa* as its sole larval host plant and has three generations per year. *R. acetosa* plants were searched systematically for eggs and larvae of *L. phlaeas* and the number of eggs and larvae on occupied plants counted. For each occupied plant a control plant was then selected. This was

the nearest plant to a metre-stick placed on the occupied plant and pointed either North, East, South or West (based on random selection of a number between 1 and 4 corresponding to these directions). Thus each occupied plant had a nearby control plant containing no *L. phlaeas* eggs. For both the occupied and control plant in each pair, a 50cm quadrat with 10cm grids was used to measure percentage cover of green vegetation, bare ground and litter.

Sward height around the focal plant was measured using the direct method following Stewart et al. (2001), reading the height below which 80% of vegetation around the plant was estimated to be growing off a ruler. The area of the focal plant was estimated as the dimensions of the tightest-fitting rectangle around each plant, i.e the longest diameter of the plant's area viewed from above, and the maximum diameter perpendicular to this. The three largest leaves without eggs of *L. phlaeas* were then removed from each plant for chemical analysis, and their length and breadth measured and multiplied to approximate their area, which was averaged across the three leaves as a measure of leaf size. The temperature on the ground directly under plants was then measured in sunny conditions between 12:00 and 14:30 (the hottest part of the day, when differences between plants were expected to be most apparent) using an ultrafine thermocouple (TC Direct Ultra Fine Wire PFA type K Thermocouple 0.08mm diameter, with handheld Type K Thermocouple Indicator, TC Direct, Uxbridge, United Kingdom). This was also used to measure ambient temperature by holding the thermocouple at approximately 6ft above the ground. This fieldwork was carried out both in late spring (10/06/2023 to 16/06/2023) and early autumn (21/09/2023 to 28/09/2023) when thermal constraints were expected to differ due to differences in ambient temperature.

Chemical Analysis

We were unable to analyse the chemistry of every plant sampled to measure host nutritional quality in terms of nitrogen content directly, owing to the costs of such analysis. We therefore aimed to identify reliable proxies for plant nitrogen content that we could use in our analysis. Observations during previous work (Chapter 2, this thesis) suggested that the area of *R. acetosa* plants and the size of their leaves were correlated with plant nitrogen content. We therefore performed a principal component analysis of average leaf size and plant area across all sampled plants and separated plants into thirty groups based on the first principal component. Within these, we made a further two groups based on sampling season (i.e. 60 groups in total, one for each season by principal component group combination). From each of these we randomly selected one plant for chemical analysis. This gave a total of 58 plants (as two group x season combinations lacked any plants). We expected that analysing leaf samples across the full range of plant morphology (plant area and leaf size) would provide the best chance of detecting relationships between these proxy measurements and plant nitrogen content.

Selected leaf samples were dried in individual glassine envelopes at 70°C for 48 hours before being transferred to microtubes and ground to a fine powder via bead beating (MP Bio FastPrep-24 5G, MP Biomedicals, Irvine, USA). Leaf samples were beaten for two minutes with three ceramic beads of diameter 2.3mm (Zirconia/Silica, Thistle Scientific, Warwickshire, England). Approximately 10mg of each leaf sample was then analysed for carbon and nitrogen content via combustion using a Thermo Flash Carlo Erba CN analyser (EA 1112 series) (Thermo Fisher Scientific, Waltham, Massachusetts, USA) by Forest Research (Alice Holt, United Kingdom).

Statistical Analysis

To identify which of plant area, average leaf size, or a combination of the two was the most appropriate proxy for plant nitrogen content, we ran models of plant nitrogen content as a function of average leaf size and plant area (separately and together) and the first and second principal components

of their PCA (alone, but not together, since this would be the equivalent to using both unmodified variables), all with a dummy 'season' (autumn or spring) variable to control for differences between seasons. We compared the performance of these models via their AIC, BIC and R^2, as well as calculating the Pearson correlation coefficients between each individual proxy and nitrogen content, looking for a combination of high explanatory power (a robust proxy) and parsimony (i.e. one variable that performs almost as well as two would be preferred, for easier interpretation of later modelling steps).

Lycaena phlaeas habitat use was analysed across seasons using a generalised linear model of the presence or absence of *L. phlaeas* eggs or larvae as a function of microhabitat temperature and our chosen host nutritional quality proxy, and their interactions with season. The model used binomial errors with a logit link and we present the exponentiated co-efficients in the results, indicating the multiplicative change in the odds ratio of egg-laying (to not egg-laying) with a one unit increase in a predictor.

Temperature measurements were made over several days with varying weather conditions in each field season, and so to account for the effect of daily variation in ambient temperature, and its influence on the temperatures measured under plants, we centred and scaled our temperature measurements by date. Thus, temperature under a plant was relative to others measured on that date, and our model indicated whether or not *L. phlaeas* was using the warmest plants, but not how warm these actually were. Our host nutritional quality proxy was also centred and scaled to match this. We also compared the results of this model to one using the scaled predicted nitrogen content of plants instead of our proxy for this, with the inverse of the scaled standard error of predicted nitrogen content as a weight.

To test the hypothesis that temperatures around host plants and host nutritional quality would be negatively related (owing to soil nitrogen levels increasing vegetation density alongside plant nitrogen content) we modelled the relationship between temperature and host nutritional quality using a linear

model. During data collection we noted that many of the plants used for *L. phlaeas* egg-laying were on bare ground created by *Talpa europaea* activity (as observed by Streitberger et al., 2014). This could produce warm microclimates under plants that would otherwise have had cool microclimates and high plant nitrogen content (in areas with high soil nitrogen and dense vegetation), thereby disrupting the hypothesised relationship. We therefore included an interaction between presence or absence of bare ground and host nutritional quality in this model. As for the egg-laying model, we also ran this model using the scaled predicted nitrogen content of plants instead of our proxy measure, with the inverse of the scaled standard error of predicted nitrogen content as a weight.

To understand what might drive the hypothesised relationship between temperature and host nutritional quality we then examined the effect of environmental variables (bare ground, vegetation cover, litter cover and sward height) on relative temperature under plants using linear models. Finally, we tested the effect of sward height and litter cover on host nutritional quality in the same way (omitting bare ground and vegetation cover and dropping plants with bare ground from the analysis since these mostly reflected *T. europaea* disturbance rather than underlying soil conditions).

All analyses were carried out in R (R Core Team, 2024) using the tidyverse, readr and Hmisc packages for data manipulation (Harrel, 2024; Wickham et al., 2019, 2024), performance for model comparison (Lüdecke et al., 2021), and GGplot2 and cowplot for plotting (Wickham, 2016; Wilke, 2024). Model fit was assessed via inspection of plots of the residuals and fitted values.

Results

Host Nutritional Quality and Proxies

Among our 58 *R. acetosa* leaf samples nitrogen content (by mass) averaged 2.54% (sd = 0.76) with a maximum of 4.39% and a minimum of 0.98%. This translated into molar content averaging 1815.79 micromoles g^{-1} (sd = 539.03) with a maximum of 3130.64 micromoles g^{-1} and a minimum of 701.09

micromoles g^{-1}. This led to molar C:N ratios of between 11.24 and 51.98, averaging 22.41. The distribution of these measurements is plotted in Figure S1, although the stratified nature of our sampling means that these are not representative of the whole population of *R. acetosa*.

Table 1: *Results of Principal Components analysis of host nutritional quality proxies.*

	PC1	**PC2**
Eigenvalue	1.59	0.42
% Variance Explained	79.26	20.74
Average Leaf Size (mm^2) loading	-0.71	-0.71
Plant Area (cm^2) loading	-0.71	0.71

Plant area and leaf size were positively correlated (Pearson's $R = 0.60$, $t = 13.54$, $df = 316$, $p < 0.001$) and the first principal component of their PCA therefore explained 79.26% of the variance in the two measurements (Table 1). Models of plant nitrogen content as a function of these proxies and principal components (results in Table S1 and Figure 1) indicated that leaf size was the best proxy for nitrogen content. Of the individual options it showed the strongest positive correlation with plant nitrogen content (Table 2), and models with just leaf size performed better than either principal component by themselves (Table 3). The model with leaf size and plant area performed only marginally better than leaf size alone (Table 3). Therefore, on the basis of parsimony and predictive power, leaf size was chosen as our proxy for plant nitrogen content. The results were qualitatively the same when C:N ratio was used instead of N content as the 'true' measure of host nutritional quality (Table S2 and Table S3).

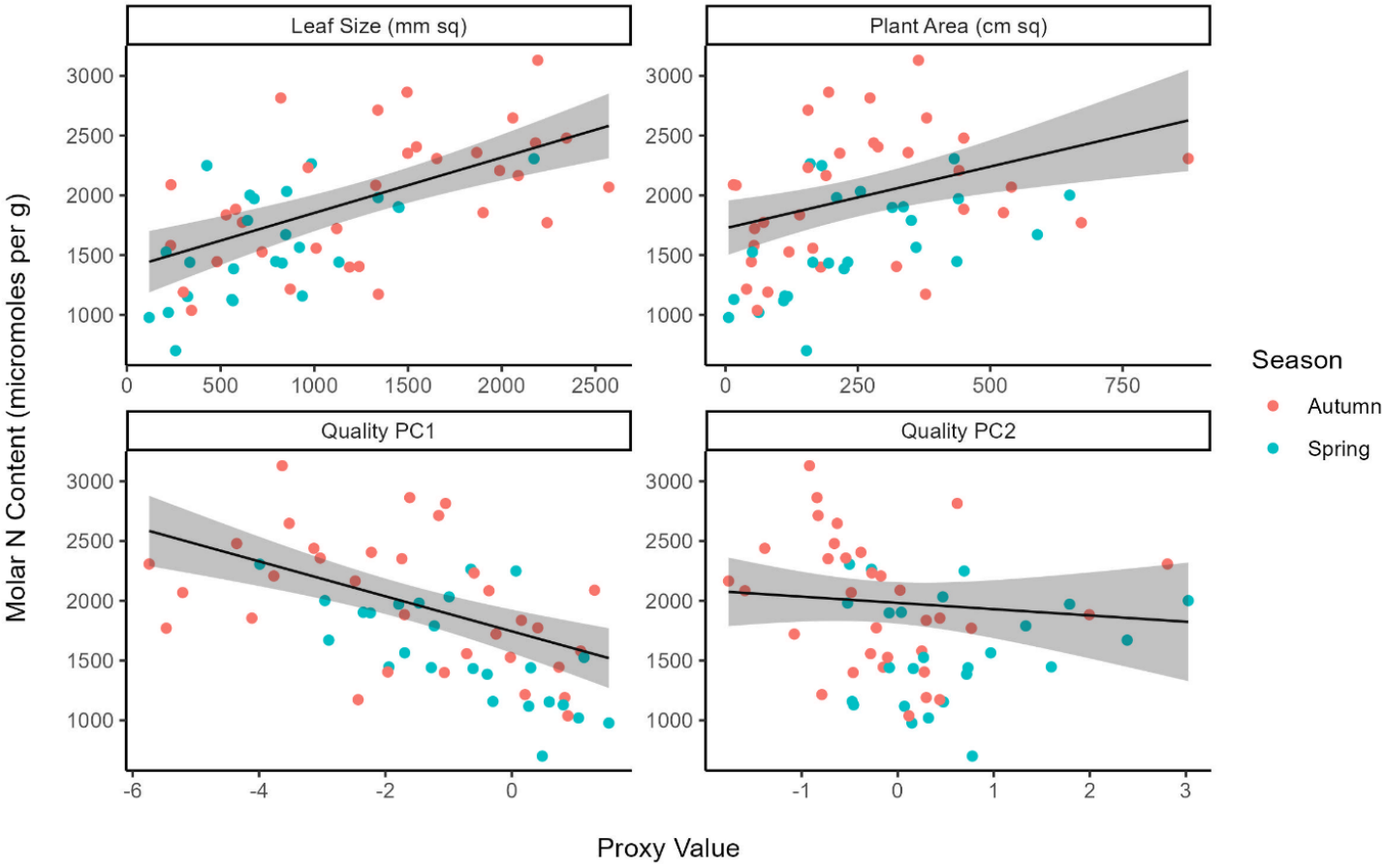


Figure 1: *Predicted relationships between host nutritional quality proxies and plant nitrogen content. Predictions are for autumn and plotted with 95% confidence intervals from models (results Table S1) alongside the underlying data.*

Table 2: *Pearson's correlation coefficients and significance tests for correlation of possible plant proxies with plant nitrogen content (measured in micromoles g^{-1})*

Proxy	R	t	DF	p	Lower Confidence Interval	Upper Confidence Interval
Average Leaf Size (mm^2)	0.63	6.13	57	<0.001	0.45	0.76
Plant Area (cm^2)	0.38	3.07	57	<0.01	0.13	0.58
Quality PC1	-0.56	-5.14	57	<0.001	-0.72	-0.36
Quality PC2	-0.23	-1.77	57	<0.05	-0.46	0.03

Table 3: *Comparison of models relating plant nitrogen content to different possible proxies*

Proxies	AIC	BIC	R^2
Average Leaf Size (mm^2) + Plant Area (cm^2)	887	897	0.42
Average Leaf Size (mm^2)	885	893	0.42
Plant Area (cm^2)	897	906	0.28
Quality PC1	888	897	0.38
Quality PC2	907	915	0.16

Microclimate under *R. acetosa*

Ambient temperatures in spring averaged 24.76°C (sd = 0.78), and in autumn 16.41°C (sd = 1.51), approximately 8.35°C lower (t = 61.38, df = 203.69, p < 0.001) (Figure 2). Matching this, temperatures measured under *R. acetosa* plants were higher in spring, averaging 36.83°C (sd = 6.2) compared to 18.37°C (sd = 1.64) in autumn, a difference of 18.50°C that was also significant (t = 39.97, df = 231.69, p < 0.001) (Figure 2). The maximum temperature recorded under *R. acetosa* plants in spring was 56°C and in autumn 23°C.

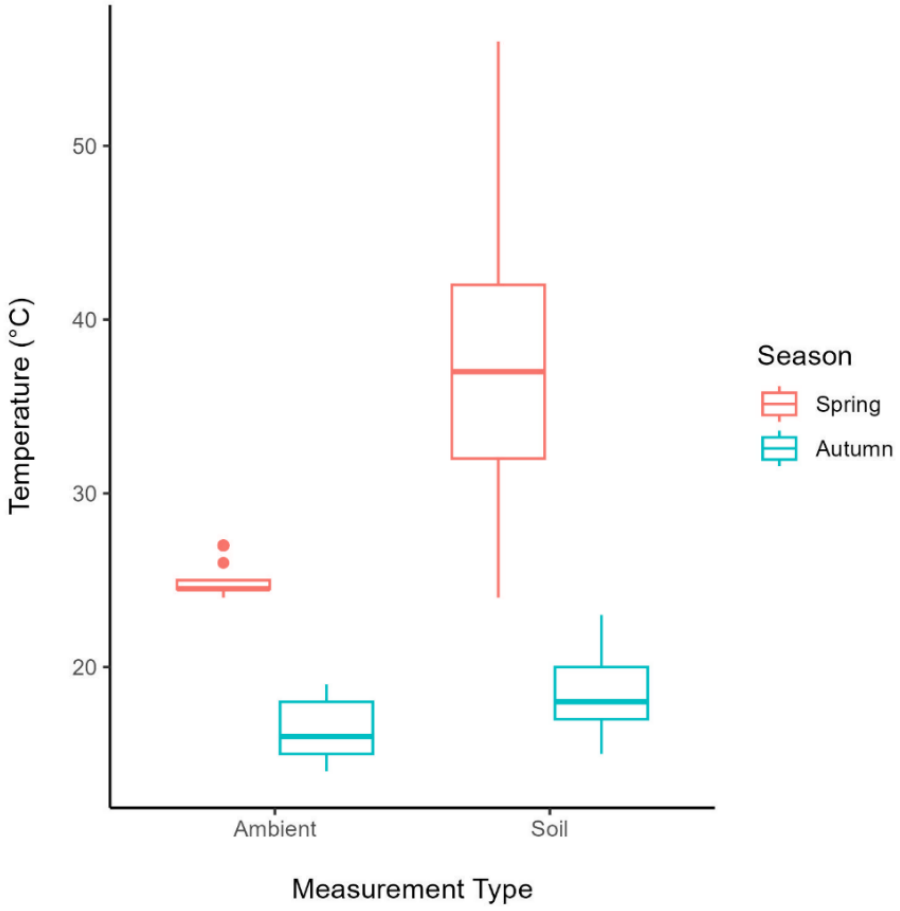


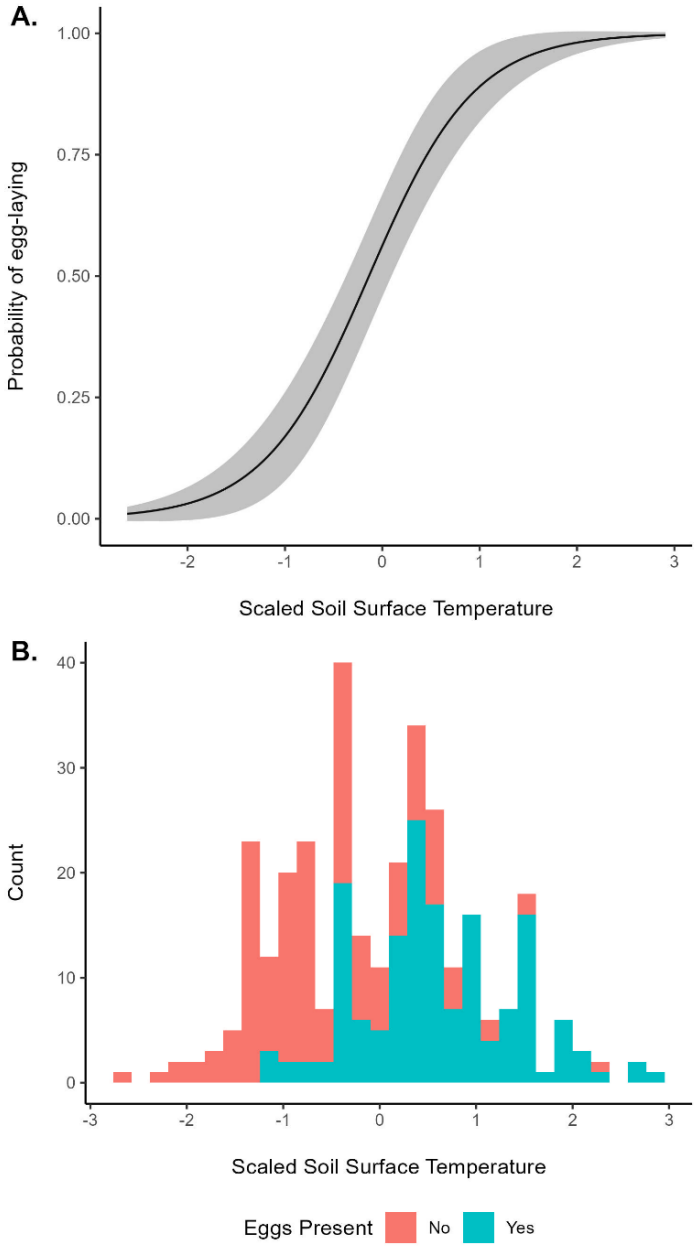
Figure 2: *Differences in ambient temperatures and temperatures under R. acetosa plants measured in spring and autumn. Boxes show the first, second (median) and third quartiles for each measurement, whiskers 1.5 x IQR above the third quartile, and below the first quartile.*

Impacts of Host Nutritional Quality and Microclimate on *L. phlaeas* egg-laying

Overall, 318 plants were sampled during our data collection, 184 in spring and 134 in the autumn (evenly divided into occupied plants, and control plants). Only scaled temperature had a significant effect on the probability of *L. phlaeas* eggs or larvae being present on *R. acetosa* across the two seasons, with a one unit increase in scaled temperature increasing the odds of egg-laying by 6.54 times (confidence interval: 3.71-12.91, $z = 5.95$, $p < 0.001$) (Figure 3).

Non-significant effects for other covariates were greater than one for scaled leaf size, indicating an increase in the probability of egg-laying with leaf size (1.40 times increase in the odds of egg-laying, confidence interval: 0.94-2.13, $z = 1.62$, $p = 0.104$), less than one for the interaction between temperature and season, indicating a reduced effect of temperature on the odds of egg-laying in spring (slope of the egg-laying temperature relationship multiplied by 0.97, confidence interval: 0.74-2.59, $z = -0.08$, $p = 0.93$) and greater than one for the interaction between leaf size and season, indicating a stronger effect of scaled leaf size on egg-laying in spring (slope of the egg-laying temperature relationship multiplied by 1.33, confidence intervals: 0.66-2.84, $z = 0.76$, $p = 0.45$). The main effect of season was not significant (with autumn as the reference level) (odds ratio = 1.38, confidence interval = 0.74-2.59, $z = 1.00$, $p = 0.31$). The McKelvey-Zavoina pseudo-R^2 for this egg-laying model was 0.46. Results did not qualitatively differ when using predicted nitrogen content as our host nutritional quality variable (see Table S4).

On the occupied plants, the mean number of eggs in spring was 2.31 (sd = 2.82) and in autumn, 1.89 (sd = 1.54), with maxima of 16 and 8 and totals of 213 and 127 eggs in autumn and spring, respectively. The median in both seasons was 1 egg.



1797

1798 **Figure 3:** *The impacts of temperature under R. acetosa on the probability of L. phlaeas egg-laying.* ***A.***

1799 *Predicted probability of L. phlaeas egg-laying with the scaled temperature under a plant. Predictions*

1800 *are for spring, at the mean of scaled leaf size, plotted with a 95% confidence interval.* ***B****. Histogram*

1801 *showing of the distribution of the underlying scaled temperature measurements and L. phlaeas eggs*

1802 *relative to these. Bars show counts of plants, stacked rather than overlapping.*

Relationships between microclimate and host nutritional quality

As expected, in the absence of bare ground, host nutritional quality (scaled leaf size) was significantly negatively related to scaled temperature under plants ($\beta = -0.33 \pm 0.08$, $t = -4.23$, $df = 332$, $p < 0.001$). At the lowest leaf sizes, plants with bare ground were significantly warmer than those without bare ground ($\beta = 0.76 \pm 0.11$, $t = 6.89$, $df = 332$, $p < 0.001$), and the presence of bare ground made the slope of the relationship between host nutritional quality and scaled temperature under plants significantly less negative, almost entirely flattening it ($\beta = 0.34 \pm 0.11$, $t = 3.13$, $df = 332$, $p < 0.005$) (Figure 4). Results did not qualitatively differ when using predicted nitrogen content as our host nutritional quality variable (see Table S5).

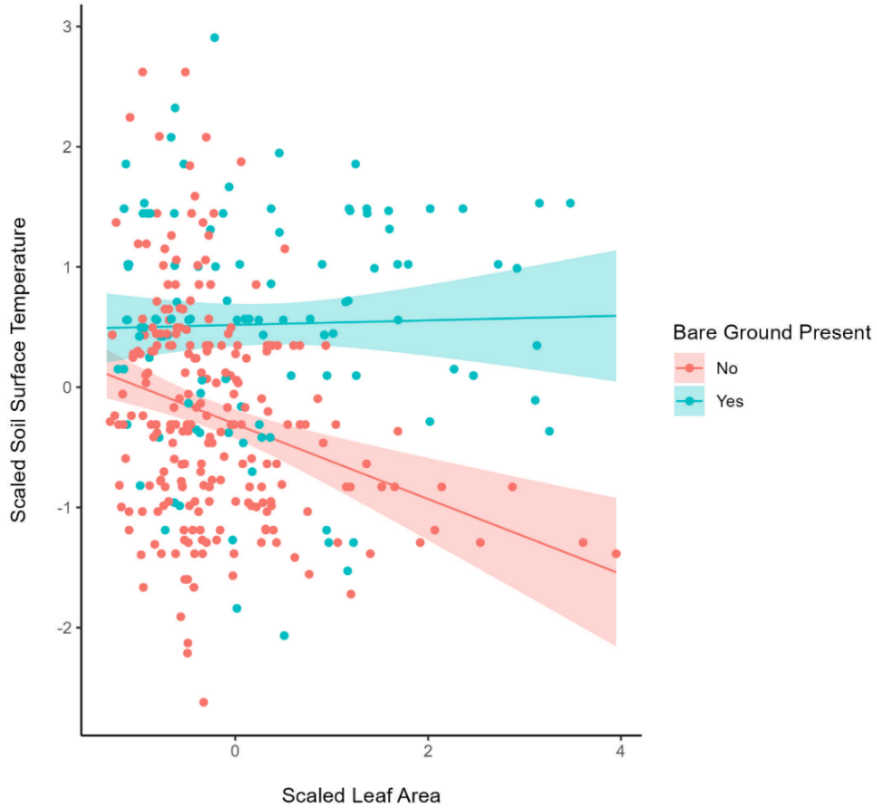


Figure 4: *The modelled relationship between plant nutritional quality (measured using the proxy of leaf size), microclimate and the presence or absence of bare ground. Predictions from linear models are shown with their 95% confidence intervals and the underlying data.*

Relationships between microclimate and vegetation structure

Variables characterising vegetation structure around *R. acetosa* plants were all significantly associated with scaled temperature under plants in the manner expected (Figure 5). Cover of bare ground (β = 0.02 ± 0.0028, t = 7.03, df = 334, $p < 0.001$) and cover of litter (β = 0.020 ± 0.0046, t = 4.26, df = 334, $p < 0.001$) were positively associated with scaled temperature under plants, and sward height (β = -0.04 ± 0.0035, t = -10.45, df = 334, $p < 0.001$) and cover of vegetation (β = -0.02 ± 0.0037, t = -6.20, df = 334, $p < 0.001$) were negatively associated. Individually, sward height explained more variation in microclimate than any other variable (R^2 = 0.25), followed by bare ground (R^2 = 0.13), vegetation cover (R^2 = 0.10) and litter cover (R^2 = 0.05).

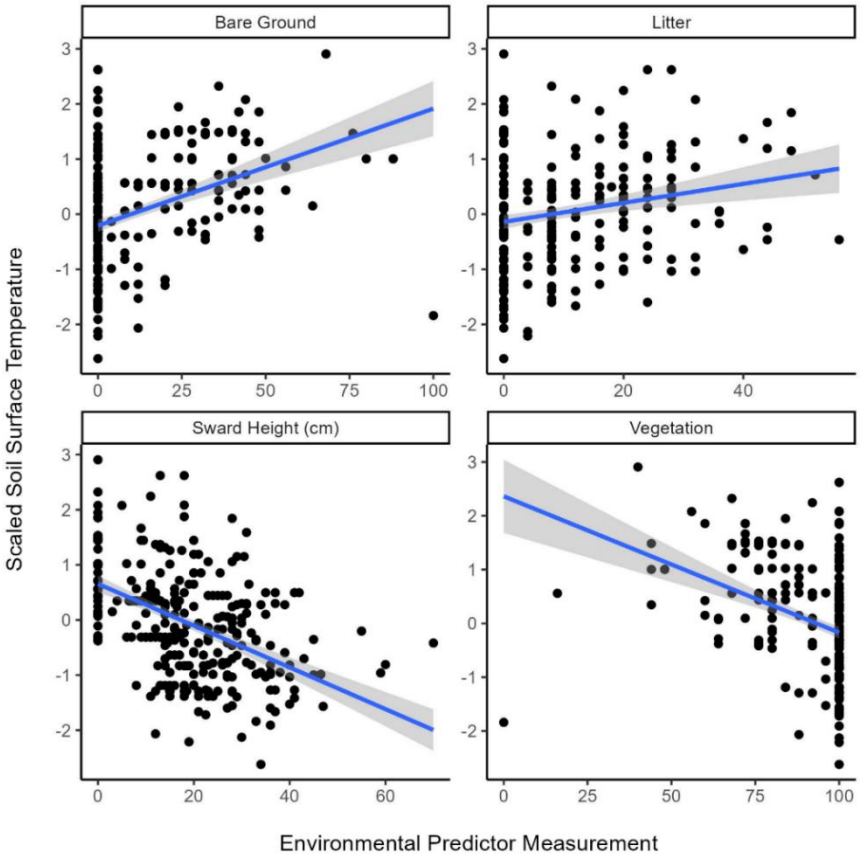


Figure 5: *Relationships between vegetation structure around hostplants and the relative temperatures measured under them. Predicted trends from linear models are shown with their 95% confidence intervals and the underlying data.*

Relationships between vegetation structure and host nutritional quality

Litter cover and sward height were both related to our host nutritional quality proxy (average leaf size) in the manner expected, the former significantly negatively associated ($\beta = -9.44 \pm 1.92$, $t = -4.91$, $df = 237$, $p < 0.001$) and the latter positively associated, but not significant ($\beta = 1.32 \pm 2.29$, $t = 0.57$, $df = 237$, $p = 0.57$). The litter model therefore explained more variation in host nutritional quality than sward height (litter $R^2 = 0.09$, sward height $R^2 = 0.0014$).

Discussion

Because of the emphasis on thermal constraints governing larval host plant use by butterflies, we wanted to examine whether host nutritional quality might play an overlooked role in egg-laying patterns of *L. phlaeas*, particularly when thermal constraints were lessened during warmer periods of the year. Our results provided no support for this. We found that egg-laying of *L. phlaeas* on *R. acetosa* was primarily determined by the fine-scale temperature around plants, and no significant effect of host nutritional quality (proxies of nitrogen content) on the probability of egg-laying, and no difference in this between a warmer period (in late spring) and a cooler one (in early autumn). Given that most plants were of low nutritional quality for *L. phlaeas* (Figure S2) (Chapter 2, this thesis), and the evidence that females of related butterfly species are able to select host plants on the basis of nutritional quality (Bourn & Thomas, 1993; Letourneau & Fox, 1989; Prudic et al., 2005), it appears that female *L. phlaeas* are missing an opportunity to maximise larval fitness by laying their eggs on the minority of plants with higher nutritional quality.

One explanation might be that host plants with higher nitrogen content are not necessarily of higher nutritional quality. Experiments increasing plant nitrogen content suggest that while under normal conditions many insects, including *L. phlaeas*, are limited by nitrogen availability, high levels of nitrogen can induce limitation by other nutrients like phosphorus, and reduce performance (Chapter 2, this thesis). These results, however, are from experiments where nitrogen is manipulated independently of phosphorus which is present at very low levels, to create very high N:P ratios. Such high N:P ratios are rare under natural conditions except on very weakly-buffered soils (Vogels et al., 2017), and we suggest unlikely here, although we did not measure phosphorus levels in *R. acetosa*. Instead, we suggest that the reason that female *L. phlaeas* do not appear to distribute eggs to afford larvae the highest food quality may be the apparent trade-off between host nutritional quality and temperatures around host plants. In the absence of soil disturbance creating bare ground under host plants there was

a significant negative correlation between host nutritional quality and temperature under hosts: plants of higher nutritional quality were located in cooler microclimates.

We expected this on the basis that higher nitrogen contents in hostplants would reflect higher soil nitrogen levels, which would in turn enhance plant productivity, leading to denser vegetation and lower temperatures around host plants. We found some support for this mechanism, with strong associations between measures of vegetation structure and temperature (shorter, more open vegetation meant higher temperatures) but only weak relationships between vegetation structure and host nutritional quality in the directions expected – higher litter cover was significantly, albeit weakly, associated with lower nutritional quality, while higher sward heights were positively related to host nutritional quality, but not significantly. In spite of this, it is difficult to see another mechanism that could alter host nutritional quality and vegetation structure to affect microclimate simultaneously. Grazing or cutting often does the latter, but the most commonly-reported plant response to grazing appears to be an increase nitrogen uptake and availability in young tissues to facilitate growth (eg. Jaramillo & Detling, 1988; Ruess, 1984), which would lead to a positive correlation between temperature and host nutritional quality, rather than the inverse observed here. The rather weak relationships detected here between host nutritional quality and vegetation structure may reflect the fact that our measures of vegetation structure, though commonly used approximations, imperfectly capture the factors driving temperature variation and responding to soil nitrogen that could create this link between host nutritional quality and temperature that we observed (for example vegetation density). Further work relating a more detailed characterisation of vegetation structure to host nutritional quality and, crucially, measuring underlying soil nitrogen levels would help to establish whether this trade-off is indeed a general phenomenon for grassland insects.

Therefore, it appears that in our system ovipositing *L. phlaeas* face a choice between high nutritional quality host plants or warm host plants, adding to previous work showing that egg-laying butterflies may have to balance nutritional requirements against thermal requirements (Krämer et al., 2012). The

apparent prioritisation of the latter implies that thermal constraints are strong for *L. phlaeas*, in line with previous work (Leon-Cortes et al., 2000; Streitberger et al., 2014), and exceed nutritional constraints. If our observed trade-off is a general phenomenon, this suggests that the strong picture of thermal constraints from previous work on habitat use by similar butterfly species has not overlooked strong nutritional constraints (eg. Thomas, Bodsworth, Wilson, Simmons, Davies, Musche, et al., 2001; Thomas, 1983; Thomas et al., 2001; Thomas, 1983; Thomas et al., 1986). Although we do not have any data relating *L. phlaeas* larval performance to temperature, previous experimental work (Chapter 2, this thesis) suggests that although most *R. acetosa* plants in our system were of low nutritional quality for *L. phlaeas* (Figure S2), they were still in a range where larval survival rarely dropped below two thirds of the maximum level, and in most treatments survival was around 80% of the maximum. Furthermore, in the work reported in Chapter 2, *L. phlaeas* larvae were unable to feed selectively on different plants or plant parts to regulate their nutrient intake as they would be able to in the field, potentially buffering impacts of low host nutritional quality. Thus, the fitness consequences of using even the hosts with the lowest nutritional quality at our study site are likely to be relatively small for *L. phlaeas* and given the previous evidence of thermal constraints for this and related butterfly species, the absence of an impact of host nutritional quality on egg-laying is perhaps not surprising. A further intriguing possibility is that the fitness costs associated with low quality food may also be reduced by high temperatures. Clissold et al. (2013) found that the efficiency of macronutrient absorption varied with temperature for *Locusta migratoria* nymphs, and that protein absorption was more efficient at higher temperatures for nymphs feeding on *Themeda triandra*. Protein is the main pool of nitrogen in plants, suggesting that increases in absorption efficiency on warm host plants could offset the cost of their lower nitrogen levels. Similar work is lacking for Lepdioptera, however, and Clissold et al. (2013) did not observe the same trend in locusts fed *Triticum aestivium*, suggesting that such increases in absorption efficiency are unlikely to be universal.

In spite of the far higher ambient temperatures and microclimatic temperatures around *R. acetosa* in late spring, we found no evidence that *L. phlaeas* shifted to use hosts with higher nutritional quality in relatively cooler microclimates in spring compared to autumn. This contrasts with previous work on another Lycaenid butterfly species *Polyommatus bellargus*, showing that higher ambient temperatures in spring allowed the use of larval hostplants growing in cooler micro-habitats, doubling habitat availability for the species (Roy & Thomas, 2003). This difference is difficult to explain and seems unlikely to reflect a difference in thermal constraints given that *L. phlaeas* occurs north into the Arctic Circle (Kudrna et al., 2011) while *P. bellargus* reaches the northern edge of its range in southern England, where it is dependent on conservation management to provide suitably warm micro-habitats (J. A. Thomas, 1983).

Swards at our study site were rather tall (mean = 23.30 cm, sd = 11.26) suggesting that in general vegetation was tall and cool, and a very small proportion of the foodplants were thermally suitable for *L. phlaeas*. It may therefore simply be that in mesotrophic grasslands like our study site, *L. phlaeas* exists mainly in small islands of disturbed ground or low fertility where host plant microclimates are suitably warm both in spring and autumn, but there is little other suitable habitat for it to move into when thermal constraints are lessened (away from these 'islands', most foodplants are still too cool). Indeed, we often observed that egg-laying took place on plants growing on bare ground produced entirely by *T. europaea* (Mole) disturbance which was positively associated with the temperature under host plants. In fact, 55% of plants with eggs had some bare ground within 25cm of them, whereas only 11% of unoccupied plants did. These results reinforce the importance of soil disturbance for creating suitable microhabitats for *L. phlaeas* and similar thermophilous grassland butterflies, in line with previous work. Streitberger et al. (2014) also observed that occupancy of foodplants on molehills by *L. phlaeas* was high, while Streitberger & Fartmann (2013) found the same for the hesperiid butterfly *Pyrgus malvae*, and de Schaetzen et al. (2018) also found that *P. malvae* egg-laying was positively related to soil disturbance by Wild Boar (*Sus scrofa*). This survival on small islands of warm

microhabitat may explain the different response to *P. bellargus* whose habitats are often heavily grazed to maintain relatively high habitat quality across sites. This suggests the potential benefits of reduced thermal constraints, either in the short term (seasonal) or the long term (due to climate change) depend partly on the extent of this reduction, but also the availability of suitable habitat the edge of species' niches for them to move into, reinforcing the importance of continued management of open habitats to create suitable microclimates for thermally-constrained species, in spite of the potential benefits of climate change to these species (O'Connor et al., 2014).

Our results indicate that habitat use by *L. phlaeas* is driven mainly by thermal requirements and not by nutritional requirements, and egg-laying females are forced to use host plants of lower nutritional quality given the trade-off between nutritional quality and microhabitat temperature that we observed. This provides useful context for laboratory experiments showing strong impacts of plant nitrogen content on the performance of *L. phlaeas* (Chapter 2). The results reported here suggest that such responses may not scale up to impacts of nitrogen pollution on wild populations via changes in plant nutritional quality because high-nitrogen plants will be thermally unsuitable. Thus, *L. phlaeas* and species with similar thermal requirements seem more likely to be negatively impacted by changes to vegetation structure under nitrogen pollution, than via changes in host nutritional quality. Interestingly, we found that soil disturbance by *T. europaea* disrupted the trade-off between host nutritional quality and temperature around plants (by creating high temperatures around plants of high nutritional quality), suggesting that soil disturbance generated in this manner, or at larger scales by grazing animals, may be able to contribute to maintaining species of early-successional habitats like *L. phlaeas* under nitrogen pollution.

Conclusions

We found that *L. phlaeas* was strongly thermally-constrained, and selection of larval host plants for egg-laying was determined by warm microclimates rather than host nutritional quality, even during

warmer periods of the year when thermal constraints might be expected to be relaxed. Indeed, these constraints may force *L. phlaeas* to use plants of lower nutritional quality, as we observed a negative association between host nutritional quality and temperatures around plants. We suggest this could be a general trade-off faced by grassland insect species, driven by an underlying gradient in soil nitrogen and its impact on host nutritional quality and vegetation structure, but further investigation is needed to confirm the mechanisms underlying this interesting pattern. If this is the case, the strong picture of thermal constraints for many threatened grassland insects like *L. phlaeas* probably does reflect weak nutritional constraints, and not an over-looked importance of host nutritional quality in shaping habitat use. Indeed, the failure of *L. phlaeas* females to shift to egg-laying on cooler plants of higher nutritional quality during a warmer period of the year when we expected these thermal constraints to be lessened reinforces the importance of thermal constraints. Overall, this places the strong responses of herbivores like *L. phlaeas* to variation in host nutritional quality in experiments in context and implies that they are more likely to be negatively impacted by nitrogen pollution via changes in vegetation structure than positively via increased host nutritional quality, and that even under a changing climate, management to maintain warm micro-habitats will be essential to their survival.

Author Contributions:

Design: WL, OL, RF

Data collection: WL

Data Analysis: WL

Manuscript: WL, OL, RF

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Supplementary Material

Proxy Model Results

Table S1: *Results of linear models relating plant nitrogen content (micromoles g^{-1}) to the possible proxies assessed.*

Model	Term	β	Standard Error	t	p
Leaf Size + Plant Area	Intercept	1386.62	141.09	9.83	<0.001
	Average Leaf Size (mm^2)	0.42	0.12	3.64	<0.001
	Plant Area (cm^2)	0.22	0.37	0.6	0.55
	Season	-191.59	125.81	-1.52	0.13
Leaf Size	Intercept	1389.57	140.2	9.91	<0.001
	Average Leaf Size (mm^2)	0.46	0.09	5.04	<0.001
	Season	-172.67	121.05	-1.43	0.16
Plant Area	Intercept	1724.81	117.11	14.73	<0.001
	Plant Area (cm^2)	1.03	0.33	3.17	<0.01
	Season	-407.63	122.4	-3.33	<0.01
Quality PC1	Intercept	1745.28	92.66	18.84	<0.001
	Quality PC1	-146.31	32.05	-4.57	<0.001
	Season	-290.92	117.12	-2.48	<0.05
Quality PC2	Intercept	1982.76	88.75	22.34	<0.001
	Quality PC2	-52.45	74.01	-0.71	0.48
	Season	-387.97	142.53	-2.72	<0.01

Relationship of host nutritional quality proxies with plant C:N ratio

These were qualitatively the same as for plant N content (micromoles per g) (Table 1 and Table 2), with average leaf size the best proxy, but plant size adding a small amount of additional explanatory power.

Table S2: *Pearson's correlation co-efficients and signifance tests for correlation of possible plant proxies with R. acetosa C:N ratio (measured in micromoles g^{-1})*

Proxy	R	t	DF	p	Lower Confidence Interval	Upper Confidence Interval
Average Leaf Size (mm^2)	-0.62	-6.03	57	<0.001	-0.76	-0.44
Plant Area (cm^2)	-0.42	-3.49	57	<0.01	-0.61	-0.18
Quality PC1	0.58	5.44	57	<0.001	0.38	0.73
Quality PC2	0.17	1.32	57	0.191	-0.09	0.41

Table S3: *Comparison of models relating plant C:N ratio to different possible proxies*

Proxies	AIC	BIC	R^2
Average Leaf Size (mm^2) + Plant Area (cm^2)	386	396	0.41
Average Leaf Size (mm^2)	385	393	0.40
Plant Area (cm^2)	395	404	0.29
Quality PC1	386	395	0.39
Quality PC2	407	416	0.13

Plant Chemistry

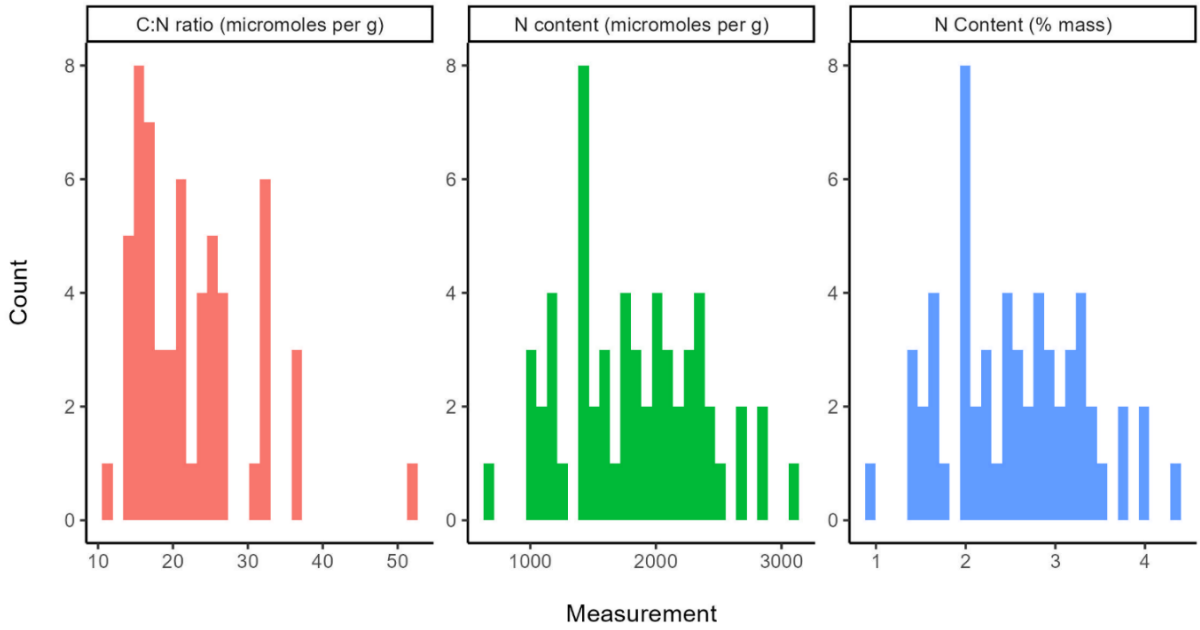


Figure S1: *Distribution of chemical measurements for R. acetosa plants that were analysed. As these were a stratified sample they do not represent the true distribution in the population.*

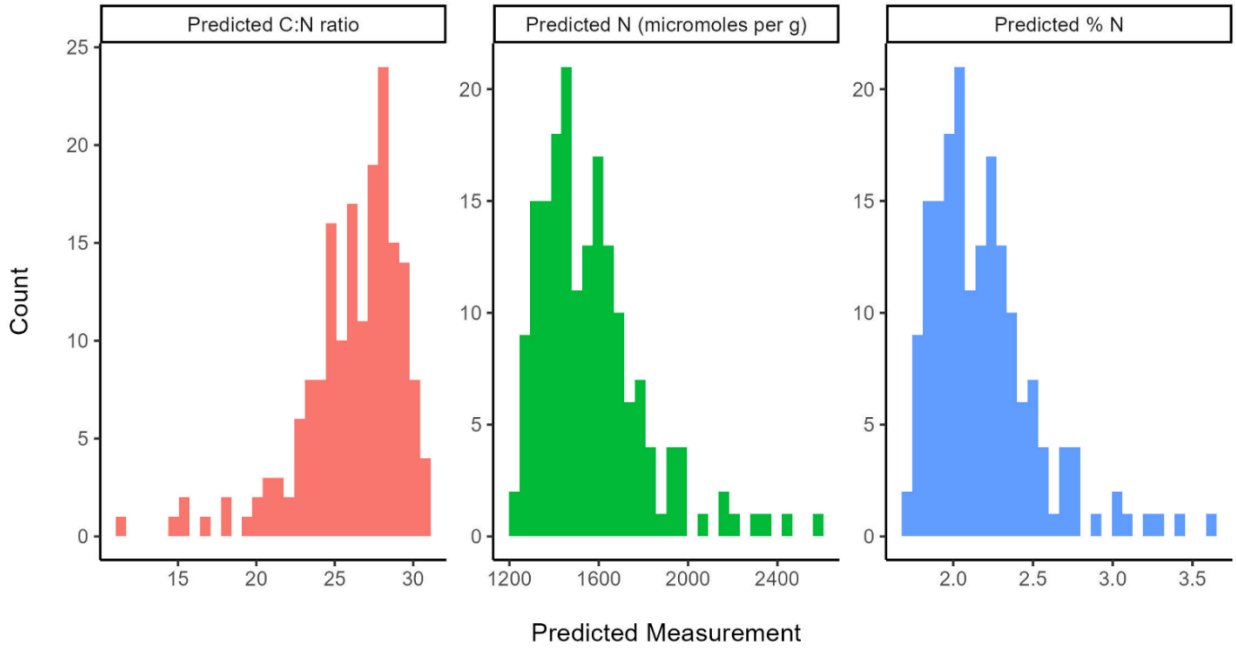


Figure S2: *Predicted C:N ratio and nitrogen contents of R. acetosa plants at Shotover Country Park, based on our models of host nutritional quality as a function of average leaf size and plant area. These are plotted for control plants, and therefore give a better sense of the likely distribution of measurements within the population, unlike figure S1 which shows a sample of plants stratified by proxies of nitrogen content, therefore giving a far more uniform distribution than is actually present. This shows that most plants were likely to be of low nutritional quality, with larvae suffering from nitrogen deficiency, as most plants are predicted to have C:N ratios higher than the value of 18.1 predicted to be optimal for L. phlaeas in previous work (Chapter 2, this thesis).*

Using predicted plant N content as a measure of host quality, rather than average leaf size

Egg-laying model

Table S4: *Results of model of L. phlaeas egg or larval presence or absence as a function of scaled temperature under R. acetosa plants, predicted N content and their interactions with season (and 1/scaled standard error around predicted N content as weights). Mckelvey-Zavoina R^2 for this model was 0.45. These were qualitatively the same as when we used our proxy for nitrogen content (leaf size) (Figure 3).*

Terms	Odds Ratios	CI	z	p
Intercept	0.90	0.53-1.52	-0.41	-
Temperature	4.85	2.90-8.90	5.56	<0.001
Season (spring)	1.59	0.99-2.65	1.86	0.06
Predicted N content	1.59	0.76-3.43	1.22	0.23
Temperature : Spring	1.10	0.51-2.29	0.25	0.81
Predicted N content: Spring	1.20	0.52-2.90	0.43	0.67

Trade-off Model

Table S5: *Results of model of scaled temperature under R. acetosa plants as a function of their predicted N content and the presence of bare ground (and 1/scaled standard error around predicted N content as weights). These were qualitatively the same as when we used our proxy for nitrogen content (leaf size) (Figure 4).*

Term	Estimate	Standard Error	t	p
Intercept	-0.3	0.06	-4.73	-
Scaled predicted N content	-0.31	0.07	-4.17	<0.01
Bare Ground Present	0.72	0.11	6.44	<0.01
Scaled predicted N content : Bare Ground Present	0.36	0.11	3.25	<0.01

Chapter 4: Modelling the effect of nitrogen levels on insect distributions

Contributors: William Langdon, Emily Dennis, Richard Fox, Owen Lewis

Fundamentally, insect responses to nitrogen pollution depend on how nitrogen levels affect the probability of their occurrence - whether rising nitrogen levels positively or negatively affect the availability of their habitat. Here we attempt to model this relationship for butterflies and moths in Great Britain to provide indices that can be used to assess the sensitivity of different species to nitrogen pollution and monitor changes in butterfly and moth communities as a result of pollution. We test whether our indices capture nutrient gradients shaping butterfly and moth communities via tests of their internal consistency and comparison with previous work and expert assessment. We find challenges with dealing with spatial bias in large citizen science datasets when deriving our indices, but that they perform reasonably well in tests. This suggests that our methods and indices could be a promising tool for understanding the sensitivity of insects to nitrogen pollution, and monitoring changes in insect community structure resulting from this sensitivity.

Introduction

Rising levels of reactive nitrogen are one of the primary anthropogenic drivers of changes to plant communities, with effects that are widespread (Smart et al., 2003, 2005), and now well-characterised (Bobbink et al., 2010). Theory, and increasingly evidence, suggests that these changes are likely to propagate up to insects that live on and among these plant communities (Nijssen et al., 2017; Stevens et al., 2018; Throop & Lerdau, 2004), but it remains a challenge to predict and detect the likely effects on insect community structure.

While there have been a number of attempts to link spatial and temporal variation in insect community structure and species' trends to spatial and temporal variation in soil eutrophication caused by nitrogen pollution (Carvalheiro, Biesmeijer, Franzén, Aguirre-Gutiérrez, et al., 2020; Hendriks et al., 2013; T. Roth et al., 2021; Vantieghem et al., 2017; WallisDeVries & van Swaay, 2013), such work is rare. This may be because nitrogen pollution has many sources that are not always mapped at the same temporal and spatial scales as insect data, making realistic analyses challenging. Even where such data are available, the relative importance of different nitrogen sources is disputed (Pescott & Jitlal, 2020), their effects are moderated by context (for example soil type) (Bobbink & Hicks, 2014), and it is challenging to control for the effects of other changes simultaneously affecting insect communities, such as rising temperatures and land-use change (Habel et al., 2019; Oliver et al., 2015).

Perhaps for these reasons, trait-based approaches have become a popular way to assess the impacts of nitrogen pollution on insect communities. These compare population trends across groups with trait differences that are expected to be strongly associated with high or low sensitivity to the effects of nitrogen pollution, and attribute differences in trends to its effect. These methods have provided valuable signals of the impacts of nitrogen pollution on insect communities, while simultaneously testing hypotheses about the likely mechanisms driving these changes. For example, differing population trends for species using nitrophilic and nitrophobic foodplants suggest that communities of insect herbivores are being affected by the dominance of nutrient-demanding plant species under eutrophication driven by nitrogen pollution (Betzholtz et al., 2012; Fox et al., 2014; Öckinger et al., 2006). However, species' sensitivities are likely to be influenced by a large number of traits (Poyry et al., 2017), reflecting the diverse mechanisms via which insects are expected to be affected by nitrogen pollution – not just changes in the abundance of foodplants, but also changes in microhabitat structure (as vegetation grows taller and denser) and food quality (as plant nitrogen content increases) (Nijssen et al., 2017; Vogels et al., 2023; WallisDeVries & van Swaay, 2006). These traits also govern insect responses to other anthropogenic pressures (WallisDeVries, 2014; WallisDeVries & van Swaay, 2017),

meaning that relating species' trends to traits may sometimes fail to fully capture the effects of nitrogen pollution (using single traits) (Figure 1, Chapter 3, this thesis) or fail to only capture the effects of nitrogen pollution (using larger numbers of traits).

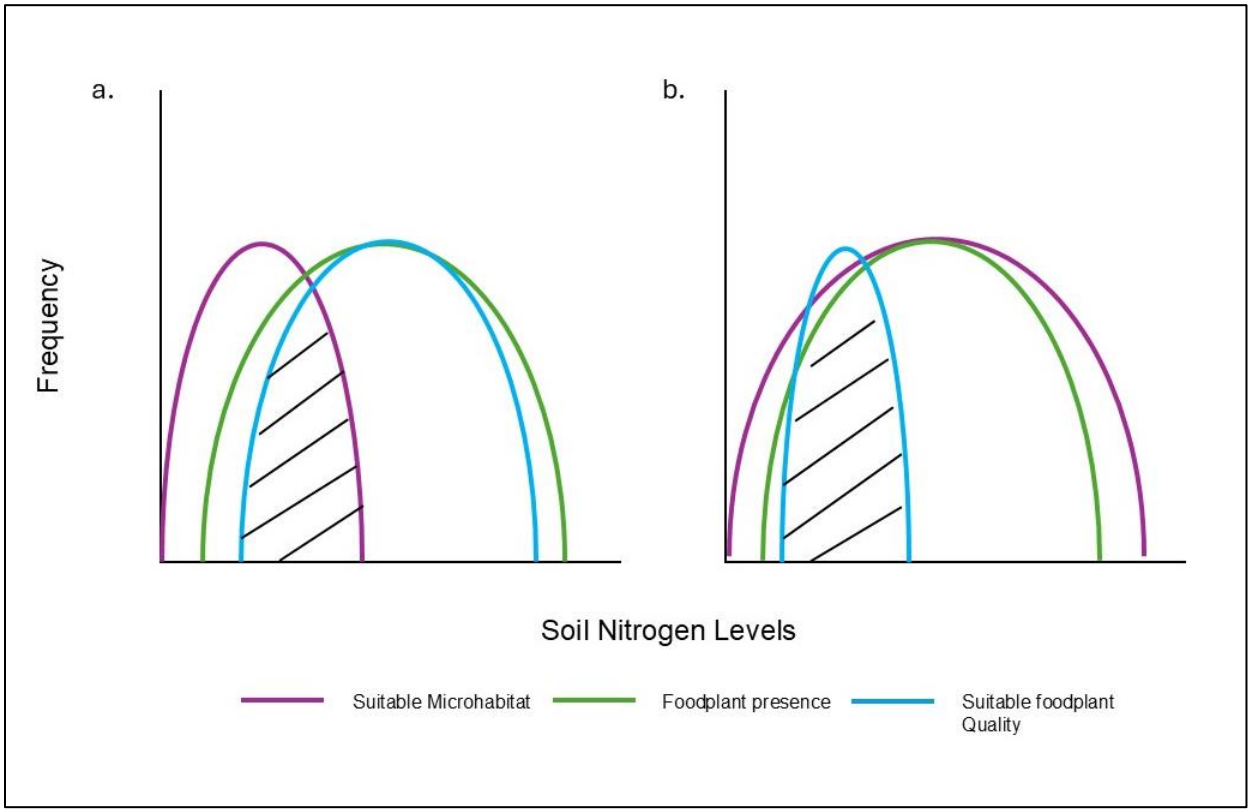


Figure 1: *Schematic showing how soil nitrogen levels could shape the availability of suitable micro-habitat, suitable foodplant quality, and the presence of foodplants for two insect species 'a' and 'b'. Each species can only occur where their requirements in all of these dimensions are met (black hashed area). Here species a and b are constrained by soil nitrogen and affected by nitrogen pollution for different reasons, a mostly by the availability of suitable microhabitat (since other aspects of its niche mostly overlap in the nitrogen levels at which they are available), and b mostly by the availability of its foodplant. Thus, using foodplant Ellenberg score as a proxy for species' sensitivity to nitrogen pollution might work well for species b but not a, while using traits associated with microhabitat preference might work well for species a, but not b. A better approach to understanding species' sensitivity may therefore be to model the 'nitrogen niche' that emerges from all of these constraints (hashed black area), as we attempt to do here.*

The use of traits as a proxy for species' sensitivity to nitrogen pollution is ultimately an attempt to capture the position or breadth of the nitrogen dimension of species' niches, based on hypotheses about the mechanisms via which soil nitrogen levels constrain insects, and how species' traits dictate these constraints. A more intuitive approach may simply be to actually measure the net effect of these constraints – the relationship between species' occurrence and soil nitrogen levels and use indices describing this relationship as a measure of species' sensitivity. A similar approach has often been used for assessing community responses to climate change, through the calculation of species temperature indices (Devictor et al., 2008). These are calculated as the mean temperature of sites occupied by a species in a study area and provide an indication of its relative temperature associations. From these, community temperature indices can be derived (as the average species temperature index of species in a community, sometimes weighted by their abundance). These have been used to understand the rate and extent of community restructuring by climate change for a wide range of taxa at large spatial scales (Devictor et al., 2008, 2012; Lindström et al., 2013; Montràs-Janer et al., 2024). Similar indices for nitrogen would therefore be extremely valuable and could complement trait-based work, providing a way to validate hypotheses about how species with different traits are constrained by soil nitrogen, and potentially highlight taxa whose sensitivity to nitrogen pollution might be overlooked using trait-based approaches. Community nitrogen indices that could be derived from these have an intuitive interpretation that could also make them a powerful way to communicate the often under-appreciated effects of nitrogen pollution to policymakers, and to assess the impact of restoration attempts.

The only attempt to develop such indices for insects has been for Dutch butterflies by Oostermeijer & van Swaay (1998). They used these indices to detect long-term shifts towards more nitrophilic species in the Dutch fauna, and fluctuations in community structure driven by annual variation in nitrogen deposition levels, showing them to be very sensitive indicators (WallisDeVries & van Swaay, 2017). The indices were derived by relating species' presence or absence along monitored transects to the Ellenberg nutrient (N) scores of vegetation on these transects. Vegetation was characterised for 954

sections of 228 butterfly monitoring transects using the Braun-Blanquet method (Ivanova, 2024), and then polynomial logistic regression was used to relate species' occurrence probabilities to vegetation Ellenberg nutrient scores. Species' preferred vegetation nitrogen levels and nitrogen indices were then derived as the peak of this regression.

Ellenberg nutrient scores were derived for most central-European plant species by Ellenberg (1979), and later extended to other areas, including the UK (Hill et al., 2000). The scores represent the nutrient levels at which plant species are most likely to occur on a scale from 1 to 8. While initially derived from expert assessments, they have since been shown to correlate with soil nitrogen levels, and the environmental gradients that they effect and insects are responding to under nitrogen pollution – productivity and its effect on vegetation structure (Hill & Carey, 1997), plant nitrogen content (Duru et al., 2010; Hodgson et al., 2011) and presence of (host) plant species (Ter Braak & Gremmen, 1987). Relating insect presence to Ellenberg scores of vegetation in this way is therefore a simple and meaningful way to summarise their responses to soil nitrogen levels via multiple mechanisms.

The disadvantage of the approach used by Oostermeijer & van Swaay (1998) is that it relies on a large monitoring scheme, with data on insect species occurrence collected systematically in a wide range of habitats, and vegetation data from each sampling site. Such extensive monitoring data are extremely rare for insects. While more such schemes are coming online (Pocock et al., 2015), their likely uptake for less well-recorded taxa is much lower than for butterflies, limiting the amount of data that will be available to support the development of indices. Furthermore, the vegetation data required for the approach of Oostermeijer & van Swaay (1998) is extremely laborious to collect.

Thus, there is a need for an approach that can use more widely available forms of insect data, and which is less demanding in terms of the associated vegetation data. Here we attempt to develop nitrogen indices using presence-only insect records from Great Britain. These are several orders of magnitude more frequent than abundance data, even for butterflies, and available for a far wider

variety of taxa. We then replace measured vegetation data with modelled Ellenberg nutrient scores of vegetation in Great Britain at the same resolution. We believe our approach provides a simple, flexible technique for developing indices that can be used to understand the sensitivity of a wide range of species to a wide range of anthropogenic changes, and to understand and predict community re-structuring in response to anthropogenic change.

Methods

Overview

In broad terms, we first model species' responses to a gradient of nutrient levels across Great Britain (GB) using generalised additive models (GAMs) relating the probability of an observer detecting a species on a visit to the modelled Ellenberg nutrient score of vegetation at the visited site, while controlling for other features of the site or visit that could affect the probability of detection. From these modelled relationships we describe species' nutrient associations by calculating the nutrient level at which detection probability is highest ('optimum'), and a measure of the range of nutrient levels over which it remains high ('tolerance'). To ensure the robustness of our indices we then test whether they are internally consistent – i.e. that species tend to occur alongside other species with similar scores, using an independent dataset. A consistent pattern implies that indices are capturing an environmental gradient shaping species' distributions (Diekmann, 1995; Ter Braak & Gremmen, 1987). We then examine the nature of this gradient (i.e. whether it is indeed related to nutrient levels) by testing whether estimated species' nutrient associations are consistent with assessments by taxonomic experts and previous similar work.

Focal Species

While a wide variety of insect taxa are likely to be directly or indirectly affected by nitrogen pollution (Nijssen et al., 2017), we chose to focus on butterflies and macro-moths (the latter hereafter 'moths').

In Great Britain these groups contain a higher proportion of thermophilous species than many other insect taxa (Bourn & Thomas, 2002; Hambler & Speight, 2004; J. A. Thomas, 1995) and almost all species are herbivorous, and are likely to be sensitive to changes in plant nitrogen content (Chapter 2, this thesis). Soil nitrogen levels are therefore likely to constrain them in several different ways. This makes them highly sensitive to the effects of nitrogen pollution (WallisDeVries & van Swaay, 2017), and of great interest in this context. These taxa are also well-studied, meaning that they have data-rich recording schemes enabling the development of robust indices, and expert assessment should provide a relatively reliable test of indices. We focus on a sub-set of these species – those butterflies and moths that feed as larvae on non-woody foodplants, as most of the proposed impacts of nitrogen pollution on lepidoptera focus on species feeding in the field layer. There is currently little evidence of strong impacts on species feeding on trees (except occasional reports of changes in food quality) (Nijssen et al., 2017). The focal taxa were selected using Cook et al. (2022), as species feeding on any non-woody plant groups (even if they also use woody foodplants). We also include species feeding on the dwarf shrubs *Calluna vulgaris* and *Vaccinium myrtillus*, on the basis that these form important parts of field-layer communities in open moorland and heathland habitats.

Species Occurrence Data

Species occurrence data were collated from separate UK recording schemes for butterflies (Fox et al., 2023) and moths (Fox et al., 2011). In both schemes, records are submitted by volunteers and verified by experts with strong identification skills and knowledge of the taxa, meaning that there is high confidence in their accuracy. Records are presence-only, representing occurrences of a given species in a given place on a given date. We retained only records that were resolved to 1km x 1km Ordnance Survey (OS) grid squares (or finer), and specific days, from Great Britain, and overlapping with the period covered by our modelled vegetation data (2015-2019). This gave a total of 4,578,993 records for butterflies and 13,792,564 records for moths.

Vegetation Data

We related the pattern of detections and non-detections for each species to nutrient levels using a map of the average cover-weighted Ellenberg nutrient (N) score for vegetation in Great Britain between 2015 and 2019 published by Jarvis et al. (2022). The map is derived from vegetation plots monitored as part of three recording schemes, the Countryside Survey (445 plots) (Smart et al., 2020), Natural England's Long-Term Monitoring Network (1287 plots) and the National Plant Monitoring Scheme (643 plots) (Pescott et al., 2020). Cover-weighted Ellenberg nutrient (N) scores were calculated for all plots, and an integrated model (accounting for the different sampling techniques and biases in each scheme) was used to explain spatial variation in scores based on a variety of covariates covering habitat type, atmospheric pollution, temperature and precipitation in each plot. The map was then formed from predictions made using this model for 1km OS grids across Great Britain in the period 2015-2019, taking the same climate, pollution and habitat data that were used as co-variates in the model as input data. These predictions have uncertainty associated with them captured by a map of standard error at the same resolution, and to minimise this we excluded grid squares with standard errors above the 95th quantile.

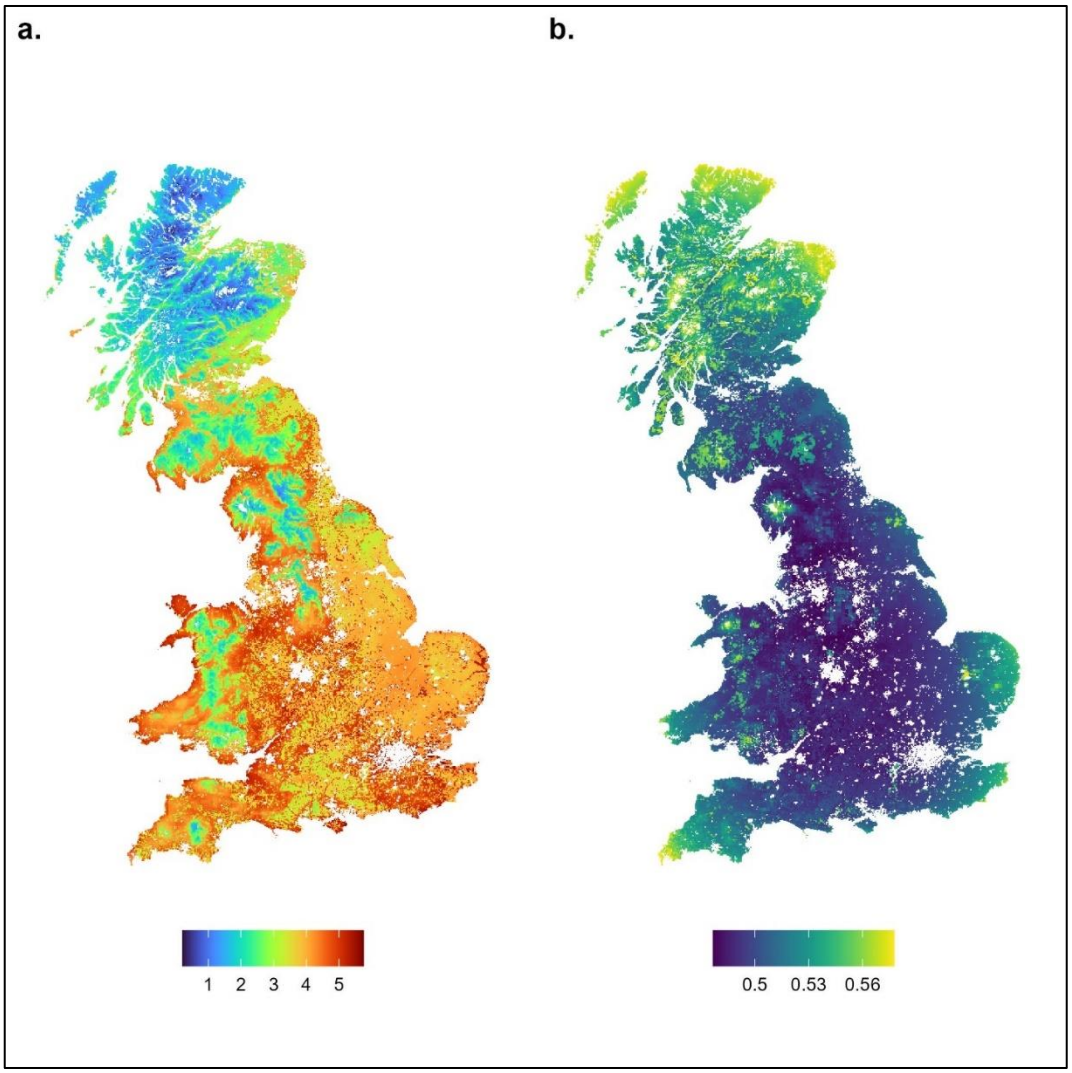


Figure 2: *a. Modelled cover-weighted Ellenberg N scores of vegetation in Great Britain at 1km resolution, from Jarvis et al. (2022), and b. the associated standard errors for predicted scores. The data shown are those used for modelling with lepidoptera occurrence data, with squares in the upper 5% quantile of standard error removed (mostly urban areas and extreme longitudes and latitudes).*

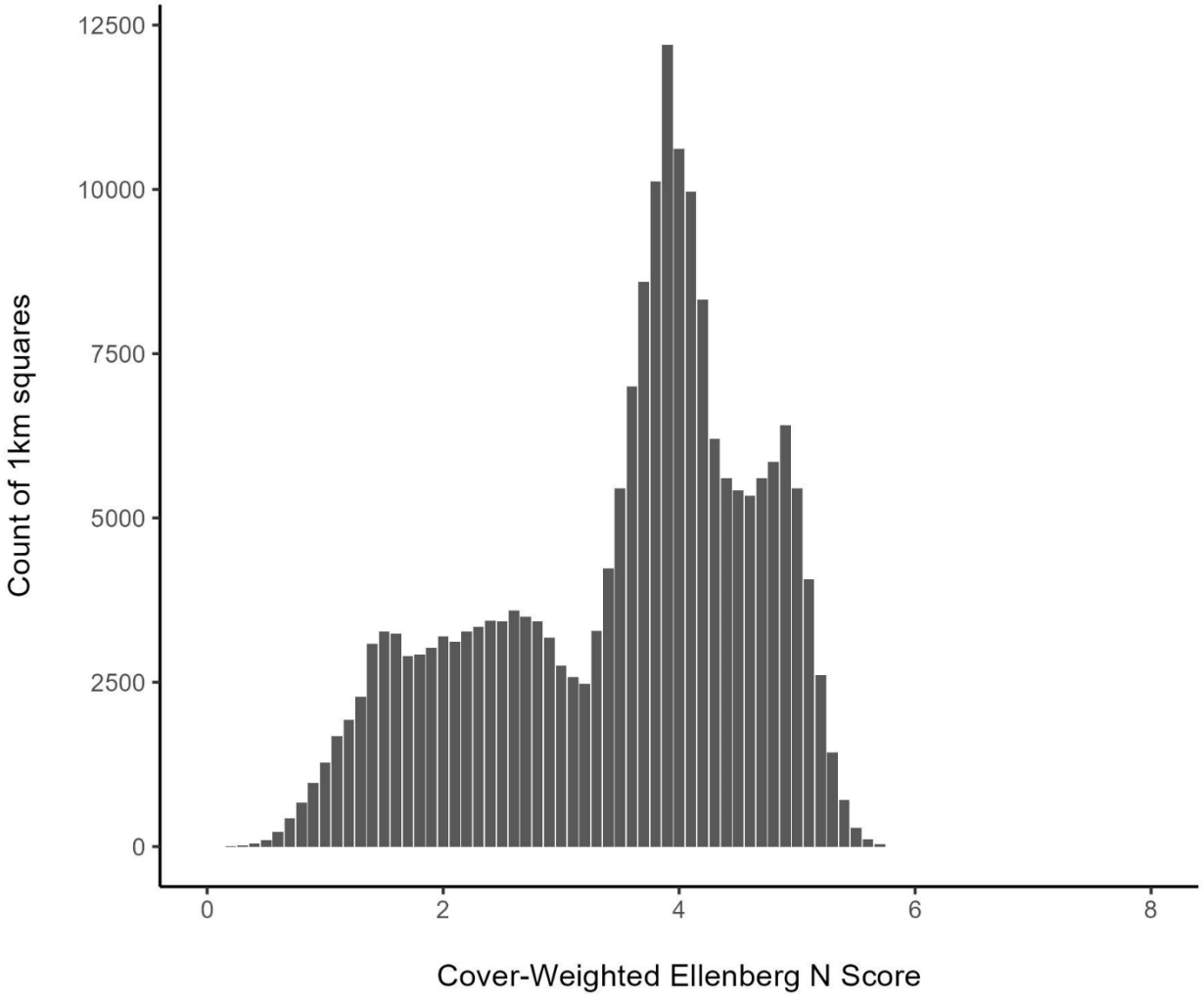


Figure 3: *Distribution of modelled cover-weighted Ellenberg N scores of vegetation in Great Britain at 1km resolution, binned into groups of 0.1 from Jarvis et al. (2022). The data shown are those used for modelling with lepidoptera occurrence data, with squares in the upper 5% quantile of standard error removed. Note that there are no squares with scores greater than 6, limiting the maximum optimum value that species could be allocated in our analysis as we did not predict their responses outside the range of the data.*

Model Choice

A variety of modelling approaches other than GAMs have been used to model species responses to environmental gradients, primarily in plant ecology, including Gaussian logistic regression (ter Braak & Looman, 1986), Beta Functions (Austin, 1976), and HOF (Huisman-Olff-Fresco) models (Huisman et al., 1993), the latter extended by Jansen & Oksanen (2013). Comparison of these techniques suggests that by allowing a larger variety of more realistic shapes, HOF models and GAMs are best at capturing species' responses to environmental gradients (Jansen & Oksanen, 2013; Oksanen & Minchin, 2002). HOF models, however, do not easily allow for covariates other than the gradient that is the object of study, being designed for plots where effort is fixed and species' detection probabilities do not vary across plots except as a function of their occurrence, governed by one or more measured environmental gradients. This is a problem for our data, where recording patterns are not fixed - the probability of a species' detection at sites is governed partly by the effect of the environmental gradient on occurrence, and partly by the effect of recording patterns on the likelihood of detection given this underlying occurrence status. One way of dealing with this is to retain only well-recorded sites where species have been reasonably well inventoried (eg. Redhead et al., 2016). To retain the maximum amount of information, however, we instead chose to follow the approach of Bowler et al. (2023) and use visit-level generalised additive models. These enabled us to retain all presence records while controlling for imperfect detection using visit-level covariates.

Data Processing

We defined 'sites' as 1km OS grid squares with at least one occurrence record of a lepidoptera species (hence grid cells where no records were ever submitted were not included in the analysis), and 'visits' as dates on which records were submitted from these sites. We then derived detection histories at each site for each species – with detections as visits when the focal species was reported, and non-detections as visits when the species was not reported but other species in the focal species' group (moth or

butterfly) were recorded. We used a relatively stringent cut-off, running models only for species with 200 detections at 30 sites in this period. These were somewhat arbitrary, but reflected the fact that previous work using the same butterfly data to model species' woodland associations required at least 100 records to derive indices that matched expert assessment, using models with fewer covariates than ours (Redhead et al., 2016).

We then retained all detections for each species and removed non-detections outside periods of the year when species were reported. Following recent work (Gaul et al., 2022; Robinson et al., 2018, 2020; Steen et al., 2021), we then randomly selected four non-detections from each 10km grid square for each year of our study period. We then randomly removed visits from the majority class (whichever was more frequent of detections or non-detections) at the end of this process (usually absences, except for extremely widespread species), down to the size of the minority class (whichever was less frequent of detections or non-detections). Such under-sampling of the majority class eliminates the class imbalance which is typically very common in detection-non-detection data. This imbalance can reduce the accuracy of modelling, meaning that under-sampling of the majority class like this is therefore often recommended (Johnston et al., 2021; Robinson et al., 2018). Doing this under-sampling of non-detections spatially (stratifying by 10km squares) also reduces spatial bias in the non-detection dataset and has been shown to improve the accuracy of predictions from species distribution models (Gaul et al., 2022; Robinson et al., 2018, 2020; Steen et al., 2021).

Modelling

For each species we used the under-sampled datasets to model species' detection on visits to sites as a function of a smoothed spline of Ellenberg score at that site. We included a number of covariates to account for variation in the probability of detecting species on visits not arising from the effect of vegetation Ellenberg score. We then included the x and y coordinates of grid cell centroids as a bivariate spatial spline to both account for variation in detection resulting from the effect of other

abiotic components of species' niche on occurrence probability (i.e. gradients in occurrence probability), and spatial auto-correlation (Bowler et al., 2023; Guélat & Kéry, 2018). To account for temporal variation in detectability resulting from species being more detectable at different times of year we included Julian day of each visit as a smoothed spline (Bowler et al., 2023). To control for variation in detection resulting from the effort of recorders on each visit, we included list length (the number of species of the focal group recorded on that visit). To deal with temporal trends in abundance, we included year of visit as a random intercept. All splines were penalised thin-plate regression splines, with parameters estimated automatically using generalised cross-validation and restricted maximum likelihood. The only exception was Ellenberg score where the spline was limited to four basis functions ('knots'), given that we did not expect shapes more complex than unimodal responses (Bowler et al., 2023). Splines otherwise used the default settings of the gamm() function in the R package mgcv (Wood, 2011). All models used binomial error distributions.

This gave a final model, run for each species of:

Detection of species q on visit i to site j ~

s(Ellenberg nutrient score of site j, k = 4) +

s(x coordinate of site j, y coordinate of site j) +

s(Julian day of visit i to site j) + list length on visit i to site j +

year of visit i to site j (random intercept)

Where Detection is 1 or 0 and k is the maximum number of basis functions in a spline.

Index Derivation

We used two metrics to summarise the modelled responses of insects to nutrient levels, deriving them for species with significant responses, following Oostermeijer & van Swaay, (1998). These metrics were (a) their 'optimum': the Ellenberg score at which their detection probability was highest, and a measure of which nutrient levels they therefore prefer; and (b) their 'tolerance': a measure of the range of Ellenberg scores at which their detection probability was close to its maximum, and therefore a measure of the specialisation of species' requirements.

To calculate these measures and the uncertainty associated with them, we used posterior simulation from our models, generating one thousand plausible sets of model coefficients by sampling from the posterior distributions of each coefficient. For each of these sets of coefficients, we predicted the shape of the response over the range of Ellenberg scores in our modelled dataset and calculated our measures of tolerance and the optimum for each predicted response shape. This imposed a range of values for species' optima that was therefore not the 1 to 8 of Ellenberg scores, but the minimum and maximum scores of grid squares in the mapped data (Figure 2), and then the subset of those visited in the butterfly and moth datasets – for moths: 1.05 to 5.64, for butterflies: 1.15 to 5.74. Other coefficients were held at their means (Julian Date, List Length, x coordinate, y coordinate) or medians (Year) for visits where the species was present. This enabled us to explore the range of possible response shapes for each species, and in turn their possible values of optima and tolerance. We calculated the value of each parameter as the mean of the values across all one thousand response curves, and their 95% confidence intervals as the 2.5^{th} and 97.5^{th} quantiles.

Although standard approaches exist for describing species' tolerance as the width of niches modelled using GAMs (Heegaard, 2002), we chose not to use them because they are based on the width of the curve at a set height below the maximum, and are therefore only applicable to species with unimodal responses, while our dataset contained a large proportion of species with linear responses. Instead we

calculated two measures of tolerance that we felt could provide meaningful information for species with linear and non-linear responses. Both were based on the area under the curve formed by the grid of Ellenberg score values and the corresponding predicted response values. The first was 'Central Area Tolerance', calculated following Cunningham & Johnson (2012) as the minimum range of Ellenberg score values that could enclose 50% of the area under the curve. As we felt this was challenging to calculate for more complex curve shapes we then also derived our own measure of 'Normal Area Tolerance' which we expected would convey the same information, while being more readily calculated using existing programs. This was the area under the curve once all response values had been divided by the maximum value of the response for that curve.

Finally, to provide a coarser classification we also divided species into groups of 'high' or 'low' optima, and 'generalist' and 'specialist' tolerance, by dividing the difference between the maximum and minimum for each measurement by three, and classifying species with values less than or equal to the minimum plus this value low, or 'specialist', and species with values greater than or equal to the maximum minus this value high or 'generalist'. The remainder were classified as 'intermediate'.

Index Assessment

Internal Consistency

Our first assessment of our indices was to check the internal consistency of the butterfly indices, whether species tended to occur alongside other species with similar scores, indicating that the scores were therefore capturing an environmental gradient structuring butterfly communities. To do this we used data from the United Kingdom Butterfly Monitoring Scheme (UKBMS) which involves fixed transects walked weekly during the butterfly season (April-September) by volunteers who count every butterfly seen within a set distance of the route. The relative annual abundance of each butterfly species on each transect is summarised as an annual site index, which accounts for variation in species' abundances through the season, and the effects of missing counts (Dennis et al., 2013). We used these

annual site indices (Botham et al., 2022, 2023) for sites in Great Britain from 2015 to 2019 to calculate annual community nitrogen indices for each site as the mean modelled optimum of the species with indices present on the site, weighted by their annual site index (cf. WallisDeVries & van Swaay, 2017).

For species with more than 70 non-zero counts during between 2015 and 2019, we then modelled annual relative abundance as a function of the community nitrogen index calculated in the same year for that site in a similar manner to the main modelling of species' response to nutrient levels based on presence/absence data but specifying Poisson errors to better reflect count data. We also dropped covariates for date and list length because site relative abundance indices integrate species' changing abundance through the season (which is entirely covered by the recording window), and because effort is fixed within sites, i.e. the same length of transect is walked at each visit. Effort is not fixed across sites, however, and we therefore added transect length as a covariate, alongside a random intercept for survey type. This was necessary because UKBMS data also includes Wider Countryside Butterfly Survey transects that are walked less frequently (2-3 times per year). Unlike other UKBMS transects, these transects are stratified to improve sampling of common but under-monitored habitats such as farmland and therefore provide potentially valuable information about butterfly communities in more eutrophic habitats. This gave a final model of:

Relative annual abundance of species i at site j in year z ~

s(community nitrogen index at site j in year z, k = 4) +

s(x coordinate of centroid of site j, y coordinate of centroid of site j) + transect length of site j +

year z (random intercept) + survey type at site j (random intercept)

Where k is the maximum number of basis functions in a spline.

We then used the same methods as the main analysis to re-derive species' indices from these models. These indices we refer to as 're-derived' indices throughout, and we then assessed the degree of agreement between these and the original occurrence data derived indices which we refer to as 'occurrence-derived' indices throughout. To do this we calculated Pearson's correlation test, checked whether the confidence intervals of the two indices for a species overlapped, and calculated the distance between the two sets of indices for each species.

We used the UKBMS to do this analysis (rather than our original dataset of occurrence records) because it provided an independent test of our indices. We used abundance indices rather than converting counts to presence-absence data to match our original occurrence-based models (cf Oostermeijer & van Swaay, 1998; Redhead et al., 2016), as counts provided extra information to better discern the nutrient preferences of common species that occur on most transects (but are more abundant in some habitats) or species that disperse and occur as occasional vagrants on transects in unsuitable habitat. Although similar moth count datasets are available, their spatial coverage was insufficient to replicate this analysis with our moth indices.

Agreement with previous work

Having tested whether our occurrence-derived indices captured an environmental gradient structuring butterfly communities, we then tested whether the mechanism driving change along this gradient was a change in nutrient levels by comparing our occurrence-derived indices to the nitrogen indices calculated for Dutch butterflies by Oostermeijer & van Swaay (1998). We calculated Pearson correlations between our occurrence-derived optima and tolerance measurements and those of Oostermeijer & van Swaay (1998) to assess the degree of agreement.

Agreement with expert assessment

Finally, for both butterflies and moths we tested whether our occurrence-derived indices matched expectation from expert assessment of species' preferred nutrient levels. Experts were recruited for

each taxon from research and conservation organisations focusing on butterflies and moths and had extensive field experience of British butterfly and moth species. Each expert (six for moths and seven for butterflies) was asked to score species' optima from 1 (prefers low nutrient levels) to 8 (prefers high nutrient levels) and their tolerance from 1 (a 'nutrient specialist' occurring at one or two nutrient levels) to 3 (a 'nutrient generalist' occurring at a variety of nutrient levels), also grouping them into coarser categories for each score type (high, intermediate or low for optima, and generalist or specialist for tolerance). This was done for all modelled butterflies, but for the more species-rich moths we binned species into 4 groups based on their occurrence-derived optima and randomly selected 12 from each group to give a total of 48 species. For each group and metric we then assessed the degree of agreement with our occurrence-derived indices by calculating the Pearson correlation between the mean of the expert scores and our occurrence-derived indices, as well as checking whether the grouping chosen by the majority of experts agreed with our index-based groupings.

Non-spatial under-sampling

Because the spatial under-sampling we applied to our occurrence data is a relatively new technique (eg. Gaul et al., 2022; Robinson et al., 2018), we compared the results of indices derived from spatially under-sampled data to indices derived from non-spatially under-sampled data. These were derived using the same modelling and analytical techniques, but with a dataset where we randomly selected the same number of absences for each species as would be achieved by spatial under-sampling, and randomly reduced the size of the majority class to that of the minority class (usually the absence dataset, except for very common species). We compared the values of indices derived from spatially and non-spatially under-sampled data using Pearson's correlation tests and paired T tests comparing the values of tolerance and optima for a given species, and applied the same validation tests. As we expected spatial under-sampling to improve the performance of our models, all reported results here are from spatially under-sampled datasets unless otherwise stated. Equivalent results for indices derived from non-spatially under-sampled datasets can be found in the supplementary material.

Software

All analysis was carried out in R (R Core Team, 2024). Spatial data were manipulated using the terra, tidyterra, sf and rnaturalearth packages (Hernangómez, 2023; Hijmans, 2024; Massicotte & South, 2023; Pebesma, 2018), and presence-only records using tidyverse and Hmisc packages (Harrel, 2024; Wickham et al., 2019). GAMs were run using mgcv (Wood, 2011) and indices derived using rootSolve (Soetaert & Herman, 2009). Results were plotted and visualised using GGplot2, cowplot, viridis and ggh4x (Garnier et al., 2024; van den Brand, 2024; Wickham, 2016; Wilke, 2024). Modelling, posterior simulation and index derivation steps were all run as array tasks (one task per species) on the University of Oxford Advanced Research Computing facility (ARC).

Results

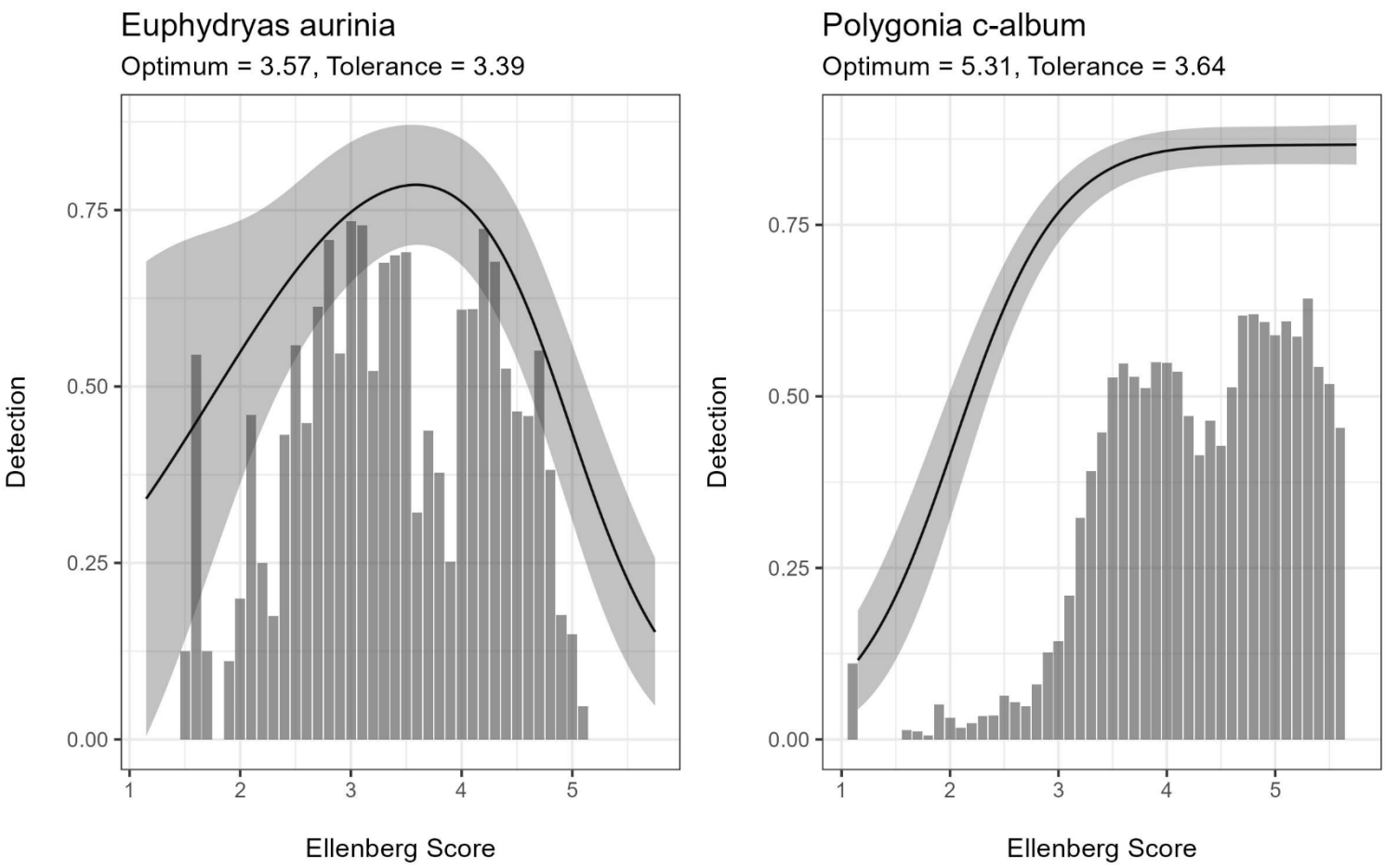


Figure 4: *Examples of the modelled responses and resultant indices for two Nymphalid butterfly species, plotted with their 95% confidence intervals and the underlying frequency of the species at different Ellenberg scores (binned into groups of 0.1). Note that no squares had Ellenberg scores exceeding 6 and we did not model species' responses outside the range of our data, hence the truncated shape of the response for P. c-album.*

Distribution of Indices

46 butterfly species and 341 moth species were modelled. Of these, we derived indices for 44 (96%) butterfly species and 279 (82%) moth species which had statistically significant responses to nutrient levels. Initial analysis showed that normal area tolerance and central area tolerance were strongly positively correlated for both moths ($r = 0.98$, $df = 277$, $p < 0.001$) and butterflies ($r = 0.93$, $df = 42$, $p < 0.001$) (Figure S1), and so for all further analyses we used normal area tolerance (hereafter referred to as tolerance). The mean optimum value for butterflies was 3.65 (sd = 1.61) and for moths 4.42 (sd = 1.55), and the mean tolerance values 3.95 (sd = 0.69) and 3.89 (sd = 0.64), respectively. These were approximately in the middle of the range of optima for butterflies (1.15 to 5.74) and moths (1.05 to 5.64) and likewise for tolerance (2.14 to 4.60 and 1.32 to 4.59, respectively). These means however did not reflect the fact that most species had intermediate optima, rather the distribution of optima was strongly bimodal (Figure 5) with more extreme optima the most common (particularly high optima for moths). This bimodal distribution still resulted in approximately equal allocations to high, intermediate and low optima for butterflies (39%, 34% and 27%), but a positive skew for moths, with 71% allocated high optima, and only 14% intermediate and 15% low. Tolerance was more positively skewed, with 75% generalist for moths, and 66% for butterflies, while only 3% of moths were modelled as specialists, against 11% of butterflies.

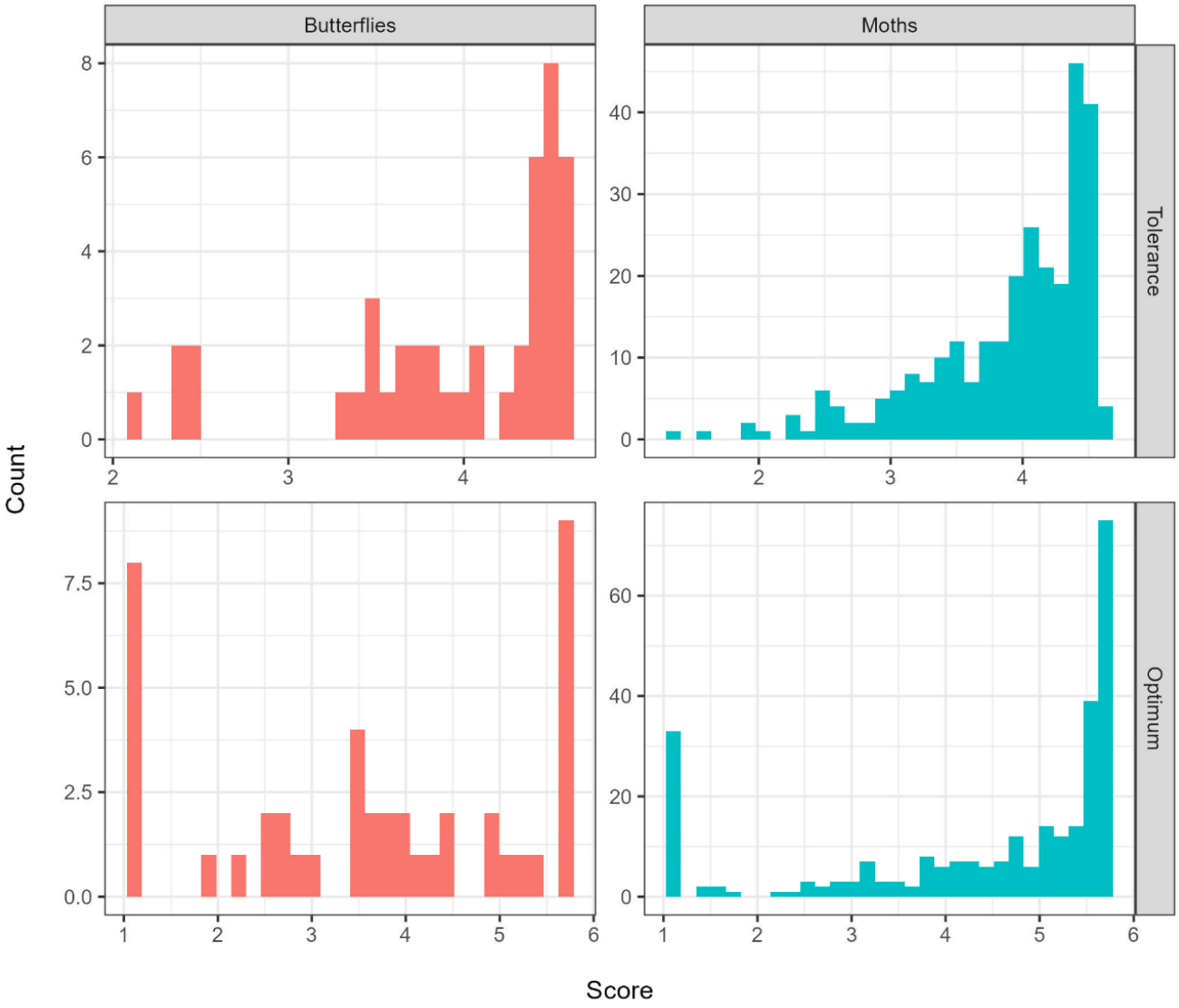


Figure 5: *Histogram of occurrence-derived optimum and tolerance values for butterflies and moths.*

Internal consistency

In our test of the indices re-derived for each butterfly species using the community nitrogen indices of UKBMS sites instead of the original Ellenberg scores (Figure 7), the re-derived optima were strongly positively correlated with the original occurrence-derived optima ($r = 0.76$, $df = 36$, $p < 0.001$) (Figure 6), and the majority were within the 95% confidence intervals of the occurrence-derived optima (52%). The mean absolute difference between the occurrence-derived optima and re-derived optima was 0.69 units (sd = 0.73) and 73% were within one unit of the occurrence-derived optima, with a maximum difference of 2.24 units. Re-derived tolerance scores and occurrence-derived tolerance scores were not significantly correlated ($r = -0.11$, $df = 36$, $p = 0.47$), with only 18% of the re-derived tolerance scores within the confidence intervals of the occurrence-derived values and a mean absolute difference of 2.12 (sd = 0.87).

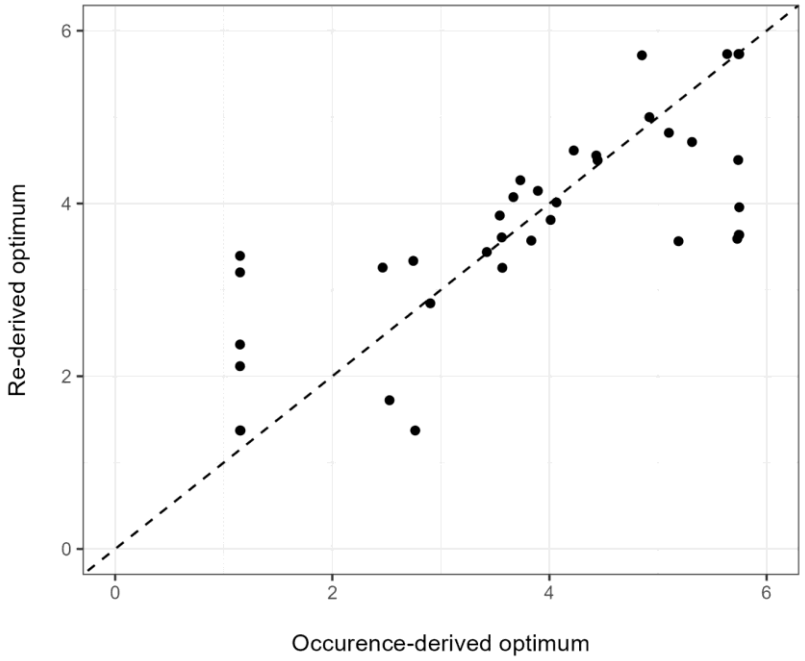


Figure 6: *Values of butterfly species' optima re-derived using community nitrogen indices calculated with UKBMS data plotted against their original occurrence-derived optima ($r = 0.76$, $df = 36$, $p < 0.001$). The dashed line is the 1:1 line.*

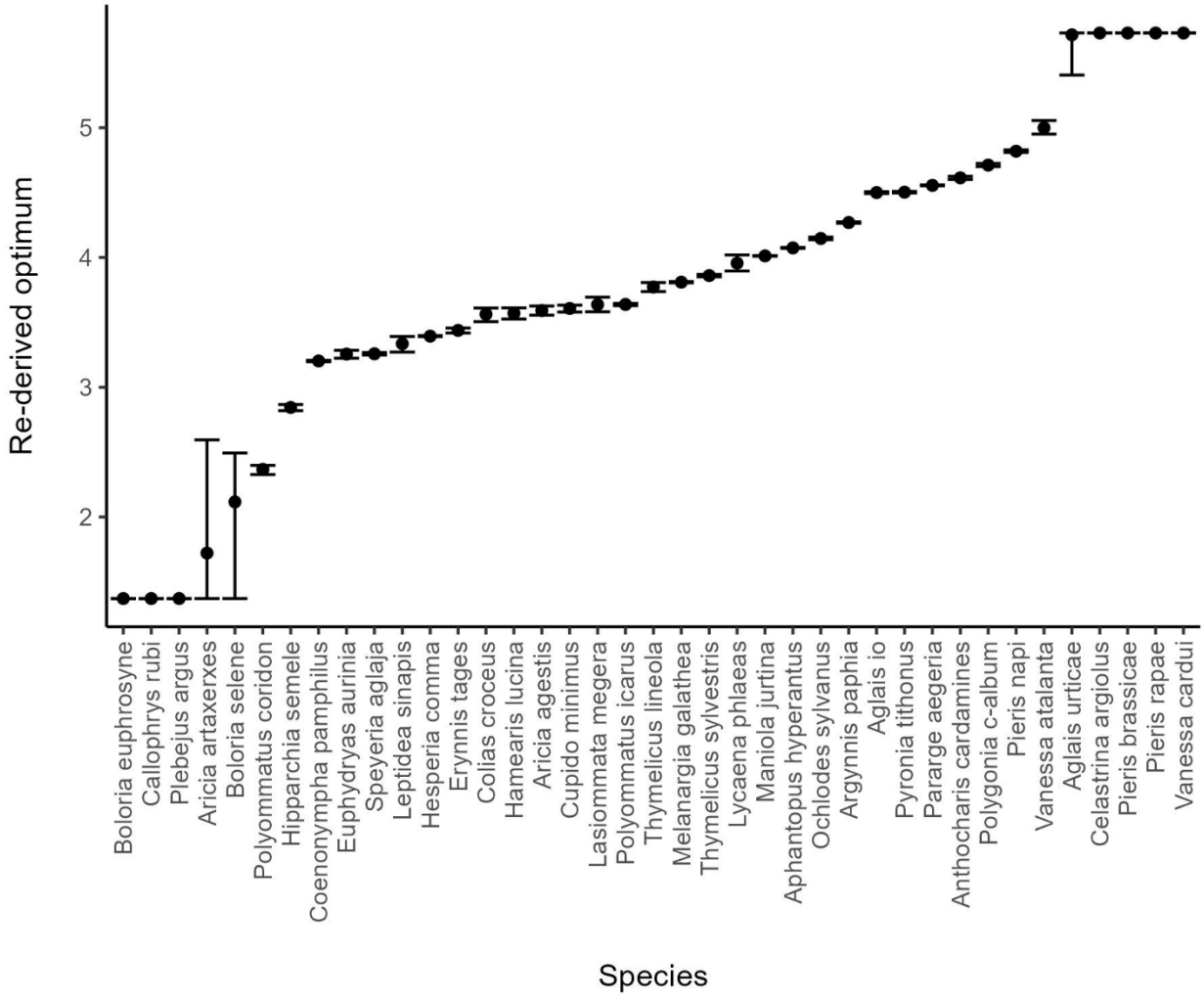


Figure 7: *Butterfly species' optima re-derived from community nitrogen indices calculated from UKBMS data using occurrence-derived optima, plotted with their 95% confidence intervals.*

Dutch Butterfly Indices

Our occurrence-derived optima for butterflies were strongly positively correlated with the optima derived for Dutch butterflies by Oostermeijer & van Swaay (1998) ($r = 0.73$, $df = 28$, $p < 0.001$) (Figure 8). As for the test of internal consistency, our occurrence-derived tolerance scores did not correlate significantly with the tolerance scores of Oostermeijer & van Swaay (1998) ($r = -0.17$, $df = 15$, $p = 0.51$).

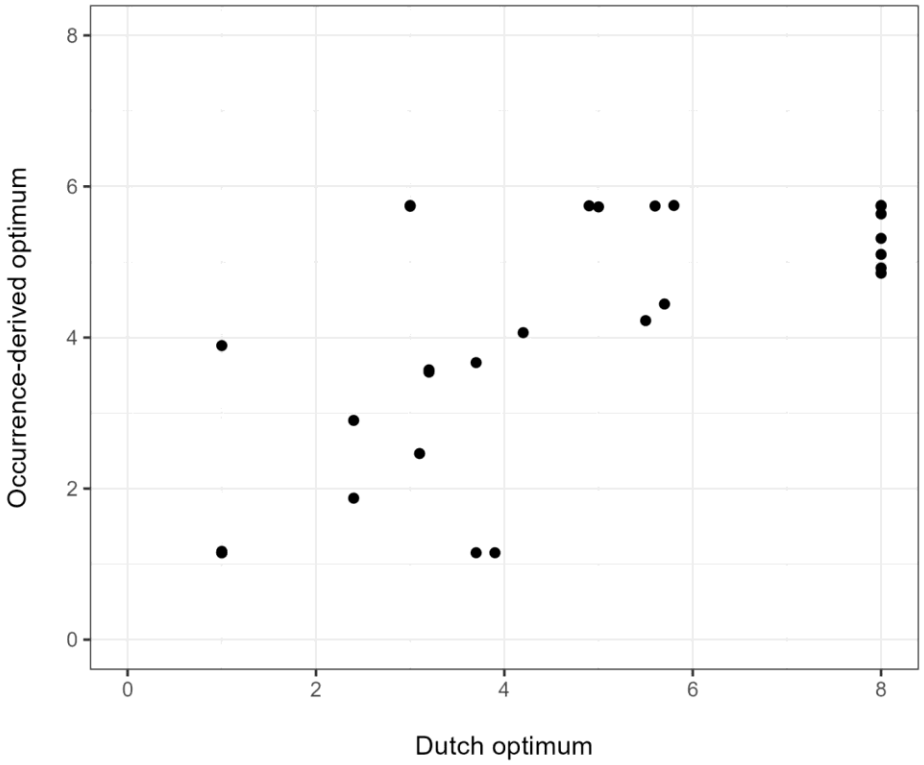


Figure 8: *Values of British butterfly species' occurrence-derived optima plotted against their optima in the Netherlands derived by Oostermeijer & van Swaay (1998) ($r = 0.73$, $df = 28$, $p < 0.001$). Oostermeijer & van Swaay (1998) did not derive optima for 14 species and tolerances for 27 species in our analysis, their optima also have a larger scale (1 to 8) than ours (1.15 to 5.74) reflecting their decision to allocate species with positive linear responses scores of 8, and negative linear responses 1, rather than the maximum and minimum in their environmental data as we did.*

Expert Scores

There was a high degree of consensus among butterfly experts on each species' optimum, with pairwise correlation coefficients all significant ($p < 0.001$, $df = 42$) and ranging between 0.69 and 0.93. For moths there was less consensus, with one of the pairwise correlations between experts not significant ($r = 0.24$, $df = 46$, $p = 0.11$), while significant correlations ($p < 0.01$, $df = 46$) between other pairs of experts ranged between 0.31 and 0.70. Expert tolerance scores showed a similar degree of consensus for butterflies and moths, with pairwise correlations in both cases all positive and significant (butterflies: $df = 42$, $p < 0.01$, moths: $df = 46$, $p < 0.05$), ranging between 0.45 and 0.93 for butterflies, and 0.34 and 0.65 for moths. There was no species of butterfly or moth where all experts agreed on optima, but 20% of butterflies, and 19% of moths had a range of just one unit, with a further 33% and 43% 2 units. For tolerance, however, 20% of butterflies and 10% of moths showed complete agreement, and a further 58% and 74% were within one unit. Moth experts tended to be less willing to allocate moth species the most extreme optima than butterfly experts, reflected in the lower standard deviation of moth optima (1.26) than butterfly optima (1.78).

Our occurrence-derived optima were positively correlated with the mean expert optima for butterflies ($r = 0.66$, $df = 42$, $p < 0.001$) and moths ($r = 0.64$, $df = 46$, $p < 0.001$) (Figure 9), but relationships between our occurrence-derived tolerances and mean expert tolerances were not significant for either group (butterflies: $r = 0.03$, $df = 42$, $p = 0.86$, moths: $r = 0.28$, $df = 46$, $p = 0.06$). At the level of individual experts, correlations between our occurrence-derived optima were all significant and for moths ranged between 0.41 and 0.68 ($df = 46$, $p < 0.01$), and for butterflies between 0.71 and 0.48 ($df = 42$, $p < 0.001$), while no correlations between our occurrence-derived tolerances and expert tolerances were significant for either moths or butterflies.

Categorisations based on our occurrence-derived optima agreed with expert categorisations for 57% of butterfly species but only 25% of moth species; for tolerance the equivalent figures were 32% and

81%. This reflected relatively high agreement for both taxa about which species had low and intermediate optima, but lower agreement about species with high optima, particularly for moths (Table 1). For tolerance, there was low agreement about which species had low tolerance for butterflies, but high agreement for both high and low tolerance for moths (Table 1).

Table 1: *Proportion of species allocated to each preference and tolerance category by our occurrence-based indices that were also placed there by expert consensus for moths and butterflies (only two categories were used by experts for tolerance).*

Group	Index	High	Intermediate	Low
Butterflies	Preference	0.41	0.67	0.67
	Tolerance	0.5	-	0
Moths	Preference	0.11	0.62	0.6
	Tolerance	0.86	-	0.67

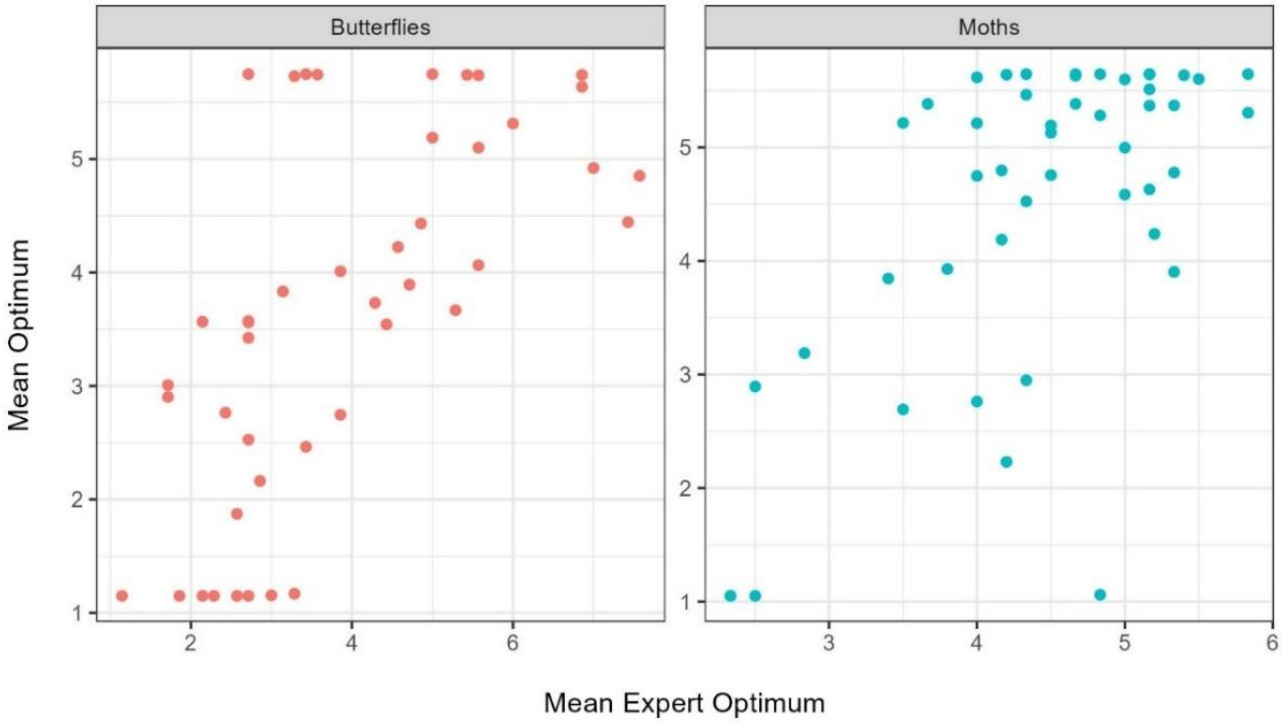


Figure 9: *Occurrence-derived optima of butterfly and moth species plotted against their mean expert-scored optima (butterflies r = 0.66, df = 42, p < 0.001, moths: r = 0.64, df = 46, p < 0.001).*

Consensus across validation methods

Optima for different butterfly species showed a high degree of consistency across validation methods, with positive correlations for optima across all sets. The strongest were between the re-derived optima and expert means (r = 0.81, df = 37, p < 0.001), followed by the re-derived optima and Dutch optima (r = 0.78, df = 25, p < 0.001) and then expert means and Dutch optima (r = 0.71, df = 25, p < 0.001).

Tolerance scores were less consistent, but there were significant positive correlations between mean expert tolerances and Dutch tolerances (r = 0.51, df = 15, p < 0.05), and Dutch tolerances and re-derived optima (r = 0.49, df = 15, p < 0.05), but not re-derived optima and mean expert optima (r = 0.19, df = 42, p = 0.22), though this weak positive correlation was an improvement on the original tolerances.

For all sets of scores, occurrence-derived optima for a given species tended to be higher than the optima allocated by the validation methods, significantly so relative to expert assessment for both moths (mean difference = 0.71, $t = 4.46$, $df = 47$, $p < 0.001$) and butterflies (mean difference = 0.51, $t = 2.79$, $df = 43$, $p < 0.01$), but not significantly for butterflies relative to the Dutch optima (mean difference = 0.47, $t = 2.00$, $df = 29$, $p = 0.054$) and the re-derived optima (mean difference = 0.11, $t = 0.70$, $df = 41$, $p = 0.49$).

Spatial vs non-spatial under-sampling

Full tests of the performance of occurrence-derived indices from non-spatially under-sampled data can be found in the supplementary material ('Indices derived from non-spatial under-sampling'). In brief, differences in the performance of occurrence-derived optima from spatially and non-spatially under-sampled data across our validation tests were relatively small. Optima derived from non-spatial under-sampling were significantly positively correlated with all validation tests, proving a slightly better match to their re-derived optima in terms of correlation, but worse in terms of overlap, and worse against Dutch scores, and mean expert optima (for both butterflies and moths). Tolerance scores derived from non-spatially under-sampled data performed somewhat better however, with positive correlations across all tests, although these were still very weak, with only mean expert tolerance for moths being significant.

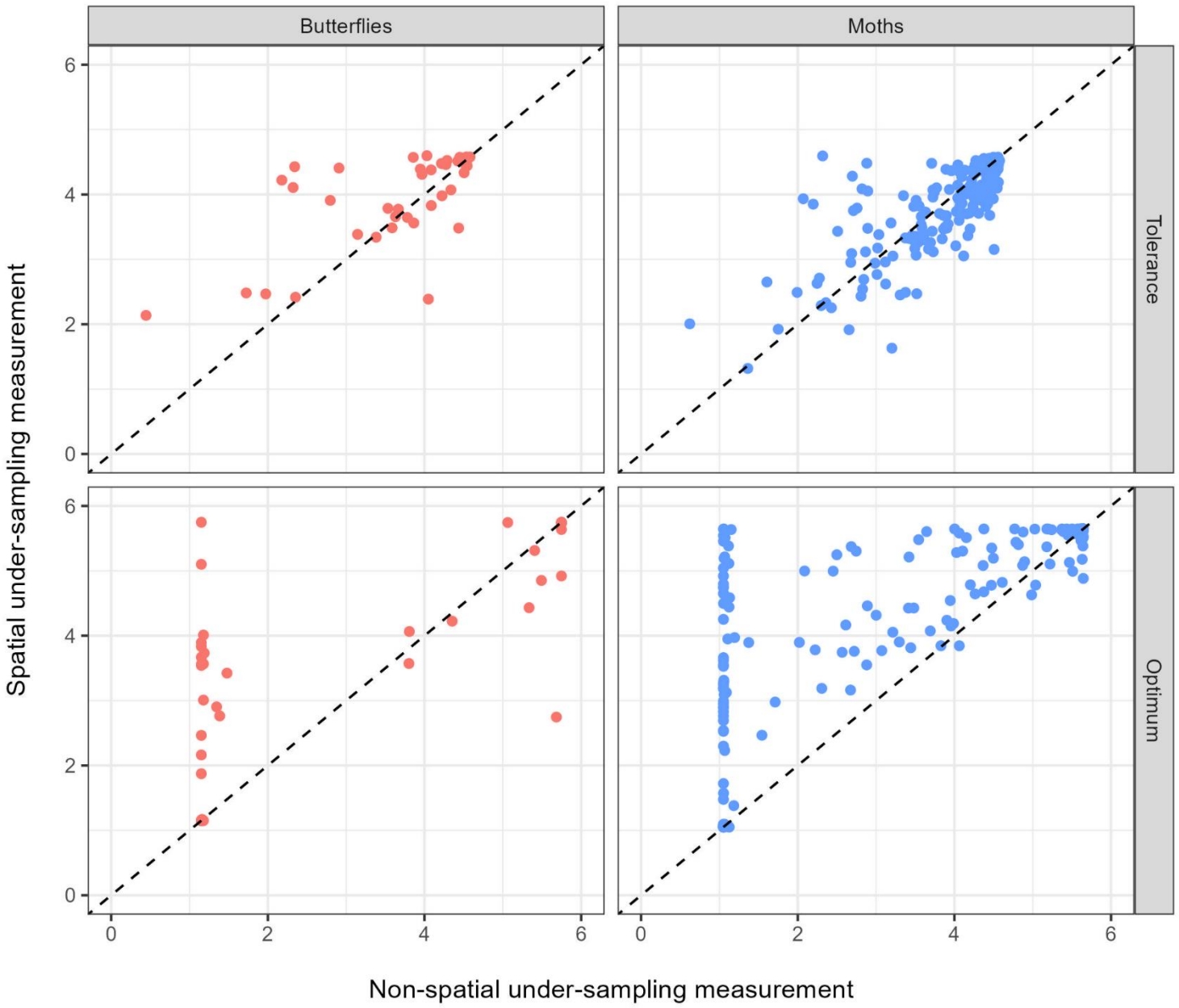
Comparison of occurrence-derived indices from spatially and non-spatially under-sampled data for butterflies and moths showed that there were strong positive correlations for both optima and tolerance scores across both taxa (Table 2, Figure 10). However, paired T-tests comparing optimum and tolerance scores derived from spatially and non-spatially under-sampled data showed that optima derived from spatially under-sampled data were significantly higher than those derived from non-spatially sampled data for both butterflies and moths, while tolerance scores were also significantly higher for butterflies, although the difference was smaller than for optima (Table 3).

Table 2: *Results of Pearson correlation tests between indices derived from spatially and non-spatially under-sampled data, for butterflies and moths.*

Group	Metric	r	t	df	p
Butterflies	Tolerance	0.66	5.47	39	<0.001
	Optimum	0.71	6.26	39	< 0.001
Moths	Tolerance	0.76	16.88	209	< 0.001
	Optimum	0.76	16.89	209	< 0.001

Table 3: *Results of paired T tests assessing the difference between indices derived from spatially and non-spatially under-sampled data for butterfly and moth species. Positive mean differences therefore indicate cases where indices derived from spatial under-sampling were larger.*

Group	Metric	Mean Difference	t	df	p
Butterflies	Tolerance	0.27	2.4	40	< 0.05
	Optimum	0.83	3.57	40	< 0.001
Moths	Tolerance	-0.05	-1.42	210	0.16
	Optimum	0.92	10.18	210	< 0.001



2603

2604 **Figure 10:** *Plots of indices derived from spatially under-sampled data against indices derived from*
2605 *non-spatially under-sampled data for butterflies and moths. The dashed line is the 1:1 line, so points*
2606 *above this are species for which indices derived from spatially under-sampled data were higher than*
2607 *those from non-spatially under-sampled data and vice versa.*

Discussion

Performance of occurrence-derived indices

Our results show that it is possible to resolve differences in the responses of insect species to nutrient levels using modelled vegetation data and occurrence records at the 1km scale, as we were able to derive indices for optima and tolerance for 96% of butterfly species assessed, and 82% of moth species. To assess the robustness of our occurrence-derived indices for butterflies we tested their internal consistency, compared them to indices derived for Dutch butterflies by Oostermeijer & van Swaay (1998) and by expert assessment of species' requirements. The latter was also used to test our occurrence-derived indices for moths.

Validation Methods

It should be noted that differences between our indices and Dutch indices could indicate genuine ecological differences rather than short-comings of our indices. For example, our optimum for the hesperiid butterfly *Ochlodes sylvanus* was 3.89, whereas Oostermeijer & van Swaay (1998) gave it a negative linear response (an optimum of 1 in their system, but the minimum in ours would have been 1.15), perhaps reflecting the fact that in the Netherlands this species is found mostly on sandy soils and absent from more nutrient-rich clay substrates (Bos et al., 2006), while in Great Britain it is widespread on humid grassy edges in a variety of woodland and scrubland habitats, on most soil types (Asher et al., 2001). Indeed, for butterflies we found that the optima of Oostermeijer & van Swaay (1998) were less well correlated with the re-derived optima for butterflies and mean expert optima than these were with each other, perhaps reflecting differences in habitat associations between Great Britain and the Netherlands.

Similarly, given the potential subjectivity of expert scoring, the relationship between our indices and expert scores needs to be viewed with some caution. We did however find a relatively high degree of

consensus among experts over the nutrient preferences of both groups studied. This was somewhat lower for moths but this is perhaps not surprising given their far greater diversity, meaning that individual species are less well-studied, and experts are unlikely to be as well-acquainted with all the species in the sample. Thus, although expert scores are inherently subjective, a relatively consistent picture emerged across our experts that suggests these scores are reasonably robust.

Given the potential for subjectivity in expert scores and ecological differences between species' habitat associations in Great Britain and the Netherlands, the analysis of the internal consistency of butterfly optima may be the most useful test of our indices. Optima performed relatively well in this test, with 52% of species' re-derived optima within the 95% confidence intervals of the original occurrence-derived optimum, and 73% within one unit, as well as strong positive correlations between re-derived and original occurrence-derived optima. Similar tests have been applied to Ellenberg moisture scores for the Dutch flora by Ter Braak & Gremmen (1987) who found that the original Ellenberg scores were within 0.5 units of the confidence intervals of re-derived scores for 74% of species. 76% of our occurrence-derived optima meet the same threshold, although Ter Braak & Gremmen (1987) do not actually provide the confidence intervals for each species, or discuss their average size, so it is hard to be sure that these statistics are really comparable. Nevertheless, this seems to suggest that our optima compare relatively favourably for internal consistency with one of the most widely-used and important sets of environmental indicators for plants (Diekmann, 2003).

Overall, there was a good consensus on butterfly species' preferred nutrient levels across the independently-derived sets of validation scores, with strong positive correlations in optima between each set, and weaker correlations for tolerance. This suggests that in spite of the potential subjectivity of expert assessment, and the potential for differences in habitat use of butterflies in the Netherlands, they do classify species along a common gradient of preferred nutrient levels and present a robust method for assessing our indices. The positive correlation of our optima with all of these validation

scores then suggests that our novel approach to modelling species' responses to nutrient levels and deriving optima to describe these is valid.

Shortcomings of Indices

Our validation tests for our occurrence-derived tolerance scores suggested that these were much less robust than our optima, and in almost all tests these tolerance scores were not supported. We suspect this reflects the strong skew to high values in our occurrence-derived tolerance scores, which did not match with expectations of the distribution of tolerance scores in our validation datasets, which approximated a normal distribution. We expected our measure of 'normal area tolerance' to be largest when species' response curves were flat, and that this pattern therefore reflected a preponderance of weak, flat responses curves in our dataset. When calculating a crude measure of effect size in species' response curves (their minimum probability of occurrence over the range of Ellenberg scores in the data subtracted from the maximum) we did indeed find a pattern consistent with this hypothesis and there was a dominance of small effect sizes, which led to higher tolerances (Figure S10). As well as being lower at large effect sizes, tolerance scores were also more varied, probably because curves can assume a wider variety of shapes when effect sizes are larger (Figure S10). When we filtered out species with very weak effect sizes, we found a tendency to more positive correlations between our occurrence-derived tolerance scores and their validation equivalents (Supplementary Material: 'what drives the distribution of tolerance scores?'), suggesting that the apparent failure of our tolerance scores may be more to do with the ability of our analysis to resolve the shapes of species' responses in real detail, than our methods for calculating tolerance.

Similarly, although our optima performed well in most validation tests, their distribution was strongly bimodal, with most species placed at the extremes of the gradient of Ellenberg scores, again reflecting a dominance of linear (mostly rather weak) responses. This is an entirely different pattern to that documented in the much larger body of work modelling plant response curves, where symmetric,

unimodal responses to environmental gradients are generally most common (eg. Lawesson & Oksanen, 2002; Minchin, 1989; Oksanen & Minchin, 2002), and as a result species' optima usually cluster somewhere in the middle of the gradient (eg. Heikkinen & Mäkipää, 2010; Lawesson & Oksanen, 2002; Minchin, 1989). It seems reasonable to expect this pattern for insect responses to ecological gradients too, given the strong relationship between plant and insect diversity. In the context of our nutrient gradient, which is effectively a productivity gradient, the frequent observation of hump-shaped relationships between biodiversity and productivity provides further support for this expectation (Chase & Leibold, 2002; Mittelbach et al., 2001; Rosenzweig & Abramsky, 1993). The absence of this pattern in our dataset could reflect a shortcoming of our optima, one that is shared with previous work using insect monitoring data to assess the responses of different species to environmental gradients. The nitrogen optima of Oostermeijer & van Swaay (1998), for example, show a similar, albeit less strongly bimodal, distribution, and Bowler et al. (2023) whose approach we largely replicated in our analysis found that across a variety of GB insect taxa, the vast majority of species showed linear responses to woodland cover at the 1km scale rather than intermediate, unimodal responses. This seems therefore to be a common finding and potentially shortcoming of these kinds of approaches.

Part of the problem could just be a simple lack of statistical power for many species. When modelling UK butterfly responses to woodland cover, Redhead et al. (2016) found that on average 5480 species records were required for species' data-derived scores to fall within the range of expert assessments for that species, though this was less for more specialised species. A high proportion of the species in our analysis were well below this threshold (36% of butterflies and 51% of moths), and plotting the structure of species' responses against their sample size suggested that a lack of power might be playing a role in this pattern (Figure S11). Intermediate responses appeared to be rare at low sample sizes, but became increasingly common at larger sample sizes, and then declined in frequency again (perhaps because species with large sample sizes are very widespread species which therefore show weak responses to nutrient levels).

This issue is probably exacerbated by the unevenness of butterfly and moth recording effort along the range of Ellenberg scores. The distribution of modelled Ellenberg scores for 1km squares was not even and did not cover the full range of scores (maximum of 5.76, minimum of 0.21) (Figure 2). Butterfly and moth recording effort is unevenly spread across the range with particularly low levels of sampling in cells with low Ellenberg scores (Figure S12, Figure S13). This gives us limited or even no information in the regions where we would expect species' occurrence probability to be declining if their responses were unimodal (eg. Figure 4) and may explain the dominance of linear responses. This effect is probably heightened by the 1km scale of our analysis. On the one hand, this might be a reasonable level at which to assess the requirements of relatively mobile lepidoptera, where smaller spatial scales might risk finding associations between species and resources required by adults (records of which dominate the dataset) (Redhead et al., 2016), rather than the more constrained larvae. Equally, vegetation and soil nutrient levels can vary at scales finer than 1km, and the Ellenberg score of a square in our analysis may often mask considerable heterogeneity in nutrient levels, supporting species requiring very different levels to the mean in that square. This may particularly be the case in areas where specialist species occur in small islands of low-nutrient habitat in an intensive agricultural matrix and will weaken the relationship between species' occurrence and nutrient levels at this scale.

The problems of spatial under-sampling

We found little difference between our indices derived from spatially under-sampled data and from non-spatially under-sampled data in terms of their performance on validation tests, barring slightly better performance of indices from non-spatially under-sampled data for tolerance. There was however a clear trend for spatial under-sampling to result in higher optima. This appears to be due to an interaction of strong spatial biases in recording with weak responses to nutrient levels of many species. Far more of the visits in the butterfly and moth datasets were to sites with high Ellenberg nutrient scores, and these were 'over-visited' relative to their frequency in Great Britain, while low-scoring sites were 'under-visited' relative to their frequency, particularly for moths (Figure S13). We suggest

this likely reflects the well-known tendency of recorders to visit sites close to where they live (Dennis & Thomas, 2000; Mair & Ruete, 2016), resulting in more visits to higher-scoring lowland areas in southern and eastern England (Figure 2). The stronger bias in the moth dataset seems likely to reflect the reliance on moth traps, which are hard to use away from mains power sources, enhancing the tendency to sample at or close to home. Spatial under-sampling removes this bias from the absence data, while retaining it in the presence data. As a result, species' frequencies at high Ellenberg scores are increased (as the number of absences at high scores are reduced) and decreased at low Ellenberg scores (as the number of absences at low scores are increased) (Figures S14, S15). Consequently, unless species had very strong negative responses to nutrient levels, spatial under-sampling usually resulted in positive responses to nutrient availability (Figure S16).

In spite of this strong positive bias, we did not find that indices from spatially under-sampled data performed much worse than indices from non-spatially under-sampled data for either butterflies or moths, except perhaps for tolerance scores, which were still a poor match with their validation equivalents. However, the absence of an impact on the relationship between moth indices and expert assessment probably reflects the fact that sample of moth species we asked experts to score was one stratified by optima derived from spatially under-sampled data, thereby under-representing the large number of species with high optima in this dataset. The very small number of species that were categorised as having high optima by the indices derived from spatially under-sampled data that were put in the same category by experts suggests that a large proportion of these high optima were indeed incorrect, and had we had taken a random sample, we would have found low agreement with these indices. Although the impact of spatial under-sampling on butterfly optima appeared to be weaker than for moths, there was a similar trend in the butterfly optima, and the lowest agreement with expert categorisations was also for species categorised as having high optima.

The lack of an impact of spatial under-sampling on the performance of the butterfly optima may partly reflect the weaker spatial bias in this dataset, larger sample sizes giving stronger responses, and a

tendency for optima from non-spatially under-sampled data to be lower than validation optima (meaning that these performed about as well as the positively-skewed optima from spatially under-sampled data). Interestingly though, they were not significantly lower than the optima produced by Oostermeijer & van Swaay (1998) who also found more species associated with low-nutrient habitats than high. Wallisdevries (2014) and WallisDeVries & van Swaay (2017) suggest that prior to nitrogen pollution induced by human activity, such habitats would have been dominant, explaining why more butterfly species are associated with them, rather than high-nutrient habitats that would historically only have been present only in areas of disturbance or on particularly deep soils. Thus, the skew towards lower values in our optima derived from non-spatial under-sampling probably reflects the fact that the majority of butterfly and moth species are associated with low-nutrient habitats.

We used spatial under-sampling for our indices because recent analyses whose approach we followed (Gaul et al., 2022; O. J. Robinson et al., 2018), have shown that it can improve performance of species distribution models, particularly for rare species, of which there were many in our analysis. Here however, by removing spatial bias from absences and retaining it in presences, spatial under-sampling seems to have accentuated rather than reduced the impacts of spatial bias on model results, leading to biased inferences. These problems suggest that in this context at least, breaking from the recommendation of matched biases in presence and absence data in species distribution modelling is not appropriate (Phillips et al., 2009) and reinforces the evidence that thinning procedures that improve model performance in some citizen science datasets may not do so in others (Steen et al., 2021). Further work to understand how the improvements in model performance resulting from spatial under-sampling reported in previous analyses come about (Gaul et al., 2022; Robinson et al., 2018, 2020), and in what context this kind of under-sampling may improve inference would be valuable.

Improving Index Derivation

Although spatial under-sampling was clearly problematic in this context, differences between validation scores and optima derived from spatially under-sampled and non-spatially under-sampled data were positively correlated (Supplementary Material: Indices derived from non-spatial under-sampling – consensus across validation methods), implying that there were common differences to validation scores in both sets of optima, and more general improvements that could be made alongside altering the way we under-sampled to derive each species' modelling dataset. As already discussed, indices seemed to differ from expectation mainly in cases where species had few occurrence records and weak modelled responses to nutrient levels. Filtering to retain only species with larger sample sizes and strong responses may therefore result in a more robust final set of indices, albeit at the expense of indices for many rare (primarily moth) species. An alternative might be to use input data covering a longer time period (we only used data from the four years overlapping with the Ellenberg score map) to increase sample size, or different data types where stronger effects are more likely. We chose to use occurrence records on the basis that they provided larger datasets for a wider variety of taxa, but count data where available, for example in the UKBMS, may provide stronger signals of species' nutrient preferences.

Nevertheless, although there are problems with spatial under-sampling, and our analysis does not resolve what we expect to be the true shape and pattern in species' responses to nutrient levels, leading to a bimodal distribution of optima, and tolerance measures that show weak relationships with validation scores, the strong positive correlations of our occurrence-derived optima with our validation optima suggest that these are at least usefully classifying species' tendencies i.e. species whose true responses might be unimodal, peaking at low Ellenberg scores are at least tending to get negative linear responses, and vice versa. Indeed, the optima of Oostermeijer & van Swaay (1998) show a similar bimodal distribution, but have proven sensitive indicators of the impacts of nitrogen deposition

on Dutch butterfly communities, and show clear relationships with traits thought to underpin butterfly responses to nutrient availability (WallisDeVries & van Swaay, 2017).

Interestingly, our re-derived optima and tolerance scores for both spatially and non-spatially under-sampled data (Figure 7, Figure S4) for butterflies performed better in all the validation tests than the original occurrence-derived indices, and they were also a closer fit with each other than the original indices. Thus, in spite of their differences, this analysis caused the two sets of indices to converge on values that were also closer to expectation, suggesting that these indices may be the most robust output from our analysis (although they are not available for moths), and that it may also be possible to improve our indices by iteratively re-deriving them in the manner that has been used to improve environmental indicators for plants (Holtland et al., 2010; Ter Braak & Gremmen, 1987).

Conclusions

Overall, our analysis suggests it should be possible to extract robust responses of butterfly and moth species to nutrient levels from occurrence records, despite the challenges of biased, incomplete sampling, and weak responses at coarse spatial scales. We therefore suggest that with some improvement, our indices could provide a powerful and objective way of assessing insect sensitivity to nitrogen pollution and what underpins this, avoiding some of the challenges of trait-based approaches. They could also provide a useful method for detecting the effects of nitrogen pollution on insect communities, either by comparing species' trends or calculating community nitrogen indices. The same approach could equally be applied to other environmental gradients and indeed other taxa, as the data revolution in ecology gathers pace.

Author Contributions

Conceptualisation and Analysis Design: WL, ED, RF, OL

Analysis: WL

Manuscript: WL, RF, OL

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Supplementary Material

Normal Area vs Central Area Tolerance

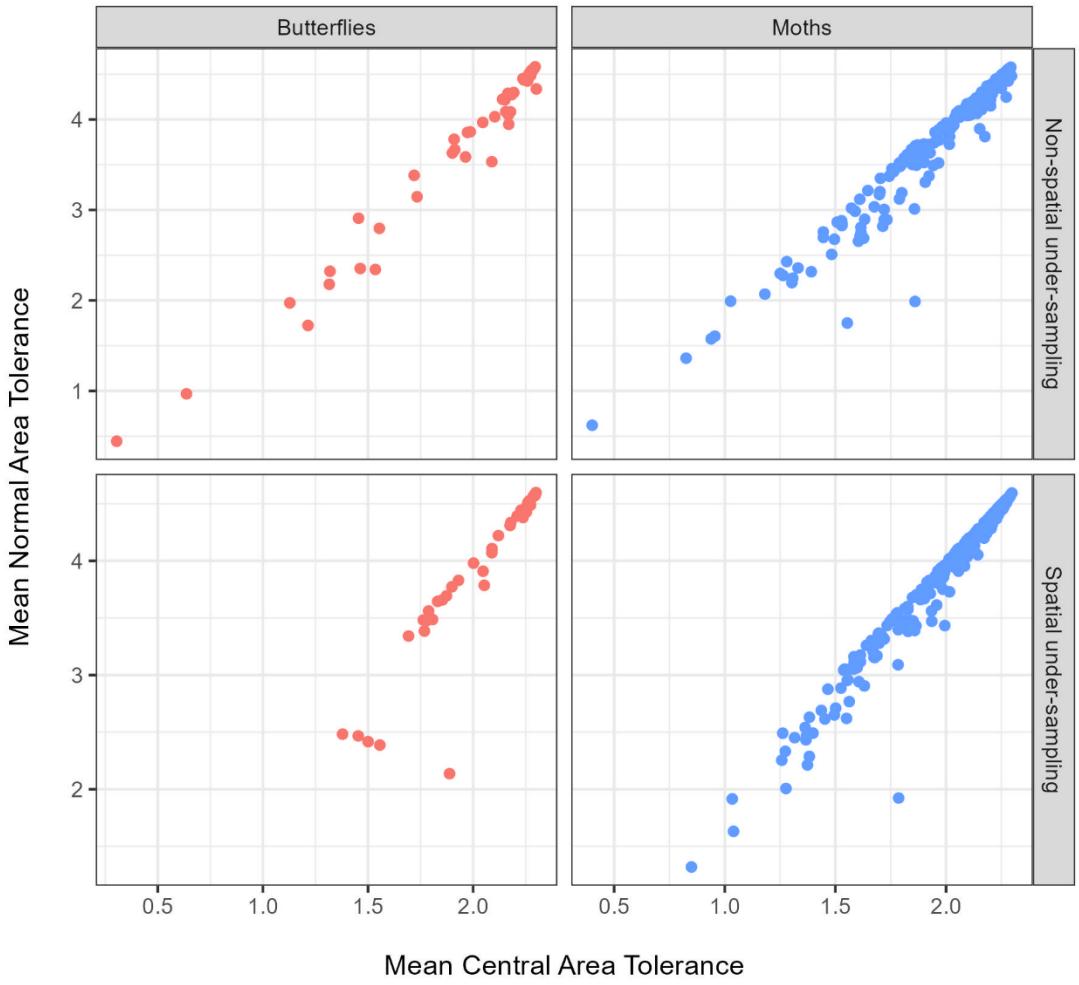


Figure S1: *Mean normal area tolerance plotted against central area tolerance for indices derived from spatially and non-spatially under-sampled occurrence data. The two measures are strongly positively correlated (spatial indices butterflies: $r = 0.93$, $df = 42$, $p < 0.001$, moths: $r = 0.98$, $df = 277$, $p < 0.001$, non-spatial indices butterflies: $r = 0.99$, $df = 41$, $p < 0.001$, moths: $r = 0.97$, $df = 230$, $p < 0.001$).*

Indices derived from non-spatial under-sampling

Distribution of Indices

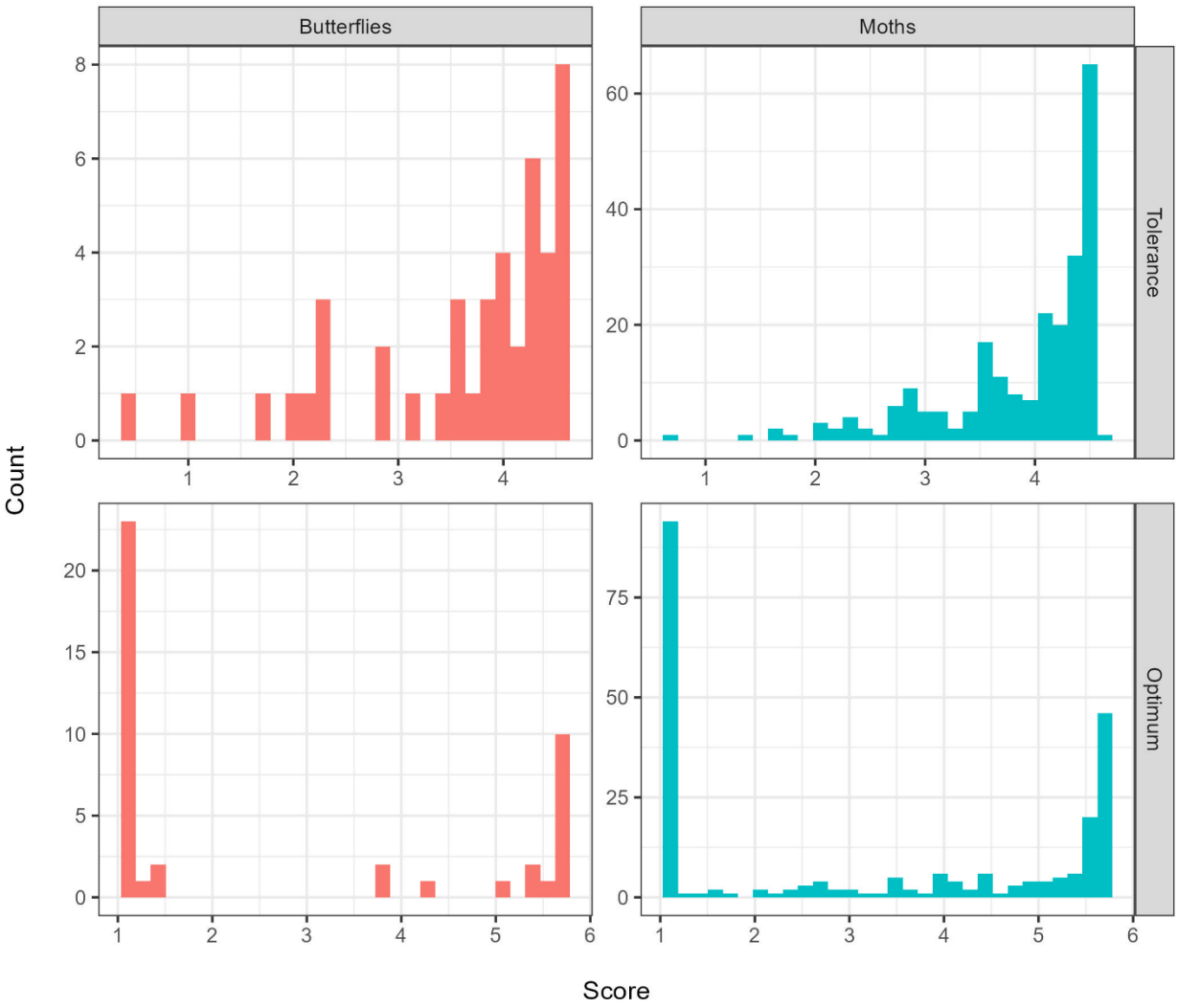


Figure S2: *Distribution of occurrence-derived indices from non-spatially under-sampled data for butterflies and moths. By comparison to indices derived from spatially under-sampled data (Figure 5), these indices show lower scores for optima for both groups, and lower scores for tolerance for butterflies.*

Internal Consistency

Non-spatially under-sampled re-derived optima showed a slightly stronger positive correlation with the original non-spatial optima ($r = 0.80$, $df = 38$, $p < 0.001$) than re-derived spatially-sampled optima did with the original spatial optima. Similarly, there was a greater tendency to a positive correlation of the occurrence-derived tolerance scores with re-derived tolerances, though this was still not significant ($r = 0.21$, $df = 38$, $p = 0.19$). A much lower proportion of the re-derived optima were within the confidence intervals of the occurrence-derived optima (16%) than for indices derived from spatially under-sampled data however, and the mean absolute difference between re-derived and occurrence-derived optima was therefore higher (1.00).

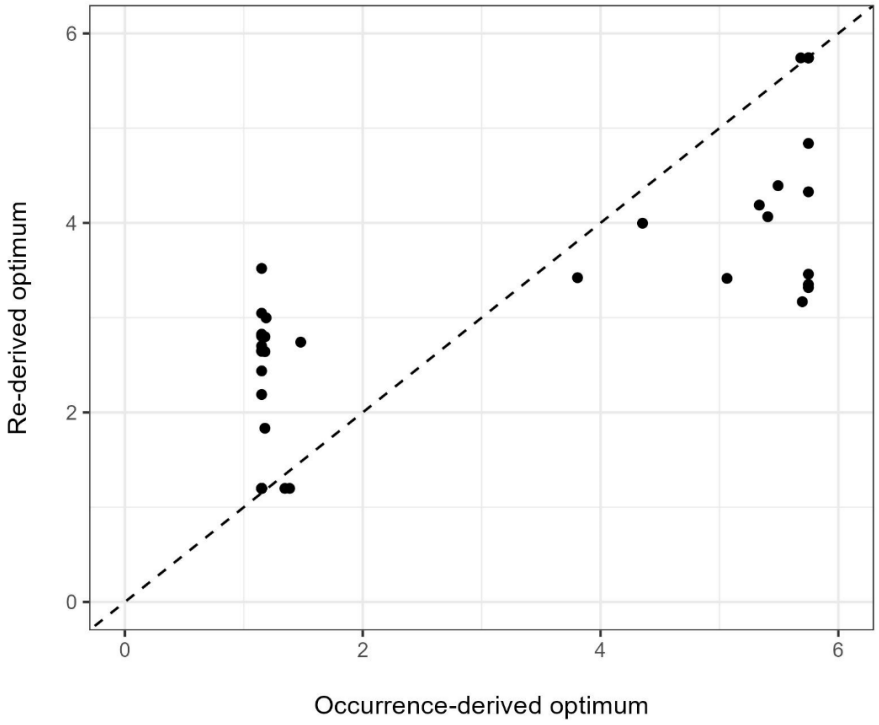
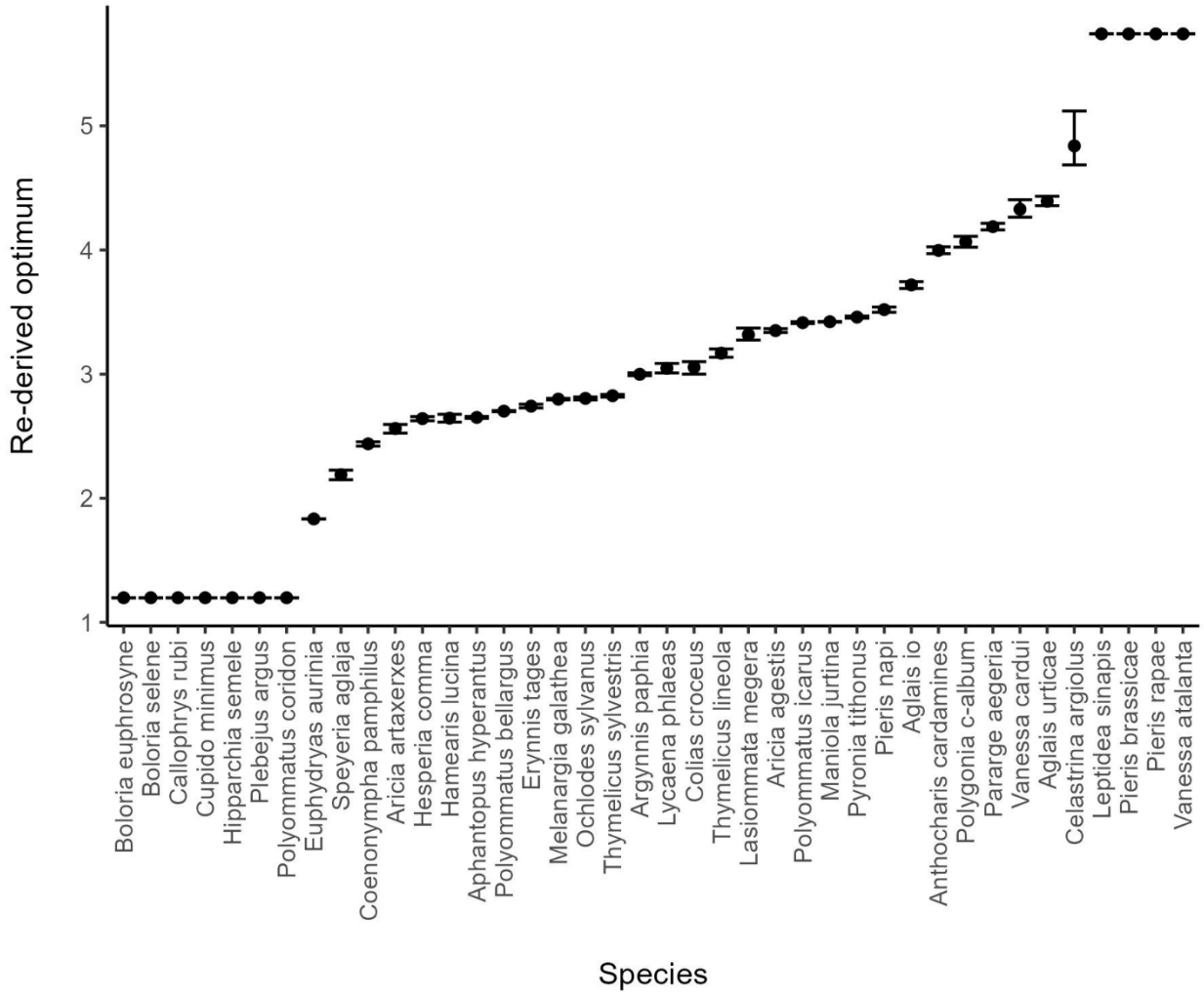


Figure S3: *Values of butterfly species' optima re-derived using community nitrogen indices calculated with UKBMS data and non-spatially under-sampled optima plotted against the original optima derived from non-spatial under-sampling of occurrence records ($r = 0.80$, $p < 0.001$). The dashed line is the 1:1 line.*



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2877 **Figure S4:** *Butterfly species' optima re-derived from community nitrogen indices calculated from*

2878 *UKBMS data using occurrence-derived optima from non-spatially under-sampled data, plotted with*

2879 *their 95% confidence intervals.*

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Dutch Butterfly Indices

The butterfly optima derived from non-spatially under-sampled data were positively correlated with the optima derived for Dutch butterflies by Oostermeijer & van Swaay (1998) ($r = 0.72$, $df = 28$, $p < 0.001$), although this was marginally weaker than for optima derived from spatial under-sampling. The butterfly tolerances derived from non-spatially under-sampled data showed a weak and non-significant positive correlation with the tolerance scores of Oostermeijer & van Swaay (1998) ($r = 0.20$, $df = 15$, $p = 0.45$).

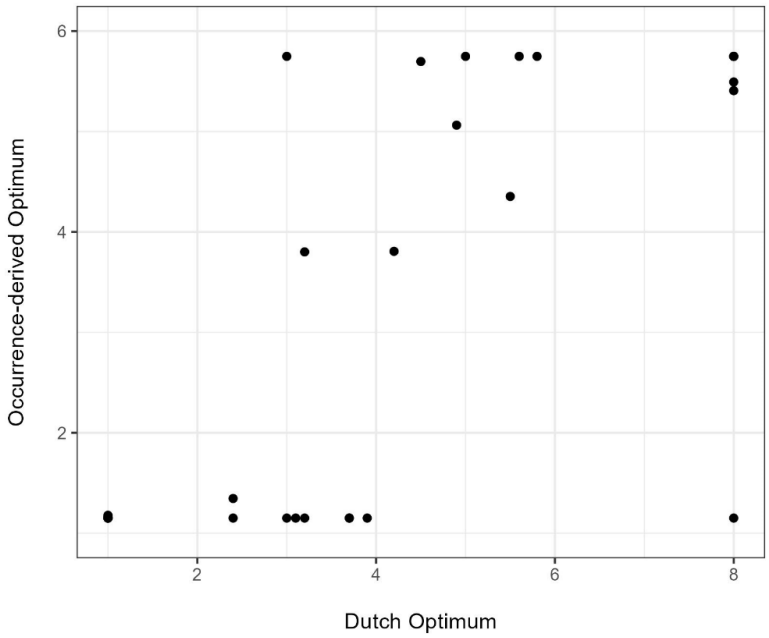


Figure S5: *Values of British butterfly species' optima from non-spatially under-sampled occurrence data plotted against their optima in the Netherlands derived by Oostermeijer & van Swaay (1998) ($r = 0.72$, $df = 28$, $p < 0.001$). Oostermeijer & van Swaay (1998) did not derive optima for 14 species and tolerances for 27 species in our analysis, their optima also have a larger scale (1 to 8) than ours (1.15 to 5.74) reflecting their decision to allocate species with positive linear responses scores of 8, and negative linear responses 1, rather than the maximum and minimum in their environmental data as we did.*

Expert Scores

Optima derived from non-spatially under-sampled occurrence data were positively correlated with mean expert optima for both butterflies ($r = 0.64$, $df = 41$, $p < 0.001$) and moths ($r = 0.57$, $df = 39$, $p < 0.001$), although this correlation was weaker than for optima derived from spatially under-sampled occurrence data. Unlike the optima derived from spatially under-sampled data, estimated tolerance from non-spatially under-sampled data was significantly positively correlated with mean expert tolerance for moths ($r = 0.36$, $df = 39$, $p < 0.05$) and positively correlated with mean expert tolerance for butterflies ($r = 0.29$, $df = 41$, $p = 0.059$), although not quite significantly.

Indices derived from non-spatially under-sampled data showed lower agreement with expert categorisations for butterfly optima (47%) than indices derived from spatially under-sample data, but agreement was marginally higher for moths (27%), in contrast to higher agreement for tolerance for butterflies (40%) but marginally lower agreement for tolerance for moths (79%). There was less agreement about which species had high and low optima for moths and butterflies than indices derived from spatially under-sample data, but more agreement about intermediate indices. Patterns were relatively similar for tolerance however, just with higher agreement.

Table S1: *Proportion of species allocated to each score category by models using non-spatially under-sampled data that were also placed there by expert consensus for moths and butterflies (only two categories were used by experts for tolerance).*

Group	Metric	High	Intermediate	Low
Butterflies	Preference	0.33	1	0.5
	Tolerance	0.62	-	0.25
Moths	Preference	0.07	0.71	0.26
	Tolerance	1	-	0.59

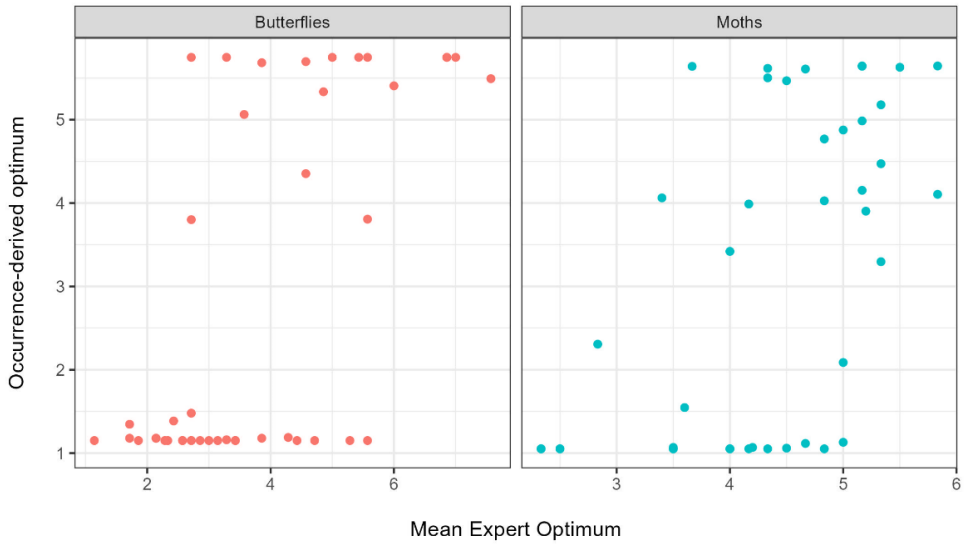


Figure S6: *Values of butterfly and moth species' optima derived from non-spatially under-sampled occurrence data plotted against their mean expert-scored values (butterflies r = 0.64, p < 0.001, moths: r = 0.57, p < 0.001).*

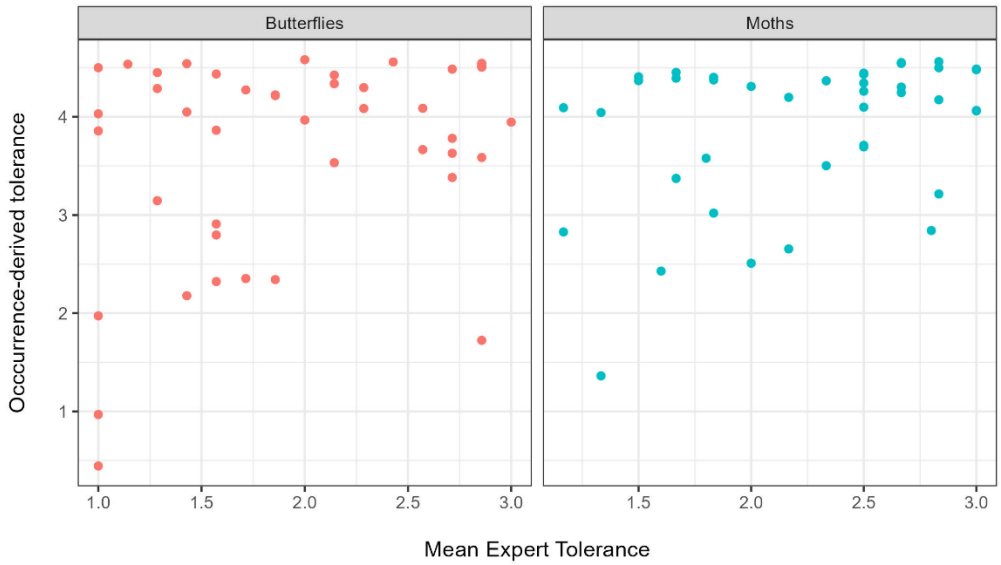


Figure S7: *Values of butterfly and moth species' tolerance derived from non-spatially under-sampled occurrence data plotted against their mean expert-scored values (butterflies r = 0.29, p = 0.059, moths: r = 0.36, p < 0.05).*

Consensus across validation methods

The re-derived butterfly optima from non-spatially under-sampled scores showed strong positive correlations with Dutch optima ($r = 0.85$, $df = 27$, $p < 0.001$) and mean expert optima ($r = 0.76$, $df = 42$, $p < 0.001$). All bar two of the optima re-derived from spatially and non-spatially under-sampled scores were within two units of one another, and if these two data points were removed (*L. sinapis* which had the smallest samples of sites in our UKBMS dataset (71) and *C. minimus*), the correlation between them was extremely strong ($r = 0.91$, $df = 38$, $p < 0.001$), with them it remained high and positive ($r = 0.80$, $df = 41$, $p < 0.001$).

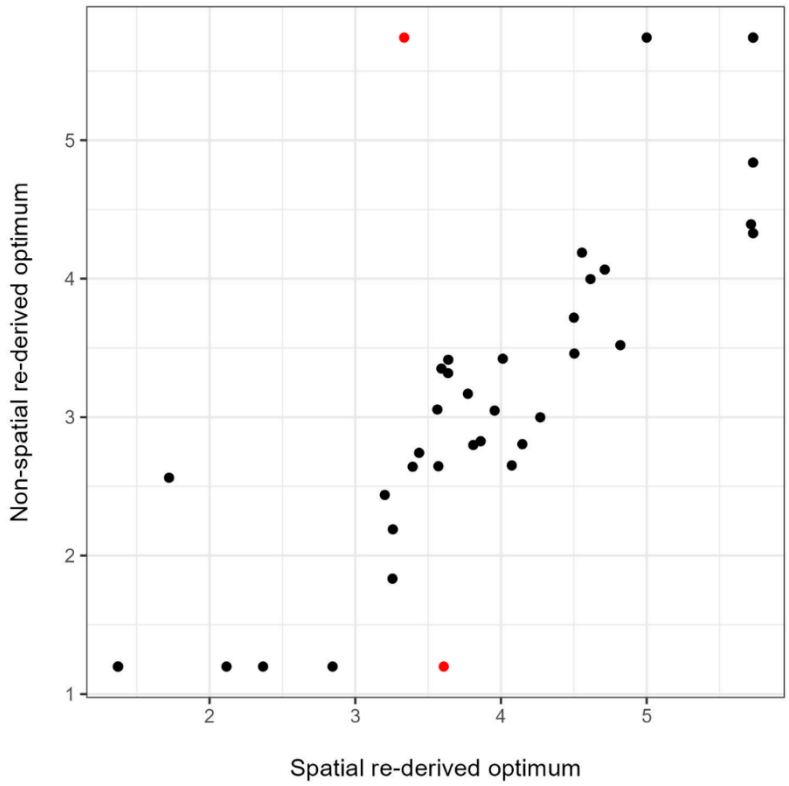


Figure S8: *Optima re-derived from spatially under-sampled scores plotted against the corresponding optimum re-derived from non-spatially under-sampled optima for the same species. Species with absolute differences > 2 are highlighted.*

Unlike optima derived from spatially under-sampled data, the directional trend in optima derived from non-spatially under-sampling relative to validation optima was negative, with significantly lower scores than re-derived butterfly optima (mean difference = -0.7, $t = -2.73$, $df = 39$, $p < 0.01$), and mean moth expert optima (mean difference = -0.5, $t = -2.22$, $df = 40$, $p < 0.05$) and non-significant differences for mean butterfly expert optima (mean difference = -0.24, $t = -0.99$, $df = 42$, $p = 0.33$) and Dutch butterfly optima (mean difference = -0.08, $t = -0.30$, $df = 29$, $p = 0.76$).

In spite of this we found that differences between optima derived from non-spatially under-sampled data and spatially under-sampled data and each and each validation method were significantly positively correlated for each validation method, mostly strongly for Dutch scores (r 0.53, $df = 27$, $p < 0.01$), followed by expert scores ($r = 0.49$, $df = 39$, $p < 0.01$) and re-derived scores ($r = 0.41$, $df = 37$, $p < 0.01$). Thus, spatial and non-spatial scores deviated from the validation methods in a fairly similar way, and there were some common failings across spatially and non-spatially under-sampled optima.

What drives the distribution of tolerance scores?

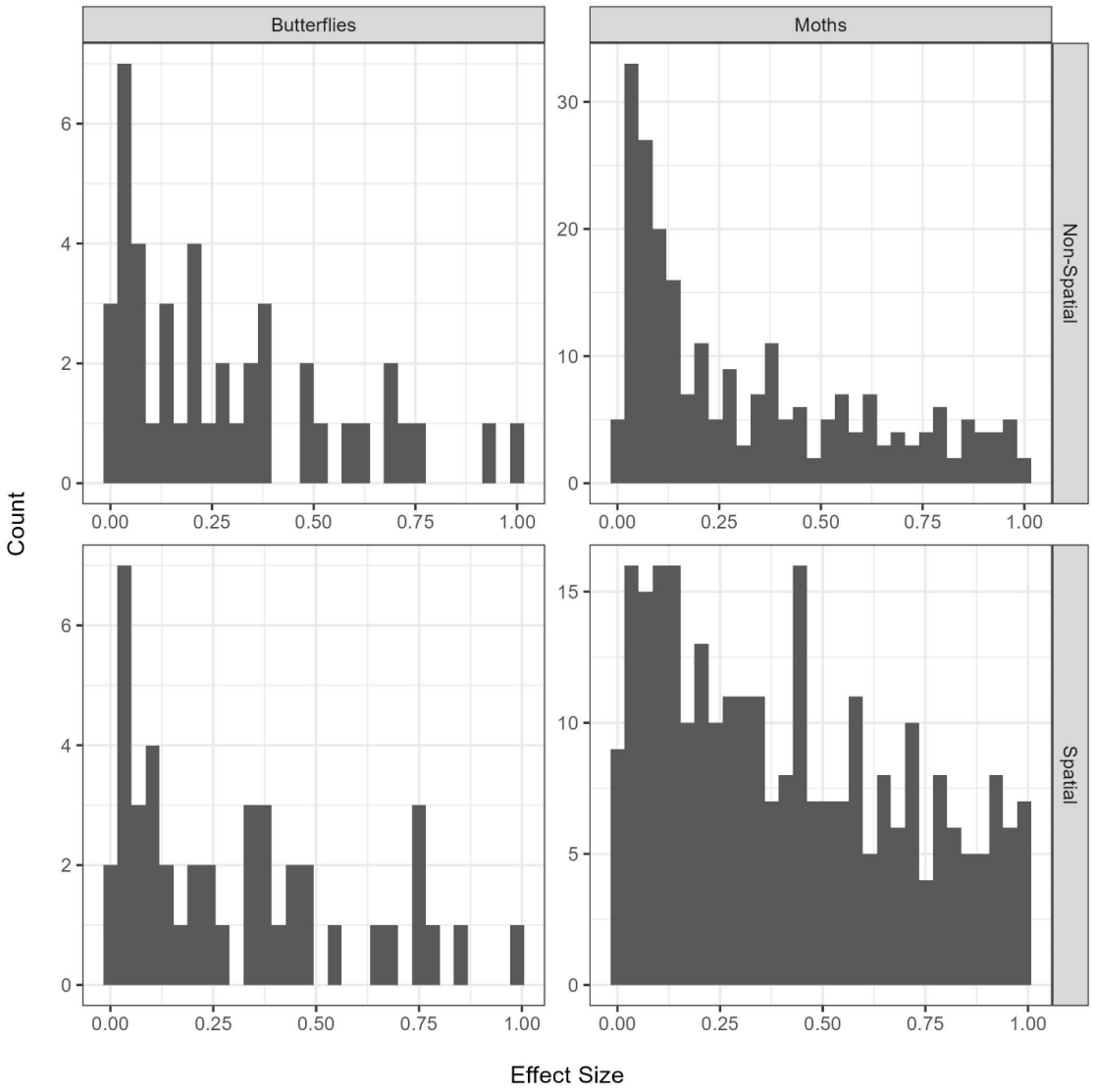


Figure S9: *Weak effects of nutrient levels on species' detection probabilities were most common in our analysis, as shown by a histogram of crude measures of effect size (the minimum probability of a species' occurrence over the range of Ellenberg scores subtracted from its maximum) across our models for butterflies and moths.*

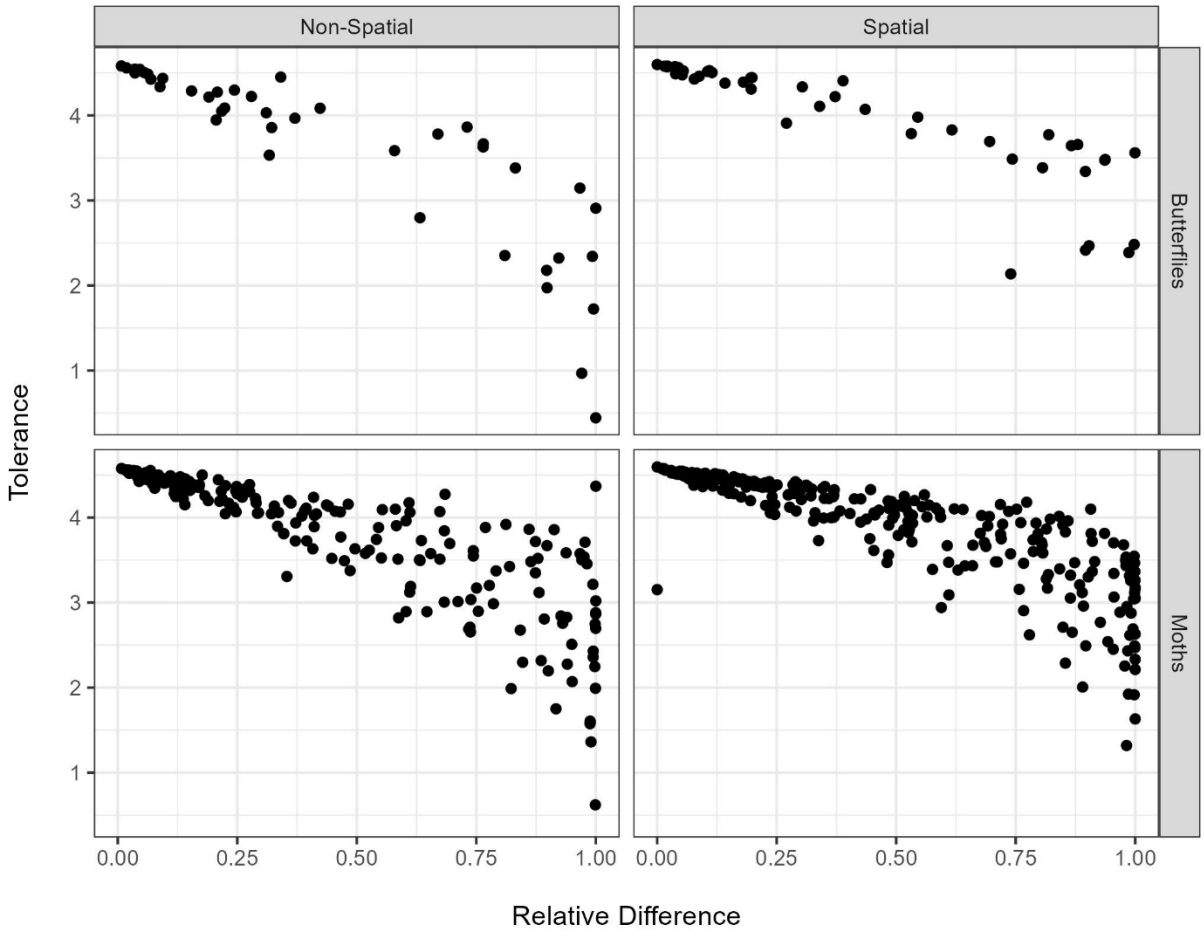


Figure S10: *Occurrence-derived tolerance scores plotted as a function of a crude measure of effect size– the minimum probability of species' occurrence over the range of Ellenberg scores, subtracted from its maximum. This shows that larger effect sizes resulted in smaller and more variable tolerance scores. Results are shown separately for indices derived from spatially and non-spatially under-sampled data for butterflies and moths.*

Because of the role of effect size in shaping tolerance scores, we assessed the match of a subset of tolerance scores for species with larger effect sizes (>0.2) against our validation scores, and found some improvement.

The internal consistency of tolerance scores derived from spatially and non-spatially under-sampled data was not much improved by dropping species with small effect sizes, and neither subsets showed significant correlations with re-derived subsets (spatial: $r = -0.17$, $df = 35$, $p = 0.32$; non-spatial: $r = 0.04$, $df = 32$, $p = 0.83$).

A noticeable improvement was seen relative to mean expert tolerance scores however, and all sub-setted tolerance scores showed positive correlations with mean expert tolerances. These were significant for moth indices derived from spatially under-sampled data ($r = 0.44$, $df = 30$, $p < 0.05$), although not for their non-spatially under-sampled equivalents ($r = 0.41$, $df = 19$, $p = 0.06$) or either set of butterfly scores (spatial: $r = 0.18$, $df = 21$, $p = 0.42$; non-spatial: $r = 0.14$, $df = 20$, $p = 0.54$).

What drives the distribution of optima?

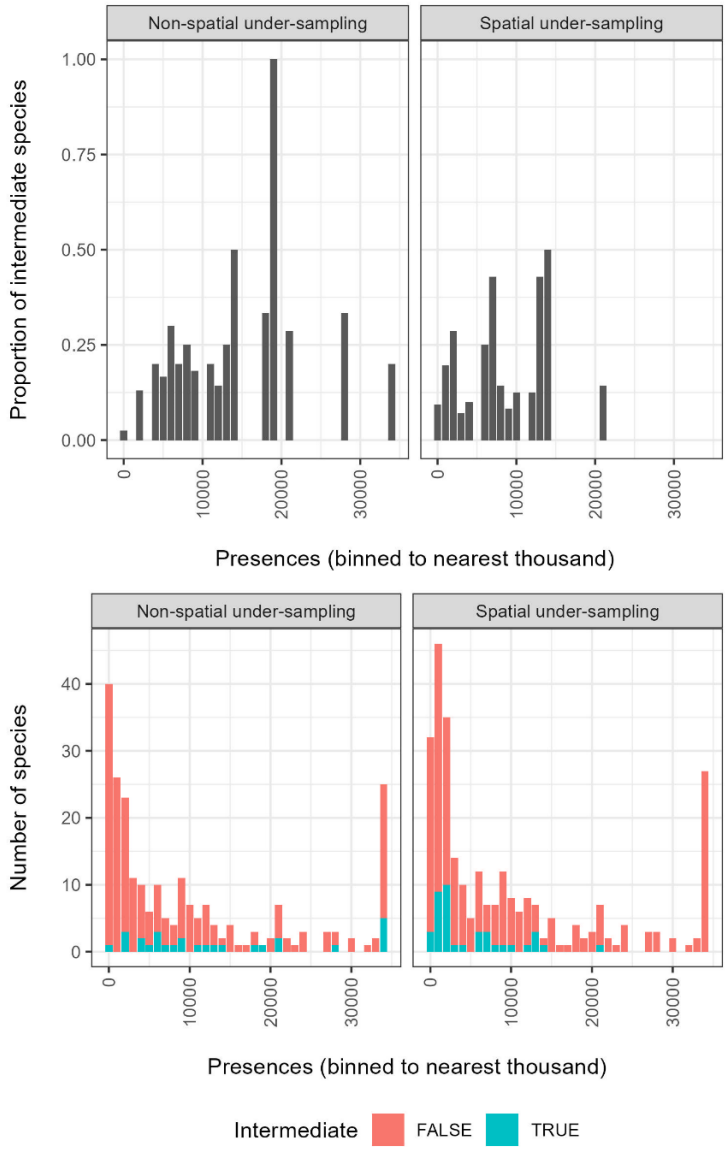


Figure S11: *The frequency of intermediate, unimodal responses at different sample sizes. Intermediate responses were more common at intermediate sample sizes, suggesting that that a lack of power may partly explain their rarity (the rarity at large sample sizes may be 'real' in that these are common species with weak responses to nutrient levels that are more or less linear). Bars are stacked rather than overlapping.*

The effects of spatial bias on optima derived from spatially under-sampled data

Sampling Bias in Butterfly and Moth Data

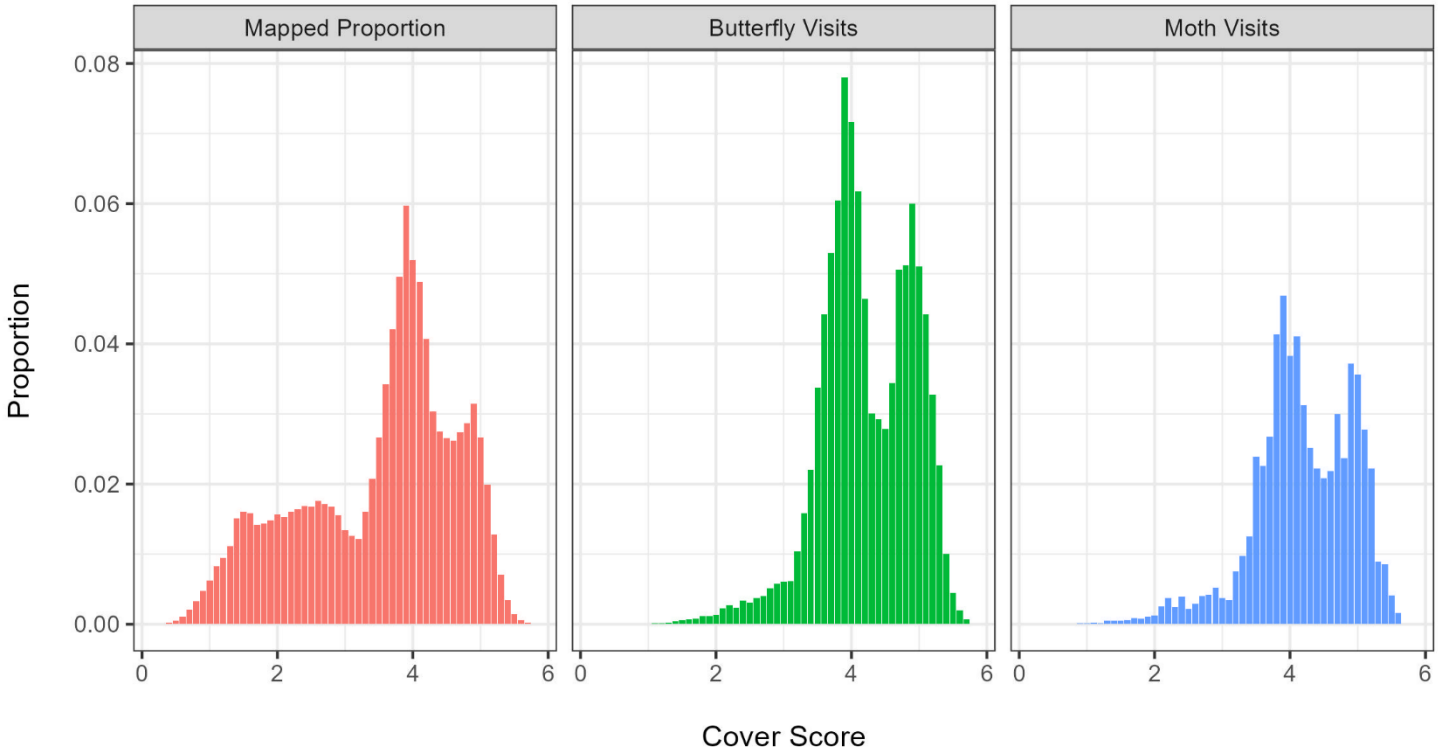


Figure S12: *The distribution of cover-weighted Ellenberg Scores in GB, in visits in the butterfly dataset and visits in the moth dataset. Scores are binned into groups of 0.1, and the proportion of grid squares or visits falling into this group are plotted. There were far more visits to sites with high Ellenberg scores in both the butterfly and moth datasets.*

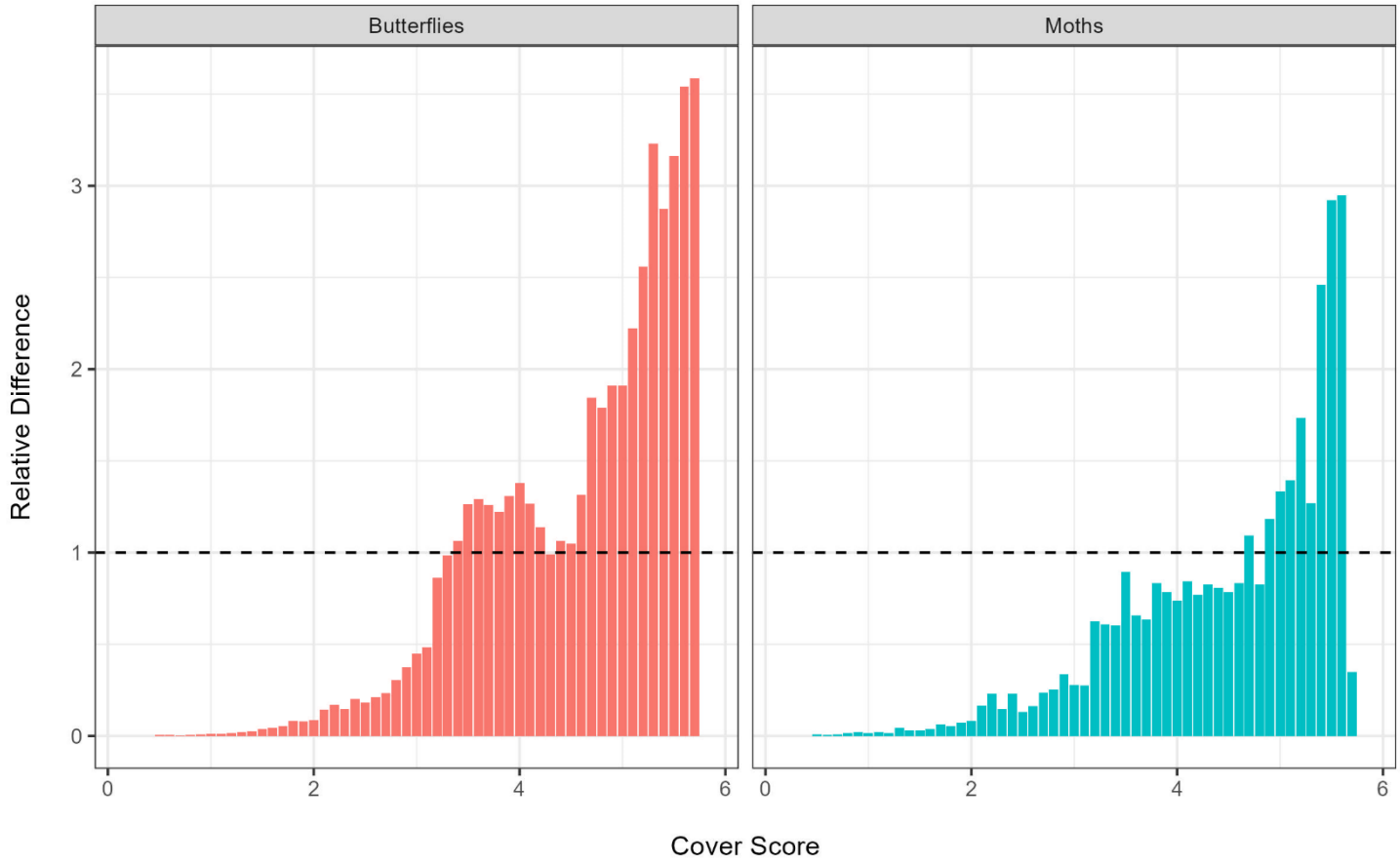


Figure S13: *The higher frequency of visits to sites with high Ellenberg scores meant that they were over-visited relative to their frequency in Great Britain, as show by this plot comparing the frequency of visits to different score levels to their frequency in Great Britain. Ellenberg scores have been binned into groups of 0.1, and bars show the proportion of visits in each dataset in each group, divided by the proportion of grid squares in GB in each group. Thus, bars greater than one (the dashed reference line) indicate that a score level is over-visited relative to its frequency in GB, and bars less than one indicate that a score level is under-visited relative to its frequency in GB.*

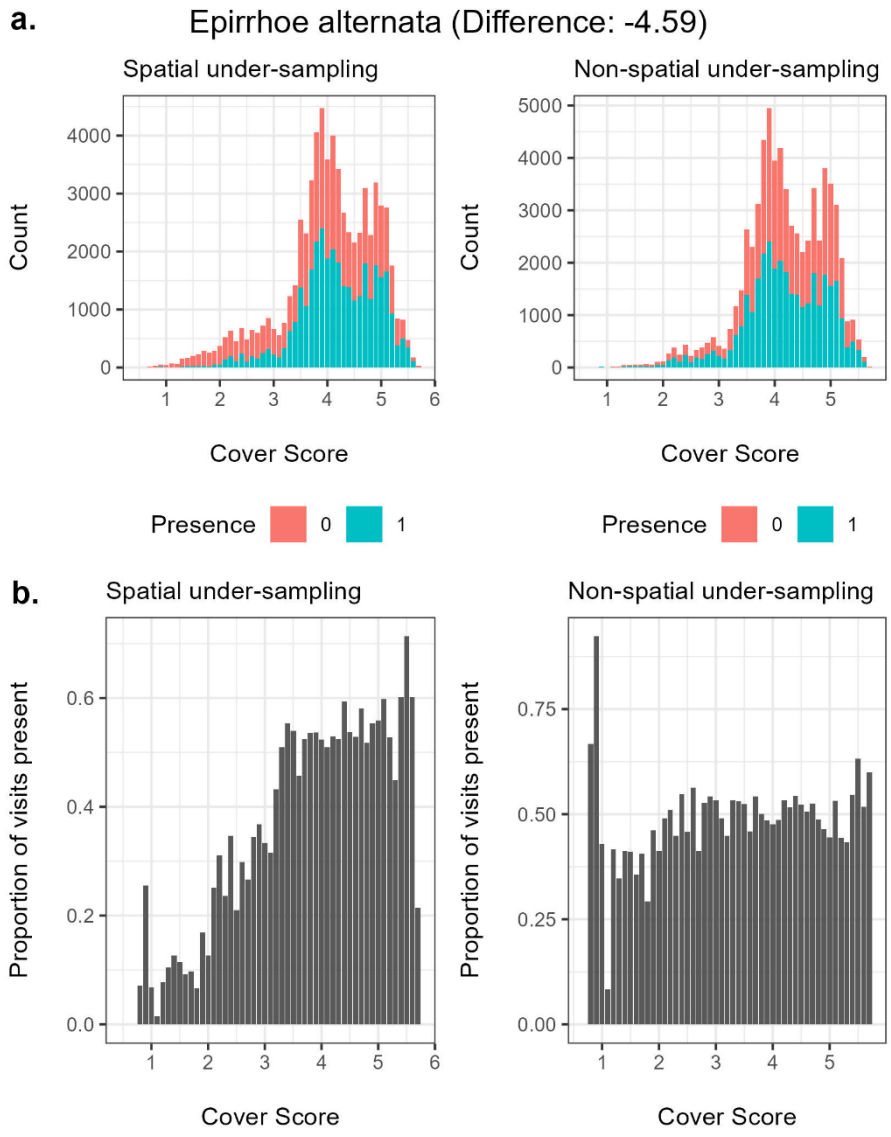
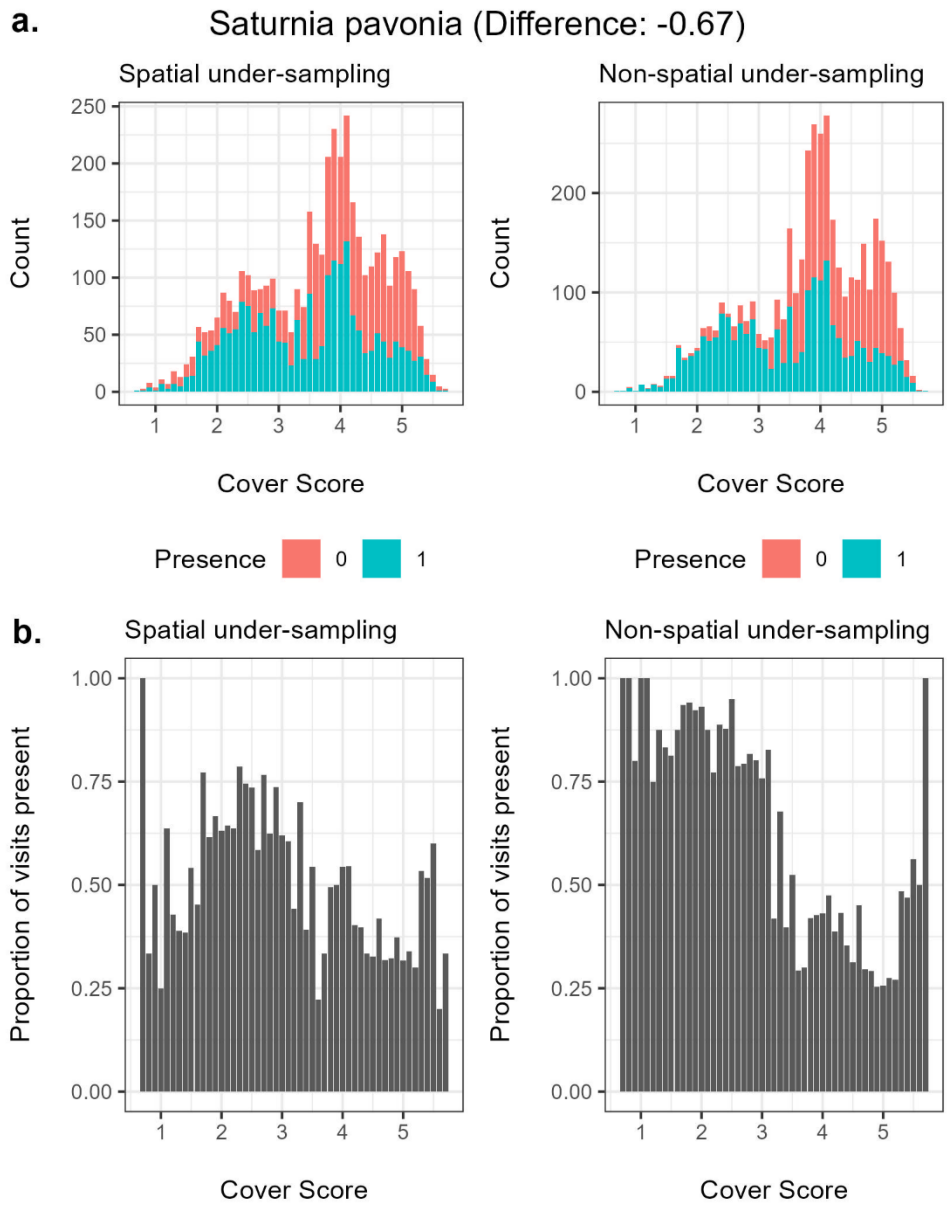


Figure S14: *Plots using the same 0.1 bins for Ellenberg score as figure S13 show an example of how spatial under-sampling inflates the optimum of a moth species, the geometrid Epirrhoe alternata: a. spatial under-sampling increases the number of absences at low scores, and decreases them at high scores (bars are stacked rather than overlapping); b. This increases the frequency of E. alternata at high scores, turning a negative or indifferent response under non-spatial under-sampling (optimum 1.05) to a strong positive response (optimum 5.64).*



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3034 **Figure S15:** *A similar plot to figure S14 for the saturniid moth, Saturnia pavonia. a. as before, spatial*
3035 *under-sampling increases the number of absences at low scores, and decreases them at high scores*
3036 *(note that bars are stacked not overlapping); b. This increases the frequency of S. pavonia at high*
3037 *scores, but because this species has a strong negative response to Ellenberg score, this is insufficient to*
3038 *reverse the direction of the trend, and the optimum for S. pavonia under non-spatial under-sampling*
3039 *(optimum 1.05) remains low under spatial under-sampling (optimum 1.72). Thus, although spatial*

under-sampling always increased species' frequencies at high scores, and decreased them at low scores, if species had very strong responses to nutrient scores, this was insufficient to alter the direction of their responses.

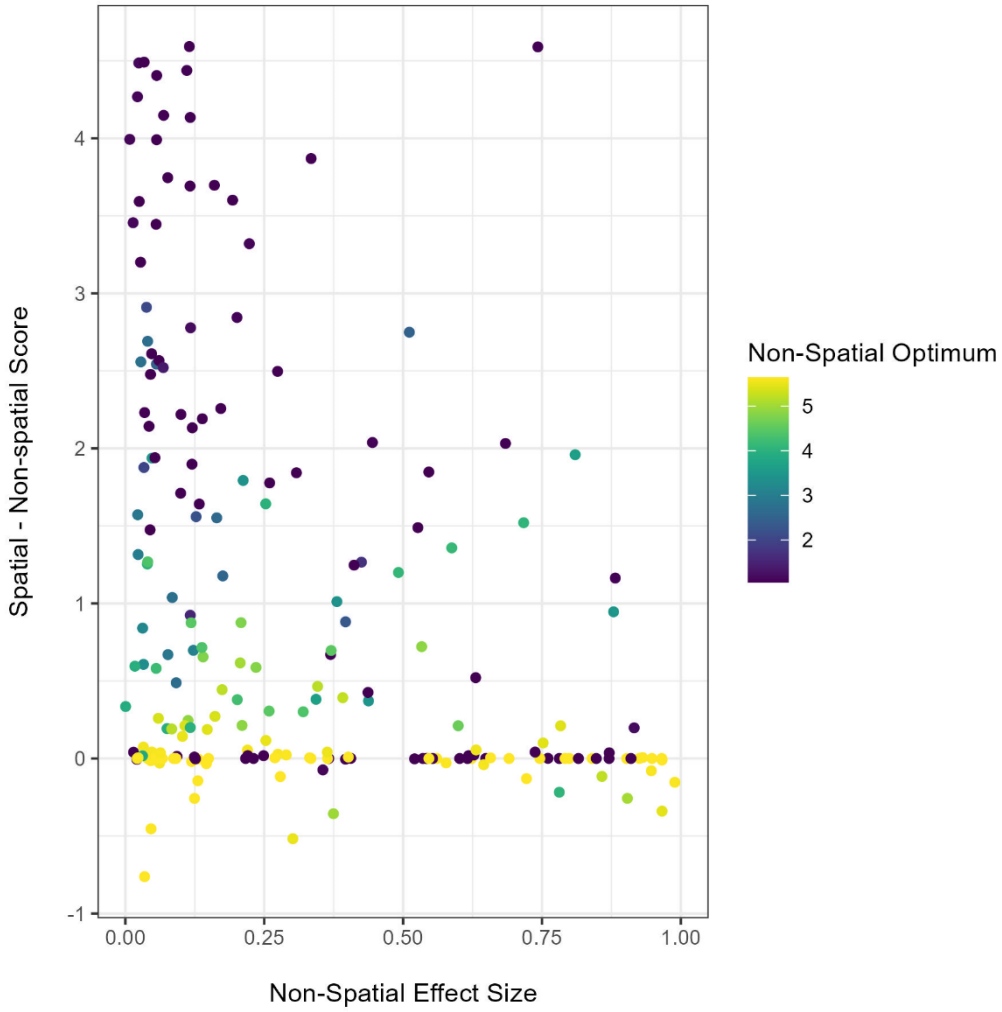


Figure S16: *Overall, spatial under-sampling led to higher optima for species with weak modelled effects and lower optima, as shown by this plot of the difference between optima derived from spatially under-sampled data and from non-spatially under-sampled data against the strength of the effect modelled with non-spatially under-sampled data (maximum probability of detection over the range of Ellenberg scores – minimum probability of detection over the range of Ellenberg scores). Larger*

differences in optima derived from spatially and non-spatially under-sampled data are seen where species had a weaker effect size in their response modelled from non-spatially under-sampled data (meaning that changes in the numbers of absences at different score levels could substantially reverse the trend in frequency) and a low optimum (if species already had a high optimum, spatial under-sampling could not further increase it).

3069 **Indices**

3070 **Table S2:** *Indices for butterflies derived from spatially under-sampled data.*

Taxon	*Optimum*	*Upper Optimum*	*Lower Optimum*	*Tolerance*	*Tolerance Upper*	*Tolerance Lower*	*Model Tolerance*	*Model Optimum*	*Presences*
Aglais io	4.44	5.75	4.08	3.69	3.81	3.57		High	39921
Aglais urticae	4.85	5.75	4.45	3.98	4.07	3.88	Generalist	High	39972
Anthocharis cardamines	4.22	4.36	4.1	4.44	4.48	4.39	Generalist	High	40450
Aphantopus hyperantus	3.67	3.83	3.51	4.5	4.53	4.47	Generalist	Intermediate	40197
Argynnis paphia	3.73	4.14	1.15	4.38	4.45	4.28	Generalist	Intermediate	26286
Aricia agestis	5.73	5.75	5.75	4.43	4.55	4.29	Generalist	High	24495
Aricia artaxerxes	2.53	3.65	1.15	4.44	4.53	4.3	Generalist	Low	1997
Boloria euphrosyne	2.76	3.47	1.15	4.57	4.59	4.55	Generalist	Intermediate	2806
Boloria selene	1.15	1.15	1.15	4.46	4.5	4.41	Generalist	Low	5031
Callophrys rubi	1.15	1.15	1.15	4.48	4.51	4.43	Generalist	Low	11502
Carterocephalus palaemon	1.17	1.15	1.15	4.11	4.51	3.46	Generalist	Low	215
Celastrina argiolus	5.75	5.75	5.75	2.48	2.62	2.35	Specialist	High	40826
Coenonympha pamphilus	1.15	1.15	1.15	3.91	4.02	3.79	Generalist	Low	40308
Coenonympha tullia	1.15	1.15	1.15	4.49	4.54	4.4	Generalist	Low	1012
Colias croceus	5.19	5.75	3.78	3.47	4	3.11		High	4462
Cupido minimus	3.56	3.9	3.2	4.58	4.59	4.55	Generalist	Intermediate	5217
Erebia aethiops	2.16	3	1.15	3.48	3.96	3.09		Low	2114
Erebia epiphron	3.01	5.75	1.15	2.14	3.32	1.16	Specialist	Intermediate	189
Erynnis tages	3.42	3.62	3.25	4.53	4.56	4.47	Generalist	Intermediate	14200
Euphydryas aurinia	3.57	3.79	3.35	3.39	3.77	2.98		Intermediate	2961
Hamearis lucina	3.83	3.96	3.68	4.22	4.46	3.8	Generalist	Intermediate	2295
Hesperia comma	1.15	1.15	1.15	4.57	4.59	4.55	Generalist	Low	1722
Hipparchia semele	2.9	3.02	2.73	2.47	2.79	2.21	Specialist	Intermediate	4437
Lasiommata megera	5.75	5.75	5.75	2.39	2.66	2.13	Specialist	High	12497
Leptidea sinapis	2.75	4.16	1.15	2.42	3.36	1.69	Specialist	Intermediate	1879
Lycaena phlaeas	5.75	5.75	5.75	3.79	3.99	3.58	Generalist	High	40702
Maniola jurtina	4.06	4.2	3.91	4.34	4.4	4.26	Generalist	Intermediate	40156
Melanargia galathea	4.01	4.28	1.15	4.58	4.59	4.56	Generalist	Intermediate	37374
Melitaea athalia	1.87	4.14	1.15	4.41	4.54	4.13	Generalist	Low	929
Ochlodes sylvanus	3.89	5.75	3.54	4.56	4.58	4.54	Generalist	Intermediate	40826

Taxon	Optimum	Upper Optimum	Lower Optimum	Tolerance	Tolerance Upper	Tolerance Lower	Model Tolerance	Model Optimum	Presences
Pararge aegeria	4.43	4.6	4.31	3.34	3.45	3.24		High	40356
Pieris brassicae	5.64	5.75	4.66	3.77	3.92	3.64		High	40270
Pieris napi	5.1	5.75	4	4.39	4.46	4.31	Generalist	High	39555
Pieris rapae	5.74	5.75	5.75	3.66	3.81	3.52		High	40126
Plebejus argus	1.15	1.15	1.15	4.52	4.56	4.48	Generalist	Low	3440
Polygonia c-album	5.31	5.75	4.52	3.64	3.81	3.49		High	40734
Polyommatus coridon	1.15	1.15	1.15	4.6	4.6	4.6	Generalist	Low	7103
Polyommatus icarus	5.74	5.75	5.75	4.07	4.21	3.92	Generalist	High	40443
Pyrgus malvae	3.57	3.71	3.46	3.56	3.93	3.32		Intermediate	6747
Pyronia tithonus	5.74	5.75	5.75	4.52	4.55	4.48	Generalist	High	40633
Speyeria aglaja	2.46	2.65	2.17	4.31	4.37	4.25	Generalist	Low	11107
Thymelicus sylvestris	3.54	4.03	3.28	4.52	4.55	4.47	Generalist	Intermediate	40818
Vanessa atalanta	4.92	5.75	4.48	3.83	4.01	3.66	Generalist	High	39748
Vanessa cardui	5.74	5.75	5.75	3.49	3.75	3.23		High	40139

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3081 **Table S3:** *Indices for butterflies derived from non-spatially under-sampled data*

Taxon	*Optimum*	*Upper Optimum*	*Lower Optimum*	*Tolerance*	*Tolerance Upper*	*Tolerance Lower*	*Model Tolerance*	*Model Optimum*	*Presences*
Aglais urticae	5.49	5.75	4.51	4.22	4.34	4.08	Generalist	High	39972
Anthocharis cardamines	4.35	4.75	4.04	4.55	4.57	4.51	Generalist	High	40450
Aphantopus hyperantus	1.15	1.15	1.15	4.49	4.53	4.44	Generalist	Low	40197
Argynnis paphia	1.19	1.15	1.15	4.08	4.29	3.96	Generalist	Low	26286
Aricia agestis	5.75	5.75	5.75	2.34	2.61	2.09		High	24495
Boloria euphrosyne	1.39	3.92	1.15	4.45	4.53	4.34	Generalist	Low	2806
Boloria selene	1.15	1.15	1.15	4.27	4.39	4.13	Generalist	Low	5031
Callophrys rubi	1.15	1.15	1.15	4.22	4.29	4.14	Generalist	Low	11502
Carterocephalus palaemon	1.16	1.15	1.15	2.32	3.75	1.38		Low	215
Celastrina argiolus	5.75	5.75	5.75	1.72	1.84	1.62	Specialist	High	40826
Coenonympha pamphilus	1.15	1.15	1.15	2.8	2.9	2.69		Low	40308
Coenonympha tullia	1.15	1.15	1.15	4.5	4.55	4.41	Generalist	Low	1012
Cupido minimus	1.15	1.15	1.15	4.54	4.55	4.51	Generalist	Low	5217
Erebia aethiops	1.15	1.15	1.15	4.44	4.55	4.22	Generalist	Low	2114
Erebia epiphron	1.18	1.15	1.15	0.44	1.75	0.17	Specialist	Low	189
Erynnis tages	1.48	3.12	1.15	4.54	4.57	4.51	Generalist	Low	14200
Euphydryas aurinia	1.18	1.15	1.15	3.15	3.58	2.7		Low	2961
Hamearis lucina	1.15	1.15	1.15	2.18	3.17	1.51		Low	2295
Hesperia comma	1.18	1.15	1.15	3.86	4.2	3.42	Generalist	Low	1722
Hipparchia semele	1.35	2.54	1.15	1.97	2.8	1.14		Low	4437
Lasiommata megera	5.75	5.75	5.75	4.05	4.31	3.82	Generalist	High	12497
Leptidea sinapis	5.68	5.75	5.75	2.35	4.02	1.39		High	1879
Lycaena phlaeas	1.15	1.15	1.15	3.53	3.85	3.24	Generalist	Low	40702
Maniola jurtina	3.81	4.07	3.49	4.51	4.55	4.45	Generalist	Intermediate	40156
Melanargia galathea	1.18	1.15	1.15	4.58	4.59	4.57	Generalist	Low	37374
Melitaea athalia	1.15	1.15	1.15	2.91	2.91	2.91		Low	929
Ochlodes sylvanus	1.15	1.15	1.15	4.56	4.58	4.54	Generalist	Low	40826
Pararge aegeria	5.34	5.75	4.39	3.38	3.53	3.2	Generalist	High	40356
Pieris brassicae	5.75	5.75	5.75	3.67	3.83	3.5	Generalist	High	40270
Pieris napi	1.15	1.15	1.15	3.95	4.16	3.75	Generalist	Low	39555
Pieris rapae	5.75	5.75	5.75	3.63	3.8	3.44	Generalist	High	40126
Plebejus argus	1.15	1.15	1.15	4.29	4.41	4.11	Generalist	Low	3440

Taxon	Optimum	Upper Optimum	Lower Optimum	Tolerance	Tolerance Upper	Tolerance Lower	Model Tolerance	Model Optimum	Presences
Polygonia c-album	5.41	5.75	4.48	3.78	3.96	3.59	Generalist	High	40734
Polyommatus bellargus	1.15	1.15	1.15	0.97	1.59	0.75	Specialist	Low	4107
Polyommatus coridon	1.15	1.15	1.15	4.03	4.17	3.87	Generalist	Low	7103
Polyommatus icarus	5.06	5.75	1.15	4.34	4.48	4.16	Generalist	High	40443
Pyrgus malvae	3.8	5.75	3.57	3.86	4.33	3.38	Generalist	Intermediate	6747
Pyronia tithonus	5.75	5.75	5.75	4.52	4.55	4.48	Generalist	High	40633
Speyeria aglaja	1.15	1.15	1.15	3.97	4.1	3.82	Generalist	Low	11107
Thymelicus lineola	5.7	5.75	5.75	4.3	4.54	3.89	Generalist	High	10589
Thymelicus sylvestris	1.15	1.15	1.15	4.43	4.48	4.35	Generalist	Low	40818
Vanessa atalanta	5.75	5.75	5.75	4.09	4.25	3.91	Generalist	High	39748
Vanessa cardui	5.75	5.75	5.75	3.59	3.87	3.33	Generalist	High	40139

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3093 **Table S4:** *Re-derived indices for butterflies from spatially under-sampled data.*

Taxon	Optimum	Upper Optimum	Lower Optimum	Tolerance	Tolerance Upper	Tolerance Lower	Presences
Aglais io	4.5	4.51	4.49	2.16	2.2	2.13	4059
Aglais urticae	5.72	5.73	5.41	2.76	2.9	2.63	3763
Anthocharis cardamines	4.61	4.63	4.6	2.26	2.31	2.2	3288
Aphantopus hyperantus	4.07	4.08	4.07	1.48	1.49	1.47	4678
Argynnis paphia	4.27	4.27	4.26	0.88	0.89	0.87	1956
Aricia agestis	3.59	3.63	3.56	2.8	2.87	2.74	2004
Aricia artaxerxes	1.72	2.59	1.37	2.4	2.52	2.13	132
Boloria euphrosyne	1.37	1.37	1.37	1.45	1.61	1.3	156
Boloria selene	2.12	2.49	1.37	2.25	2.3	2.14	256
Callophrys rubi	1.37	1.37	1.37	1.8	2	1.61	1083
Celastrina argiolus	5.73	5.73	5.73	1.71	1.79	1.63	3115
Coenonympha pamphilus	3.2	3.21	3.2	1.7	1.71	1.69	2695
Colias croceus	3.56	3.61	3.51	1.82	1.96	1.68	760
Cupido minimus	3.61	3.63	3.58	1.71	1.78	1.65	495
Erynnis tages	3.44	3.46	3.42	1.89	1.94	1.85	1223
Euphydryas aurinia	3.26	3.28	3.22	1.46	1.52	1.41	104
Hamearis lucina	3.57	3.61	3.53	1.39	1.51	1.27	88
Hesperia comma	3.39	3.4	3.39	0.81	0.81	0.8	140
Hipparchia semele	2.84	2.87	2.82	1.97	2.01	1.93	440
Lasiommata megera	3.64	3.69	3.58	2.67	2.79	2.55	751
Leptidea sinapis	3.34	3.39	3.27	1.83	1.93	1.74	71
Lycaena phlaeas	3.96	4.02	3.9	3.01	3.09	2.93	3289
Maniola jurtina	4.01	4.01	4.01	1.68	1.69	1.68	4948
Melanargia galathea	3.81	3.81	3.8	1.64	1.65	1.63	2797
Ochlodes sylvanus	4.15	4.16	4.14	2.19	2.23	2.15	4103
Pararge aegeria	4.56	4.56	4.55	1.66	1.68	1.65	4349
Pieris brassicae	5.73	5.73	5.73	1.68	1.72	1.65	4754
Pieris napi	4.82	4.83	4.81	1.97	2	1.95	4373
Pieris rapae	5.73	5.73	5.73	1.17	1.19	1.16	4640
Plebejus argus	1.37	1.37	1.37	1	1.02	0.98	278
Polygonia c-album	4.71	4.72	4.7	1.76	1.8	1.73	4187
Polyommatus coridon	2.37	2.4	2.33	2.04	2.05	2.03	598
Polyommatus icarus	3.64	3.65	3.63	2.51	2.53	2.49	4119
Pyronia tithonus	4.5	4.51	4.5	2.86	2.88	2.83	4463
Speyeria aglaja	3.26	3.27	3.25	1.29	1.31	1.27	1164
Thymelicus lineola	3.77	3.81	3.74	1.92	2	1.84	1575
Thymelicus sylvestris	3.86	3.87	3.85	1.62	1.64	1.6	3663
Vanessa atalanta	5	5.06	4.95	2.4	2.45	2.35	4487
Vanessa cardui	5.73	5.73	5.73	1.95	2.02	1.88	3347

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3096 **Table S5:** *Re-derived indices for butterflies from non-spatially under-sampled data.*

Taxon	*Optimum*	*Upper Optimum*	*Lower Optimum*	*Tolerance*	*Tolerance Upper*	*Tolerance Lower*	*Presences*
Aglais io	3.72	3.75	3.69	3.07	3.09	3.04	4059
Aglais urticae	4.39	4.43	4.36	3.06	3.1	3.02	3763
Anthocharis cardamines	4	4.03	3.97	3.33	3.38	3.28	3288
Aphantopus hyperantus	2.65	2.66	2.64	2.37	2.38	2.37	4678
Argynnis paphia	3	3.01	2.99	2.02	2.05	1.99	1956
Aricia agestis	3.35	3.37	3.34	2.19	2.22	2.16	2004
Aricia artaxerxes	2.56	2.59	2.53	2.17	2.24	2.1	132
Boloria euphrosyne	1.2	1.2	1.2	1.27	1.34	1.19	156
Boloria selene	1.2	1.2	1.2	0.77	0.81	0.73	256
Callophrys rubi	1.2	1.2	1.2	1.79	1.94	1.65	1083
Celastrina argiolus	4.84	5.12	4.69	2.87	2.91	2.83	3115
Coenonympha pamphilus	2.44	2.45	2.42	2.27	2.28	2.26	2695
Colias croceus	3.06	3.1	3	2.12	2.29	1.97	760
Cupido minimus	1.2	1.2	1.2	1.85	1.86	1.84	495
Erynnis tages	2.74	2.76	2.73	1.86	1.9	1.83	1223
Euphydryas aurinia	1.83	1.83	1.83	1.82	1.82	1.82	104
Hamearis lucina	2.65	2.68	2.61	1.51	1.58	1.45	88
Hesperia comma	2.64	2.66	2.62	1.65	1.68	1.62	140
Hipparchia semele	1.2	1.2	1.2	1.72	1.83	1.61	440
Lasiommata megera	3.32	3.37	3.27	2.67	2.75	2.58	751
Leptidea sinapis	5.74	5.74	5.74	0.95	0.95	0.95	71
Lycaena phlaeas	3.05	3.09	3.01	3.11	3.17	3.06	3289
Maniola jurtina	3.42	3.42	3.42	1.91	1.92	1.91	4948
Melanargia galathea	2.8	2.8	2.79	2.04	2.05	2.03	2797
Ochlodes sylvanus	2.8	2.82	2.79	2.34	2.36	2.31	4103
Pararge aegeria	4.19	4.22	4.16	3.1	3.12	3.08	4349
Pieris brassicae	5.74	5.74	5.74	2.63	2.69	2.58	4754
Pieris napi	3.52	3.54	3.5	3.31	3.34	3.28	4373
Pieris rapae	5.74	5.74	5.74	2.03	2.06	2	4640
Plebejus argus	1.2	1.2	1.2	0.71	0.72	0.7	278
Polygonia c-album	4.07	4.11	4.02	3.06	3.1	3.01	4187
Polyommatus bellargus	2.7	2.71	2.7	1.02	1.03	1.01	324
Polyommatus coridon	1.2	1.2	1.2	1.21	1.22	1.2	598
Polyommatus icarus	3.41	3.42	3.41	2.32	2.34	2.31	4119
Pyronia tithonus	3.46	3.47	3.45	2.79	2.8	2.77	4463
Speyeria aglaja	2.19	2.23	2.15	1.9	1.91	1.88	1164
Thymelicus lineola	3.17	3.2	3.14	2.59	2.67	2.51	1575
Thymelicus sylvestris	2.83	2.84	2.82	2.29	2.31	2.27	3663
Vanessa atalanta	5.74	5.74	5.74	2.83	2.93	2.74	4487
Vanessa cardui	4.33	4.41	4.26	3.19	3.24	3.15	3347

3097

3098 **Table S6:** *Indices for moths derived from spatially under-sampled data.*

Taxon	Optimum	Upper Optimum	Lower Optimum	Tolerance	Tolerance Upper	Tolerance Lower	Model Tolerance	Model Optimum	Presences
Abraxas grossulariata	5.63	5.65	5.21	4.05	4.2	3.88	Generalist	High	10448
Abrostola tripartita	5.64	5.65	5.65	4.01	4.12	3.89	Generalist	High	34086
Abrostola triplasia	5.64	5.65	5.65	2.47	2.79	2.18		High	3915
Acronicta menyanthidis	1.05	1.05	1.05	2.94	3.78	2.01		Low	388
Acronicta psi	5.62	5.65	5.22	3.48	3.83	3.13		High	2197
Acronicta rumicis	5.51	5.65	3.85	4.22	4.37	4.05	Generalist	High	11674
Agriopis marginaria	5.64	5.65	5.65	4.56	4.59	4.53	Generalist	High	9359
Agrius convolvuli	5.65	5.65	5.65	3.47	4.06	2.85		High	898
Agrochola lychnidis	5.28	5.65	3.62	4.1	4.4	3.84	Generalist	High	17597
Agrotis clavis	5.63	5.65	5.29	4.42	4.52	4.27	Generalist	High	14071
Agrotis exclamationis	5.65	5.65	5.65	3.5	3.63	3.36		High	33973
Agrotis puta	5.65	5.65	5.65	2.33	2.43	2.23	Specialist	High	34201
Agrotis segetum	4.82	5.65	4.58	3.3	3.61	3.02		High	19812
Agrotis trux	5.65	5.65	5.65	2.45	3.51	1.65		High	235
Agrotis vestigialis	5.44	5.65	4.26	3.46	4.22	2.7		High	748
Alcis repandata	4.58	5.65	4.09	4.46	4.49	4.42	Generalist	High	23313
Alsophila aescularia	4.15	4.58	3.83	4.36	4.48	4.19	Generalist	High	10774
Amphipoea crinanensis	1.09	1.05	1.05	3.82	4.51	2.82	Generalist	Low	123
Amphipoea lucens	2.98	3.59	2.57	4.01	4.42	3.37	Generalist	Intermediate	551
Amphipyra tragopoginis	4.43	5.65	3.55	3.68	3.86	3.52	Generalist	High	11638
Anaplectoides prasina	5.63	5.65	5.65	4.47	4.53	4.38	Generalist	High	5633
Anarta myrtilli	1.05	1.05	1.05	3.39	3.85	2.93		Low	403
Anarta trifolii	5.1	5.65	4.25	3.16	3.59	2.92		High	8678
Anchoscelis helvola	1.06	1.05	1.05	4.52	4.57	4.45	Generalist	Low	1118
Anchoscelis litura	3.81	5.65	3.44	3.7	3.95	3.5	Generalist	Intermediate	8257
Anchoscelis lunosa	5.65	5.65	5.65	4.18	4.4	3.93	Generalist	High	24398
Antitype chi	5.62	5.65	5.65	4.44	4.56	4.27	Generalist	High	821
Apamea anceps	1.05	1.05	1.05	4.58	4.58	4.57	Generalist	Low	4131
Apamea crenata	5.54	5.65	4.42	4.48	4.52	4.42	Generalist	High	12995
Apamea epomidion	5.65	5.65	5.65	4.52	4.56	4.46	Generalist	High	2345
Apamea lithoxylaea	5.4	5.65	4.49	3.94	4.12	3.74	Generalist	High	12705
Apamea monoglypha	5.65	5.65	5.65	4.37	4.43	4.3	Generalist	High	33799
Apamea remissa	5.61	5.65	4.91	4.54	4.57	4.51	Generalist	High	7322
Apamea scolopacina	5	5.65	4.22	4.48	4.54	4.38	Generalist	High	5095
Apamea sordens	5.04	5.65	3.45	4.27	4.4	4.11	Generalist	High	12701
Apamea unanimis	5.63	5.65	5.65	3.81	4.2	3.45	Generalist	High	2398
Aplocera efformata	3.93	4.31	1.05	2.89	3.87	2.3		Intermediate	1452
Aplocera plagiata	5.65	5.65	5.65	3.47	4	2.97		High	2582
Aporophyla lutulenta	5.55	5.65	5.65	4.56	4.59	4.5	Generalist	High	2015
Aporophyla nigra	5.13	5.65	3.91	4.5	4.54	4.44	Generalist	High	18768
Archanara dissoluta	5.61	5.65	5.65	4.12	4.51	3.44	Generalist	High	389
Arctia caja	5.65	5.65	5.65	3.95	4.11	3.78	Generalist	High	8751
Arctia villica	5.54	5.65	5.65	2.9	4.36	1.68		High	631
Arenostola phragmitidis	5.65	5.65	5.65	4.47	4.56	4.31	Generalist	High	1818
Autographa bractea	3.29	5.65	2.97	4.5	4.56	4.4	Generalist	Intermediate	1787
Autographa gamma	5.65	5.65	5.65	3.47	3.6	3.33		High	33501
Autographa jota	5.44	5.65	4.51	4.06	4.26	3.85	Generalist	High	8672
Autographa pulchrina	3.9	5.65	3.53	4.42	4.48	4.36	Generalist	Intermediate	11634

Taxon	Optimum	Upper Optimum	Lower Optimum	Tolerance	Tolerance Upper	Tolerance Lower	Model Tolerance	Model Optimum	Presences
Axylia putris	5.63	5.65	5.65	4.07	4.23	3.91	Generalist	High	34094
Biston betularia	5	5.65	4.58	4.36	4.43	4.27	Generalist	High	28437
Callimorpha dominula	5.64	5.65	5.65	4.52	4.57	4.46	Generalist	High	3551
Callistege mi	3.24	5.65	2.76	4.39	4.53	4.18	Generalist	Intermediate	1929
Calophasia lunula	5.62	5.65	5.65	4.15	4.54	3.42	Generalist	High	674
Camptogramma bilineata	5.65	5.65	5.65	3.68	3.83	3.53	Generalist	High	16784
Caradrina morpheus	5.65	5.65	5.65	3.26	3.37	3.17		High	23596
Catarhoe cuculata	3.84	4.29	1.05	2.25	3.34	1.59	Specialist	Intermediate	369
Celaena haworthii	1.05	1.05	1.05	4.27	4.45	4.01	Generalist	Low	693
Ceramica pisi	1.05	1.05	1.05	4.37	4.45	4.27	Generalist	Low	5130
Cerapteryx graminis	3.19	3.52	2.93	4.55	4.57	4.52	Generalist	Intermediate	10338
Cerastis leucographa	3.74	4.14	1.05	4.22	4.56	3.55	Generalist	Intermediate	789
Cerastis rubricosa	3.55	3.92	3.15	4.36	4.47	4.22	Generalist	Intermediate	7423
Charanyca trigrammica	5.37	5.65	4.52	4.1	4.38	3.79	Generalist	High	21395
Chesias legatella	4.29	5.65	3.69	4.22	4.49	3.79	Generalist	High	1129
Chiasmia clathrata	4.75	5.65	3.03	4.13	4.36	3.87	Generalist	High	4462
Chilodes maritima	5.53	5.65	4.5	4.28	4.52	3.87	Generalist	High	1007
Chloroclystis v-ata	4.68	5.65	4.43	4.05	4.26	3.83	Generalist	High	12391
Cirrhia icteritia	4.88	5.65	3.78	4.16	4.33	3.97	Generalist	High	10477
Coenotephria salicata	1.05	1.05	1.05	1.32	1.81	1	Specialist	Low	469
Colostygia multistrigaria	2.76	3.2	1.88	4.44	4.53	4.29	Generalist	Intermediate	3289
Colostygia pectinataria	4.92	5.65	3.4	4.09	4.23	3.9	Generalist	High	33903
Colotois pennaria	4.99	5.65	3.66	4.48	4.54	4.39	Generalist	High	12310
Conistra ligula	4.24	5.65	3.53	2.69	2.99	2.36		High	5331
Cosmorhoe ocellata	1.05	1.05	1.05	4.05	4.34	3.81	Generalist	Low	7691
Crocallis elinguaria	5.65	5.65	5.65	4.36	4.42	4.29	Generalist	High	20749
Cucullia chamomillae	5.41	5.65	3.55	2.21	2.78	1.59	Specialist	High	227
Cucullia lychnitis	1.05	1.05	1.05	4.59	4.59	4.59	Generalist	Low	200
Cucullia umbratica	4.99	5.65	4.33	4	4.3	3.65	Generalist	High	1947
Cucullia verbasci	5.37	5.65	4.57	2.88	3.35	2.61		High	1920
Cyclophora puppillaria	5.65	5.65	5.65	3.6	4.28	2.84	Generalist	High	462
Dasypolia templi	5.25	5.65	2.73	1.92	2.95	1.07	Specialist	High	398
Deilephila elpenor	5.62	5.65	5.02	4.15	4.26	4.03	Generalist	High	34008
Deilephila porcellus	5.22	5.65	4.04	4.39	4.5	4.22	Generalist	High	6272
Denticucullus pygmina	3.97	5.65	2.87	4.38	4.48	4.25	Generalist	Intermediate	6335
Diachrysia chrysitis	5.48	5.65	4.41	4.14	4.24	4.03	Generalist	High	28169
Diacrisia sannio	2.89	3.18	2.62	3.95	4.25	3.56	Generalist	Intermediate	809
Diaphora mendica	4.78	5.65	4.47	4.11	4.32	3.88	Generalist	High	14602
Diarsia brunnea	4.76	5.65	3.49	4.53	4.56	4.49	Generalist	High	8946
Diarsia dahlii	2.69	3.58	1.05	4.55	4.58	4.51	Generalist	Intermediate	1603
Diarsia mendica	5.04	5.65	3.39	4.52	4.55	4.47	Generalist	High	19723
Diarsia rubi	5.56	5.65	4.81	3.05	3.23	2.87		High	34034
Dicallomera fascelina	1.09	1.05	1.05	2.01	3.79	1.13	Specialist	Low	375
Dypterygia scabriuscula	5.58	5.65	5.65	4.39	4.58	4.11	Generalist	High	1309
Dyscia fagaria	1.08	1.05	1.05	3.38	4.35	2.27		Low	211
Dysstroma truncata	5.65	5.65	5.65	2.49	2.66	2.35		High	34011
Ecliptopera silaceata	4.44	5.65	4.17	3.71	3.83	3.57	Generalist	High	21797
Eilema complana	4.26	4.52	3.93	4.52	4.58	4.4	Generalist	High	14920
Ematurga atomaria	1.05	1.05	1.05	4.08	4.22	3.91	Generalist	Low	3253
Entephria caesiata	1.38	2.16	1.05	2.49	3.04	1.99		Low	373
Epirrhoe alternata	5.64	5.65	5.65	4.08	4.2	3.95	Generalist	High	33751

Taxon	Optimum	Upper Optimum	Lower Optimum	Tolerance	Tolerance Upper	Tolerance Lower	Model Tolerance	Model Optimum	Presences
Epirrhoe tristata	1.09	1.85	1.05	2.96	3.55	2.34		Low	562
Epirrita autumnata	1.62	3.42	1.05	4.21	4.49	3.69	Generalist	Low	926
Epirrita filigrammaria	1.05	1.05	1.05	4.37	4.55	3.98	Generalist	Low	235
Erannis defoliaria	4.17	4.53	3.86	3.67	3.98	3.37	Generalist	High	9418
Eugnorisma depuncta	4.68	5.65	3.21	4.25	4.46	3.92	Generalist	High	998
Eugnorisma glareosa	1.07	1.05	1.05	4.56	4.59	4.53	Generalist	Low	5716
Eulithis populata	1.48	2.55	1.05	4.37	4.47	4.25	Generalist	Low	3098
Euphyia biangulata	3.09	4.21	1.05	4.52	4.58	4.35	Generalist	Intermediate	984
Eupithecia absinthiata	5.65	5.65	5.65	4.28	4.42	4.12	Generalist	High	3822
Eupithecia centaureata	5.64	5.65	5.65	3.12	3.35	2.93		High	12427
Eupithecia icterata	5.1	5.65	4.24	4.25	4.45	3.98	Generalist	High	2656
Eupithecia linariata	5.6	5.65	5.65	4.32	4.56	3.99	Generalist	High	744
Eupithecia nanata	1.07	1.05	1.05	4.46	4.55	4.35	Generalist	Low	2254
Eupithecia pulchellata	5.38	5.65	4.48	4.42	4.48	4.34	Generalist	High	10453
Eupithecia satyrata	1.57	2.82	1.05	3.66	4.12	3.06	Generalist	Low	489
Eupithecia simpliciata	5.64	5.65	5.65	3.99	4.44	3.34	Generalist	High	512
Eupithecia subfuscata	4.82	5.65	4.19	4.47	4.52	4.4	Generalist	High	6993
Eupithecia succenturiata	4.52	5.65	3.85	4.02	4.52	3.42	Generalist	High	983
Eupithecia tripunctaria	5.53	5.65	4.66	3.28	3.65	2.95		High	4199
Eupithecia virgaureata	5.22	5.65	3.74	3.4	3.8	2.91		High	1695
Eupithecia vulgata	5.65	5.65	5.65	3.9	4.04	3.77	Generalist	High	23736
Euplagia quadripunctaria	5.65	5.65	5.65	3.75	4.24	3.17	Generalist	High	2548
Euplexia lucipara	5.22	5.65	4.43	4.47	4.52	4.41	Generalist	High	10969
Euproctis similis	5.65	5.65	5.65	3.05	3.14	2.97		High	15266
Euthrix potatoria	5.19	5.65	3.42	4.45	4.52	4.36	Generalist	High	15003
Euxoa nigricans	5.43	5.65	3.83	2.62	3.32	1.87		High	514
Euxoa tritici	5.49	5.65	4.31	3.92	4.3	3.56	Generalist	High	1917
Gandaritis pyraliata	4.5	5.65	3.15	4.52	4.55	4.48	Generalist	High	17551
Globia sparganii	5.64	5.65	5.65	4.43	4.56	4.17	Generalist	High	929
Graphiphora augur	5.64	5.65	5.65	4.24	4.47	3.93	Generalist	High	1496
Gymnoscelis rufifasciata	5.65	5.65	5.65	2.77	2.93	2.61		High	30460
Hada plebeja	4.65	5.65	3.07	4.37	4.51	4.18	Generalist	High	5893
Hadena bicruris	5.63	5.65	5.65	3.07	3.25	2.88		High	8976
Hadena compta	5.59	5.65	5.65	4.4	4.57	4.15	Generalist	High	1034
Hadena confusa	5.52	5.65	4.29	2.95	3.61	2.35		High	572
Hecatera bicolorata	5.55	5.65	4.69	3.82	4.26	3.41	Generalist	High	1707
Hecatera dysodea	4.47	5.65	1.05	3.04	4.37	2.44		High	615
Helicoverpa armigera	5.64	5.65	5.65	3.61	4.26	2.86	Generalist	High	1546
Heliothis peltigera	5.65	5.65	5.65	2.62	3.54	1.89		High	1063
Helotropha leucostigma	5.58	5.65	4.58	4.44	4.54	4.3	Generalist	High	2048
Hemaris tityus	3.19	3.67	2.6	2.54	3.34	1.9		Intermediate	219
Hepialus humuli	5.6	5.65	4.66	3.87	4.06	3.67	Generalist	High	8650
Hoplodrina ambigua	5.65	5.65	5.65	2.63	2.83	2.43		High	26649
Hoplodrina blanda	5.6	5.65	4.84	3.91	4.14	3.72	Generalist	High	20590
Hoplodrina octogenaria	5.65	5.65	5.65	3.55	3.72	3.42	Generalist	High	34171
Hydraecia micacea	5.5	5.65	4.25	4.2	4.3	4.08	Generalist	High	23932
Hydriomena furcata	3.55	3.77	3.36	4.36	4.42	4.3	Generalist	Intermediate	21193
Hydriomena ruberata	3.13	5.65	2.51	4.1	4.47	3.48	Generalist	Intermediate	239
Hypena proboscidalis	5.5	5.65	4.69	3.93	4.06	3.77	Generalist	High	33933
Hyppa rectilinea	1.06	1.05	1.05	4.39	4.56	3.96	Generalist	Low	219
Idaea aversata	5.65	5.65	5.65	4.25	4.34	4.15	Generalist	High	33961

Taxon	Optimum	Upper Optimum	Lower Optimum	Tolerance	Tolerance Upper	Tolerance Lower	Model Tolerance	Model Optimum	Presences
Idaea dimidiata	5.65	5.65	5.65	3.37	3.49	3.27		High	27196
Idaea emarginata	5.64	5.65	5.65	4.58	4.59	4.55	Generalist	High	1113
Idaea rusticata	5.65	5.65	5.65	4.57	4.58	4.56	Generalist	High	8792
Korscheltellus fusconebulosa	3.62	5.65	3.12	4.54	4.57	4.51	Generalist	Intermediate	5649
Korscheltellus lupulina	5.65	5.65	5.65	3.47	3.63	3.34		High	18149
Lacanobia contigua	1.12	1.05	1.05	4	4.48	3.31	Generalist	Low	206
Lacanobia oleracea	5.65	5.65	5.65	4.24	4.33	4.15	Generalist	High	34091
Lacanobia suasa	5.63	5.65	5.65	3.09	4.01	2.28		High	1619
Lacanobia thalassina	4.65	5.65	3.81	4.41	4.47	4.33	Generalist	High	7955
Lampropteryx otregiata	2.23	4.01	1.05	3.43	4.09	2.57		Low	1255
Lampropteryx suffumata	4.74	5.65	3	4.44	4.53	4.32	Generalist	High	6034
Lasiocampa quercus	1.05	1.05	1.05	3.73	3.94	3.48	Generalist	Low	3105
Lateroligia ophiogramma	5.18	5.65	4.2	3.93	4.34	3.56	Generalist	High	2839
Leptologia macilenta	2.53	5.65	1.05	3.15	4.59	0.02		Low	15055
Leucania comma	5.65	5.65	5.65	3.98	4.23	3.76	Generalist	High	11205
Leucania obsoleta	5.46	5.65	3.55	3.17	3.5	2.94		High	962
Litoligia literosa	5.57	5.65	4.66	3.74	4.08	3.41	Generalist	High	2918
Luperina testacea	5.65	5.65	5.65	4.15	4.3	4.01	Generalist	High	27209
Lycophotia porphyrea	1.05	1.05	1.05	4.51	4.53	4.48	Generalist	Low	8337
Lygephila pastinum	5.48	5.65	3.88	3.96	4.35	3.62	Generalist	High	1954
Macroglossum stellatarum	4.64	5.65	4.27	3.37	3.56	3.18		High	10888
Macrothylacia rubi	1.05	1.05	1.05	2.29	2.49	2.08	Specialist	Low	2563
Mamestra brassicae	5.55	5.65	4.87	3.32	3.6	3.1		High	9589
Melanchra persicariae	5.14	5.65	4.55	4.28	4.46	4.05	Generalist	High	12416
Mesapamea didyma	4.17	5.65	3.2	4.03	4.43	3.57	Generalist	High	1101
Mesapamea secalis	5.48	5.65	4.56	3.99	4.21	3.75	Generalist	High	5991
Mesoligia furuncula	5.62	5.65	4.9	4.37	4.52	4.15	Generalist	High	8340
Mesotype didymata	3.18	5.65	2.6	4.47	4.54	4.38	Generalist	Intermediate	2453
Minoa murinata	5.65	5.65	5.65	1.63	2.12	1.1	Specialist	High	258
Mniotype adusta	2.47	2.69	2.05	4.36	4.47	4.22	Generalist	Low	1378
Mormo maura	5.64	5.65	5.65	3.36	3.74	3.11		High	4505
Mythimna albipuncta	3.88	5.65	1.05	4.38	4.53	4.05	Generalist	Intermediate	13112
Mythimna conigera	3.9	5.65	3.46	4.42	4.53	4.26	Generalist	Intermediate	7310
Mythimna ferrago	5.51	5.65	3.97	4.01	4.21	3.82	Generalist	High	22243
Mythimna impura	4.32	5.65	3.47	4.52	4.54	4.49	Generalist	High	33956
Mythimna l-album	5.65	5.65	5.65	3.96	4.38	3.37	Generalist	High	3014
Mythimna pallens	5.6	5.65	5.61	3.49	3.62	3.38		High	34117
Mythimna straminea	5.63	5.65	5.65	4.48	4.57	4.35	Generalist	High	2209
Mythimna turca	3.89	5.65	1.05	4.53	4.59	4.36	Generalist	Intermediate	1667
Naenia typica	5.61	5.65	4.95	4.05	4.27	3.8	Generalist	High	2413
Noctua comes	5.65	5.65	5.65	4.15	4.24	4.04	Generalist	High	34057
Noctua fimbriata	5.35	5.65	4.57	4.26	4.39	4.09	Generalist	High	21017
Noctua interjecta	5.64	5.65	5.65	4.46	4.52	4.36	Generalist	High	7122
Noctua janthe	5.6	5.65	4.85	4.38	4.44	4.32	Generalist	High	34000
Noctua pronuba	5.65	5.65	5.65	4.1	4.2	3.99	Generalist	High	33434
Nonagria typhae	5.65	5.65	5.65	4.41	4.52	4.25	Generalist	High	1763
Ochropleura plecta	5.65	5.65	5.65	3.86	4	3.71	Generalist	High	33723
Odezia atrata	2.95	3.2	2.72	4.47	4.52	4.39	Generalist	Intermediate	2479
Odontopera bidentata	5.64	5.65	5.65	4.35	4.44	4.25	Generalist	High	12602
Oligia fasciuncula	5.31	5.65	4.53	4.47	4.5	4.43	Generalist	High	18189

Taxon	Optimum	Upper Optimum	Lower Optimum	Tolerance	Tolerance Upper	Tolerance Lower	Model Tolerance	Model Optimum	Presences
Oligia latruncula	4.07	4.24	3.85	4.01	4.41	3.55	Generalist	Intermediate	2393
Oligia strigilis	4.63	5.65	4.35	3.75	4.06	3.45	Generalist	High	4311
Oligia versicolor	1.05	1.05	1.05	4.48	4.53	4.4	Generalist	Low	1887
Operophtera brumata	4.25	5.65	3.57	3.48	3.93	3.11		High	10519
Orgyia antiqua	5.65	5.65	5.65	4.28	4.43	4.1	Generalist	High	3242
Orthonama vittata	5.2	5.65	4.17	3.16	3.76	2.47		High	1393
Orthosia gothica	4.54	5.65	4.32	4.19	4.28	4.1	Generalist	High	34083
Orthosia gracilis	5.57	5.65	4.63	4.11	4.29	3.9	Generalist	High	8411
Orthosia incerta	4.78	5.65	4.38	4.34	4.41	4.25	Generalist	High	34158
Ourapteryx sambucaria	5.64	5.65	5.65	3.81	4.04	3.63	Generalist	High	15947
Papestra biren	2.3	2.68	1.05	4.14	4.4	3.75	Generalist	Low	658
Parasemia plantaginis	1.05	1.05	1.05	3.33	3.81	2.8		Low	270
Perconia strigillaria	1.07	1.05	1.05	4	4.36	3.52	Generalist	Low	273
Peridroma saucia	5.65	5.65	5.65	3.56	4.19	2.94	Generalist	High	793
Perizoma affinitata	5.58	5.65	4.57	3.34	3.6	3.11		High	5625
Perizoma albulata	4.79	5.65	4.3	3.87	4.16	3.55	Generalist	High	2441
Perizoma alchemillata	4.46	5.65	3.71	4.39	4.48	4.28	Generalist	High	9459
Perizoma bifaciata	5.59	5.65	5.65	4.48	4.58	4.29	Generalist	High	526
Perizoma flavofasciata	5.65	5.65	5.65	3.32	3.49	3.14		High	6645
Petrophora chlorosata	3.27	3.44	3.12	4.43	4.48	4.35	Generalist	Intermediate	13135
Phibalapteryx virgata	1.09	1.05	1.05	2.65	3.91	1.73		Low	295
Phlogophora meticulosa	5.64	5.65	5.65	3.21	3.35	3.07		High	29764
Photedes minima	2.99	3.75	1.05	4.57	4.59	4.56	Generalist	Intermediate	5891
Phragmatobia fuliginosa	4.8	5.65	4.47	4.52	4.55	4.49	Generalist	High	20592
Phymatopus hecta	3.31	5.65	2.87	4.32	4.48	4.09	Generalist	Intermediate	920
Phytometra viridaria	1.05	1.05	1.05	2.71	3.31	2.14		Low	482
Plusia festucae	5.65	5.65	5.65	4.36	4.44	4.27	Generalist	High	11733
Plusia putnami	3.77	5.65	3.07	4.54	4.58	4.48	Generalist	Intermediate	1568
Polia nebulosa	5	5.65	3.92	4.53	4.57	4.48	Generalist	High	3909
Polychrysia moneta	4.86	5.65	4.1	3.58	4.42	2.77	Generalist	High	198
Polymixis flavicincta	5.64	5.65	5.65	4.45	4.56	4.28	Generalist	High	1756
Polymixis lichenea	5.38	5.65	4.57	1.92	2.33	1.4	Specialist	High	2868
Protodeltote pygarga	3.66	4.06	3.33	4.45	4.56	4.25	Generalist	Intermediate	6939
Pseudopanthera macularia	3.76	4.11	3.35	4.03	4.33	3.66	Generalist	Intermediate	1784
Pyrrhia umbra	4.91	5.65	3.59	3.05	3.48	2.71		High	614
Rheumaptera undulata	1.06	1.05	1.05	4.49	4.56	4.42	Generalist	Low	1489
Rhizedra lutosa	4.78	5.65	3.75	3.54	3.84	3.3	Generalist	High	5047
Rhodometra sacraria	5.65	5.65	5.65	4.2	4.45	3.82	Generalist	High	2303
Rivula sericealis	5.6	5.65	4.75	4.14	4.26	4	Generalist	High	33917
Rusina ferruginea	3.16	3.28	3.06	4.33	4.42	4.21	Generalist	Intermediate	13155
Saturnia pavonia	1.72	2.31	1.05	4.07	4.21	3.88	Generalist	Low	2092
Scopula floslactata	4.19	4.75	3.81	4.27	4.47	4.03	Generalist	High	1934
Scopula ternata	2.54	5.65	2	4.04	4.35	3.58	Generalist	Low	468
Scotopteryx chenopodiata	5.19	5.65	3.85	3.83	4	3.67	Generalist	High	10367
Scotopteryx luridata	3.95	4.25	3.6	3.71	4.29	3.05	Generalist	Intermediate	587
Sideridis rivularis	5.08	5.65	4.07	3.8	4.06	3.52	Generalist	High	2967
Sideridis turbida	5.61	5.65	5.65	3.12	4.25	2.23		High	113
Simyra albovenosa	5.65	5.65	5.65	3.93	4.42	3.22	Generalist	High	534
Sphinx ligustri	5.65	5.65	5.65	4.51	4.56	4.45	Generalist	High	9818
Spilosoma lubricipeda	5.3	5.65	4.3	4.12	4.22	4.02	Generalist	High	33961

Taxon	Optimum	Upper Optimum	Lower Optimum	Tolerance	Tolerance Upper	Tolerance Lower	Model Tolerance	Model Optimum	Presences
Spilosoma lutea	5.63	5.65	5.24	4.13	4.24	4.01	Generalist	High	33995
Spilosoma urticae	5.61	5.65	5.65	3.57	4.45	2.61	Generalist	High	273
Stilbia anomala	1.05	1.05	1.05	4.06	4.41	3.53	Generalist	Low	573
Syngrapha interrogationis	1.05	1.05	1.05	3.67	4.17	3.06	Generalist	Low	447
Thalpophila matura	5.13	5.65	4.5	4.48	4.57	4.29	Generalist	High	9203
Tholera decimalis	4.19	5.65	3.73	3.81	4.28	3.45	Generalist	High	4243
Thyatira batis	4.27	4.57	4.06	4.4	4.5	4.28	Generalist	High	7712
Timandra comae	5.3	5.65	4.28	3.26	3.46	3.1		High	21339
Triodia sylvina	4.88	5.65	4.35	4.43	4.51	4.32	Generalist	High	10621
Tyria jacobaeae	5.37	5.65	4.03	4.22	4.35	4.07	Generalist	High	18611
Xanthia togata	3.53	5.65	3.16	4.33	4.44	4.22	Generalist	Intermediate	8511
Xanthorhoe decoloraria	1.05	1.05	1.05	3.79	4.21	3.31	Generalist	Low	361
Xanthorhoe designata	5.65	5.65	5.65	3.43	3.61	3.25		High	20342
Xanthorhoe ferrugata	4.43	5.65	3.46	3.48	3.72	3.21		High	6040
Xanthorhoe fluctuata	5.65	5.65	5.65	2.43	2.52	2.35		High	32613
Xanthorhoe montanata	5.46	5.65	3.53	4.34	4.41	4.27	Generalist	High	27860
Xestia agathina	2.9	5.65	2.51	3.91	4.26	3.47	Generalist	Intermediate	905
Xestia baja	5.11	5.65	3.56	4.52	4.55	4.48	Generalist	High	9509
Xestia c-nigrum	5.65	5.65	5.65	3.43	3.61	3.28		High	34053
Xestia castanea	2.83	3.04	2.62	4.03	4.38	3.55	Generalist	Intermediate	760
Xestia ditrapezium	3.84	4.07	3.59	4.38	4.49	4.22	Generalist	Intermediate	3567
Xestia sexstrigata	4.05	4.29	3.76	4.43	4.49	4.34	Generalist	Intermediate	13764
Xestia triangulum	5.08	5.65	4.54	4.2	4.29	4.1	Generalist	High	32390
Xestia xanthographa	5.65	5.65	5.65	4.37	4.42	4.32	Generalist	High	34089
Xylena solidaginis	1.05	1.05	1.05	3.98	4.49	3.14	Generalist	Low	204
Xylena vetusta	3.78	5.65	3.27	3.17	3.62	2.77		Intermediate	1828
Zygaena filipendulae	5.21	5.65	4	3.71	3.9	3.52	Generalist	High	5558
Zygaena lonicerae	4.2	5.65	3.42	3.72	4.23	3.35	Generalist	High	1567
Zygaena trifolii	1.05	1.05	1.05	3.85	4.18	3.42	Generalist	Low	584

3106 **Table S7:** *Indices for moths derived from non-spatially under-sampled data.*

Taxon	*Optimum*	*Upper Optimum*	*Lower Optimum*	*Tolerance*	*Tolerance Upper*	*Tolerance Lower*	*Model Tolerance*	*Model Optimum*	*Presences*
Abraxas grossulariata	5.65	5.65	5.65	4.33	4.43	4.21	Generalist	High	10448
Abrostola tripartita	5.2	5.65	4.41	4.31	4.42	4.18	Generalist	High	34086
Abrostola triplasia	5.64	5.65	5.65	3.52	4.18	2.95	Generalist	High	3915
Acronicta menyanthidis	1.05	1.05	1.05	2.99	3.71	2.2		Low	388
Acronicta rumicis	1.07	1.05	1.05	4.22	4.44	4.02	Generalist	Low	11674
Agriopis marginaria	1.15	2.61	1.05	4.52	4.58	4.48	Generalist	Low	9359
Agrochola lychnidis	4.03	4.33	3.73	4.55	4.58	4.48	Generalist	Intermediate	17597
Agrotis clavis	5.37	5.65	4.61	4.51	4.56	4.44	Generalist	High	14071
Agrotis exclamationis	5.65	5.65	5.65	3.88	4.09	3.7	Generalist	High	33973
Agrotis puta	5.65	5.65	5.65	2.36	2.49	2.23		High	34201
Agrotis segetum	4.61	5.65	4.44	3.61	3.98	3.28	Generalist	High	19812
Agrotis trux	5.18	5.65	1.05	3.31	4.32	1.93	Generalist	High	235
Alcis repandata	1.13	1.05	1.05	4.56	4.59	4.54	Generalist	Low	23313
Alsophila aescularia	3.96	4.24	1.05	4.48	4.56	4.35	Generalist	Intermediate	10774
Amphipoea crinanensis	1.05	1.05	1.05	3.55	4.19	2.87	Generalist	Low	123
Amphipoea fucosa	5.61	5.65	5.65	1.58	2.96	0.84	Specialist	High	172
Amphipoea lucens	1.71	2.86	1.05	4.15	4.49	3.64	Generalist	Low	551
Amphipyra tragopoginis	3.48	5.65	3.33	3.86	4.08	3.65	Generalist	Intermediate	11638
Anarta myrtilli	1.05	1.05	1.05	3.62	4.02	3.15	Generalist	Low	403
Anarta trifolii	5.22	5.65	4.08	3.67	4.31	3.16	Generalist	High	8678
Anchoscelis helvola	1.05	1.05	1.05	4.46	4.53	4.35	Generalist	Low	1118
Anchoscelis litura	3.44	3.67	3.19	4.16	4.43	3.82	Generalist	Intermediate	8257
Anchoscelis lunosa	4.77	5.65	4.08	4.44	4.54	4.28	Generalist	High	24398
Apamea anceps	1.06	1.05	1.05	4.55	4.57	4.54	Generalist	Low	4131
Apamea crenata	1.05	1.05	1.05	4.52	4.55	4.49	Generalist	Low	12995
Apamea epomidion	5.57	5.65	5.65	4.52	4.58	4.43	Generalist	High	2345
Apamea furva	1.12	1.05	1.05	3.52	4.37	2.62	Generalist	Low	174
Apamea lithoxylaea	4.82	5.65	3.96	4.36	4.49	4.2	Generalist	High	12705
Apamea monoglypha	5.65	5.65	5.65	4.49	4.52	4.44	Generalist	High	33799
Apamea scolopacina	2.45	4.12	1.05	4.51	4.56	4.43	Generalist	Low	5095
Aplasta ononaria	1.07	1.05	1.05	4.07	4.4	3.61	Generalist	Low	149
Arctia caja	5.5	5.65	1.05	4.25	4.41	4.04	Generalist	High	8751
Arenostola phragmitidis	5.64	5.65	5.65	4.48	4.56	4.35	Generalist	High	1818
Autographa bractea	1.06	1.05	1.05	4.53	4.57	4.48	Generalist	Low	1787
Autographa gamma	4	4.27	3.7	4.2	4.32	4.07	Generalist	Intermediate	33501
Autographa jota	4.79	5.65	3.96	4.42	4.52	4.28	Generalist	High	8672
Autographa pulchrina	2.02	3.68	1.05	4.54	4.57	4.48	Generalist	Low	11634
Axylia putris	5.58	5.65	4.84	4.37	4.46	4.25	Generalist	High	34094
Bembecia ichneumoniformis	1.08	1.05	1.05	4.05	4.4	3.64	Generalist	Low	390

Taxon	Optimum	Upper Optimum	Lower Optimum	Tolerance	Tolerance Upper	Tolerance Lower	Model Tolerance	Model Optimum	Presences
Biston betularia	2.09	4.51	1.05	4.48	4.54	4.4	Generalist	Low	28437
Callistege mi	1.05	1.05	1.05	4.35	4.44	4.25	Generalist	Low	1929
Camptogramma bilineata	5.6	5.65	5.65	4.45	4.56	4.33	Generalist	High	16784
Caradrina morpheus	5.65	5.65	5.65	3.54	3.75	3.37	Generalist	High	23596
Catarhoe cuculata	4.06	4.35	3.76	2.43	3.55	1.78		Intermediate	369
Catarhoe rubidata	5.57	5.65	5.65	2.9	4.32	1.87		High	298
Celaena haworthii	1.05	1.05	1.05	4.09	4.33	3.8	Generalist	Low	693
Cepphis advenaria	1.06	1.05	1.05	3.5	4.36	2.4	Generalist	Low	340
Ceramica pisi	1.05	1.05	1.05	4.22	4.35	4.06	Generalist	Low	5130
Cerapteryx graminis	1.05	1.05	1.05	4.52	4.54	4.49	Generalist	Low	10338
Cerastis leucographa	2.57	4.17	1.05	4.5	4.57	4.33	Generalist	Low	789
Cerastis rubricosa	1.05	1.05	1.05	4.06	4.2	3.89	Generalist	Low	7423
Charanyca trigrammica	5.18	5.65	4.23	4.5	4.56	4.4	Generalist	High	21395
Chiasmia clathrata	1.05	1.05	1.05	4.2	4.36	4.04	Generalist	Low	4462
Chilodes maritima	5.61	5.65	5.65	2.7	3.2	2.32		High	1007
Chloroclystis v-ata	4.38	4.61	4.15	4.17	4.39	3.95	Generalist	High	12391
Coenotephria salicata	1.05	1.05	1.05	1.36	1.89	0.99	Specialist	Low	469
Colostygia multistrigaria	1.05	1.05	1.05	4.45	4.53	4.33	Generalist	Low	3289
Colostygia pectinataria	1.05	1.05	1.05	2.82	3.11	2.57		Low	33903
Conistra ligula	3.9	4.28	3.63	2.84	3.41	2.48		Intermediate	5331
Cosmorhoe ocellata	1.05	1.05	1.05	2.89	3.15	2.64		Low	7691
Crocallis elinguaria	5.65	5.65	5.65	4.46	4.5	4.41	Generalist	High	20749
Cucullia lychnitis	1.12	1.05	1.05	2.32	4.03	1.42		Low	200
Cucullia umbratica	5.51	5.65	4.58	4.13	4.39	3.79	Generalist	High	1947
Cyclophora puppillaria	5.64	5.65	5.65	4.06	4.51	3.32	Generalist	High	462
Dasypolia templi	2.5	5.65	1.05	1.75	2.85	0.87	Specialist	Low	398
Deilephila elpenor	5.5	5.65	4.51	4.34	4.44	4.23	Generalist	High	34008
Deilephila porcellus	1.07	1.05	1.05	4.39	4.53	4.25	Generalist	Low	6272
Denticucullus pygmina	1.19	2.33	1.05	4.3	4.42	4.14	Generalist	Low	6335
Diachrysia chrysitis	3.54	4.36	1.05	4.51	4.57	4.43	Generalist	Intermediate	28169
Diacrisia sannio	1.05	1.05	1.05	4.04	4.33	3.65	Generalist	Low	809
Diaphora mendica	4.47	5.65	4.09	4.3	4.47	4.08	Generalist	High	14602
Diarsia dahlii	1.05	1.05	1.05	4.38	4.47	4.24	Generalist	Low	1603
Diarsia mendica	1.05	1.05	1.05	4.48	4.5	4.45	Generalist	Low	19723
Diarsia rubi	5.59	5.65	4.78	4.12	4.37	3.88	Generalist	High	34034
Dicallomera fascelina	1.05	1.05	1.05	0.62	1.95	0.28	Specialist	Low	375
Dyscia fagaria	1.06	1.05	1.05	3.03	4.18	1.93		Low	211
Dysstroma citrata	1.05	1.05	1.05	4.48	4.53	4.42	Generalist	Low	4673
Dysstroma truncata	5.65	5.65	5.65	3.38	3.58	3.19	Generalist	High	34011
Ecliptopera silaceata	1.12	1.12	1.05	3.81	4.32	3.38	Generalist	Low	21797
Elaphria venustula	1.08	1.05	1.05	4.48	4.57	4.28	Generalist	Low	183
Ematurga atomaria	1.05	1.05	1.05	3.94	4.11	3.73	Generalist	Low	3253

Taxon	Optimum	Upper Optimum	Lower Optimum	Tolerance	Tolerance Upper	Tolerance Lower	Model Tolerance	Model Optimum	Presences
Entephria caesiata	1.18	1.89	1.05	1.99	2.47	1.49		Low	373
Epirrhoe alternata	1.05	1.05	1.05	4.15	4.34	3.97	Generalist	Low	33751
Epirrhoe tristata	1.05	1.05	1.05	3.12	3.63	2.57		Low	562
Epirrita filigrammaria	1.05	1.05	1.05	3.96	4.32	3.52	Generalist	Low	235
Erannis defoliaria	2.61	4.19	1.05	3.9	4.27	3.2	Generalist	Intermediate	9418
Euclidia glyphica	1.05	1.05	1.05	4.37	4.37	4.37	Generalist	Low	3868
Eugnorisma glareosa	1.05	1.05	1.05	4.44	4.49	4.39	Generalist	Low	5716
Eulithis populata	1.05	1.05	1.05	4.13	4.29	3.93	Generalist	Low	3098
Euphyia biangulata	1.06	1.05	1.05	4.27	4.46	4.05	Generalist	Low	984
Eupithecia absinthiata	4.38	5.65	1.05	4.42	4.52	4.3	Generalist	High	3822
Eupithecia centaureata	5.65	5.65	5.65	2.87	3.04	2.71		High	12427
Eupithecia nanata	1.05	1.05	1.05	4.04	4.22	3.83	Generalist	Low	2254
Eupithecia pulchellata	1.12	1.05	1.05	4.54	4.59	4.51	Generalist	Low	10453
Eupithecia satyrata	1.05	1.05	1.05	3.58	4.04	3.02	Generalist	Low	489
Eupithecia tripunctaria	5.65	5.65	5.65	3.49	3.89	3.12	Generalist	High	4199
Eupithecia venosata	5.58	5.65	3.88	2.75	3.53	2.14		High	202
Eupithecia vulgata	5.65	5.65	5.65	4.35	4.42	4.28	Generalist	High	23736
Euplagia quadripunctaria	5.65	5.65	5.65	2.71	3.44	2.09		High	2548
Euproctis chrysorrhoea	5.57	5.65	5.65	4.54	4.59	4.47	Generalist	High	4036
Euproctis similis	5.65	5.65	5.65	3.21	3.44	3.03		High	15266
Euthrix potatoria	1.06	1.05	1.05	4.26	4.38	4.15	Generalist	Low	15003
Euxoa tritici	5.64	5.65	5.65	4.33	4.53	4.06	Generalist	High	1917
Gandaritis pyraliata	1.05	1.05	1.05	4.45	4.49	4.41	Generalist	Low	17551
Gymnoscelis rufifasciata	5.65	5.65	5.65	3.01	3.26	2.77		High	30460
Hada plebeja	1.05	1.05	1.05	4.07	4.29	3.82	Generalist	Low	5893
Hadena bicruris	5.24	5.65	4.53	3.51	3.78	3.22	Generalist	High	8976
Hadena confusa	5.65	5.65	5.65	2.68	3.67	1.83		High	572
Hadena perplexa	1.05	1.05	1.05	3.01	3.76	2.43		Low	1278
Hecatera bicolorata	5.45	5.65	4.11	3.48	4.03	3.02	Generalist	High	1707
Heliothis peltigera	5.62	5.65	5.65	3.12	4.18	2.19		High	1063
Hemaris tityus	2.31	3.54	1.05	2.83	3.74	1.75		Low	219
Hepialus humuli	4.88	5.65	3.56	4.1	4.29	3.89	Generalist	High	8650
Hoplodrina ambigua	5.65	5.65	5.65	2.25	2.49	2		High	26649
Hoplodrina blanda	5.63	5.65	5.65	4.06	4.29	3.82	Generalist	High	20590
Hoplodrina octogenaria	5.65	5.65	5.65	3.92	4.14	3.72	Generalist	High	34171
Horisme tersata	1.06	1.05	1.05	4.49	4.55	4.4	Generalist	Low	1911
Hydriomena furcata	2.88	3.19	2.12	4.47	4.51	4.41	Generalist	Intermediate	21193
Hydriomena ruberata	1.09	1.05	1.05	3.77	4.47	2.77	Generalist	Low	239
Hyppa rectilinea	1.06	1.05	1.05	3.89	4.44	3.12	Generalist	Low	219
Idaea aversata	5.65	5.65	5.65	4.48	4.52	4.42	Generalist	High	33961
Idaea dimidiata	5.64	5.65	5.65	3.46	3.63	3.32	Generalist	High	27196
Idaea rusticata	5.65	5.65	5.65	4.56	4.57	4.53	Generalist	High	8792

Taxon	Optimum	Upper Optimum	Lower Optimum	Tolerance	Tolerance Upper	Tolerance Lower	Model Tolerance	Model Optimum	Presences
Korscheltellus fusconebulosa	1.05	1.05	1.05	4.49	4.53	4.44	Generalist	Low	5649
Korscheltellus lupulina	5.65	5.65	5.65	3.86	4.13	3.61	Generalist	High	18149
Lacanobia oleracea	5.65	5.65	5.65	4.41	4.45	4.36	Generalist	High	34091
Lacanobia suasa	5.64	5.65	5.65	2.69	3.82	1.82		High	1619
Lacanobia thalassina	1.05	1.05	1.05	4.54	4.58	4.51	Generalist	Low	7955
Lampropteryx otregiata	1.07	1.05	1.05	2.51	3.15	2.02		Low	1255
Lampropteryx suffumata	1.05	1.05	1.05	4.28	4.37	4.19	Generalist	Low	6034
Lasiocampa quercus	1.05	1.05	1.05	3.63	3.82	3.42	Generalist	Low	3105
Lateroligia ophiogramma	5.63	5.65	5.65	4.5	4.58	4.39	Generalist	High	2839
Leptologia macilenta	1.05	1.05	1.05	4.51	4.54	4.47	Generalist	Low	15055
Leucania comma	5.65	5.65	5.65	4.46	4.51	4.39	Generalist	High	11205
Leucania obsoleta	5.62	5.65	5.65	3.02	3.68	2.65		High	962
Litoligia literosa	5.53	5.65	4.56	4.03	4.35	3.61	Generalist	High	2918
Luperina testacea	5.64	5.65	5.65	4.39	4.48	4.27	Generalist	High	27209
Lycophotia porphyrea	1.05	1.05	1.05	4.36	4.42	4.3	Generalist	Low	8337
Macroglossum stellatarum	4.26	4.51	3.99	4.17	4.4	3.94	Generalist	High	10888
Macrothylacia rubi	1.05	1.05	1.05	2.3	2.58	2.03		Low	2563
Mamestra brassicae	5.59	5.65	4.77	3.42	3.77	3.12	Generalist	High	9589
Melanchra persicariae	4.9	5.65	4.47	4.44	4.54	4.28	Generalist	High	12416
Mesotype didymata	1.05	1.05	1.05	4.39	4.45	4.3	Generalist	Low	2453
Minoa murinata	1.06	1.05	1.05	3.2	3.93	2.45		Low	258
Mniotype adusta	1.54	2.32	1.05	4.33	4.45	4.16	Generalist	Low	1378
Mormo maura	5.64	5.65	5.65	3.5	3.94	3.2	Generalist	High	4505
Mythimna conigera	3.3	3.88	1.05	4.55	4.58	4.5	Generalist	Intermediate	7310
Mythimna ferrago	4.15	5.65	3.55	4.17	4.35	3.99	Generalist	High	22243
Mythimna impura	3	3.2	2.74	4.56	4.57	4.54	Generalist	Intermediate	33956
Mythimna l-album	5.62	5.65	5.65	3.73	4.29	3.07	Generalist	High	3014
Mythimna pallens	3.64	3.73	3.56	3.58	3.76	3.44	Generalist	Intermediate	34117
Mythimna pudorina	5.06	5.65	1.05	1.99	3.27	1.01		High	936
Mythimna straminea	5.64	5.65	5.65	2.88	3.52	2.54		High	2209
Mythimna turca	1.37	3.77	1.05	4.55	4.58	4.48	Generalist	Low	1667
Naenia typica	5.65	5.65	5.65	4.32	4.44	4.17	Generalist	High	2413
Noctua comes	5.65	5.65	5.65	4.46	4.49	4.42	Generalist	High	34057
Noctua fimbriata	4.48	5.65	3.89	4.44	4.53	4.31	Generalist	High	21017
Noctua interjecta	5.65	5.65	5.65	4.49	4.54	4.42	Generalist	High	7122
Noctua janthe	5.39	5.65	4.41	4.49	4.53	4.44	Generalist	High	34000
Noctua pronuba	5.37	5.65	4.66	4.39	4.45	4.31	Generalist	High	33434
Ochropleura plecta	5.64	5.65	5.65	4.43	4.54	4.31	Generalist	High	33723
Odezia atrata	1.05	1.05	1.05	4.41	4.46	4.34	Generalist	Low	2479
Oligia latruncula	3.69	4.16	1.05	4.08	4.46	3.55	Generalist	Intermediate	2393
Oligia strigilis	4.99	5.65	3.52	4.06	4.41	3.69	Generalist	High	4311
Oligia versicolor	1.05	1.05	1.05	4.33	4.45	4.16	Generalist	Low	1887

Taxon	Optimum	Upper Optimum	Lower Optimum	Tolerance	Tolerance Upper	Tolerance Lower	Model Tolerance	Model Optimum	Presences
Operophtera brumata	1.05	1.05	1.05	2.89	3.54	2.41		Low	10519
Orthosia gothica	3.95	4.31	1.05	4.56	4.58	4.53	Generalist	Intermediate	34083
Orthosia incerta	4.2	4.43	3.92	4.53	4.56	4.48	Generalist	High	34158
Orthosia opima	1.55	5.65	1.05	3.58	4.22	2.76	Generalist	Low	204
Ourapteryx sambucaria	5.64	5.65	5.65	4.07	4.3	3.83	Generalist	High	15947
Pachycnemia hippocastanaria	1.06	1.05	1.05	4.35	4.51	4.08	Generalist	Low	356
Panemeria tenebrata	5.1	5.65	1.05	4.11	4.48	3.42	Generalist	High	998
Papestra biren	1.05	1.05	1.05	4.04	4.36	3.59	Generalist	Low	658
Parasemia plantaginis	1.05	1.05	1.05	3.37	3.89	2.82	Generalist	Low	270
Pelurga comitata	5.63	5.65	5.65	3.63	4.41	2.76	Generalist	High	351
Perconia strigillaria	1.05	1.05	1.05	4.28	4.49	3.92	Generalist	Low	273
Peridroma saucia	5.64	5.65	5.65	3.19	4.01	2.5		High	793
Perizoma affinitata	4.06	4.29	3.81	3.52	3.84	3.26	Generalist	Intermediate	5625
Perizoma alchemillata	2.89	3.75	1.05	4.56	4.58	4.52	Generalist	Intermediate	9459
Perizoma flavofasciata	5.65	5.65	5.65	3.84	4.15	3.55	Generalist	High	6645
Petrophora chlorosata	1.05	1.05	1.05	4.41	4.46	4.37	Generalist	Low	13135
Phibalapteryx virgata	1.07	1.05	1.05	1.61	3.03	0.86	Specialist	Low	295
Phlogophora meticulosa	5.03	5.65	4.45	4.02	4.2	3.84	Generalist	High	29764
Photedes minima	1.05	1.05	1.05	4.5	4.53	4.46	Generalist	Low	5891
Phragmatobia fuliginosa	1.05	1.05	1.05	4.34	4.4	4.27	Generalist	Low	20592
Phymatopus hecta	1.06	1.05	1.05	4.26	4.42	4.06	Generalist	Low	920
Phytometra viridaria	1.05	1.05	1.05	2.28	2.87	1.74		Low	482
Plusia festucae	5.65	5.65	5.65	4.5	4.55	4.44	Generalist	High	11733
Plusia putnami	3.07	3.54	1.96	4.44	4.54	4.3	Generalist	Intermediate	1568
Polymixis lichenea	5.64	5.65	5.65	2.65	3.87	1.83		High	2868
Protodeltote pygarga	1.05	1.05	1.05	4.5	4.53	4.47	Generalist	Low	6939
Pseudopanthera macularia	2.72	3.71	1.05	4.46	4.54	4.32	Generalist	Intermediate	1784
Rheumaptera undulata	1.05	1.05	1.05	4.37	4.48	4.22	Generalist	Low	1489
Rhizedra lutosa	5.04	5.65	3.6	3.58	4.06	3.26	Generalist	High	5047
Rusina ferruginea	2.67	2.91	2.25	4.4	4.47	4.32	Generalist	Intermediate	13155
Saturnia pavonia	1.05	1.05	1.05	3.73	3.91	3.48	Generalist	Low	2092
Scopula ternata	1.05	1.05	1.05	3.74	4.19	3.15	Generalist	Low	468
Scotopteryx chenopodiata	4.5	5.65	3.46	4.24	4.41	4.05	Generalist	High	10367
Scotopteryx luridata	1.11	1.05	1.05	4.19	4.52	3.72	Generalist	Low	587
Scotopteryx mucronata	1.05	1.05	1.05	4.22	4.46	3.89	Generalist	Low	298
Sideridis rivularis	4.87	5.65	3.78	4.24	4.5	3.95	Generalist	High	2967
Sideridis turbida	5.55	5.65	5.65	3.73	4.54	2.5	Generalist	High	113
Simyra albovenosa	5.65	5.65	5.65	2.07	3.13	1.29		High	534
Sphinx ligustri	5.61	5.65	5.65	4.43	4.56	4.29	Generalist	High	9818
Spilosoma lubricipeda	2.75	3.78	1.05	4.54	4.57	4.49	Generalist	Intermediate	33961
Spilosoma lutea	5.44	5.65	4.64	4.38	4.46	4.29	Generalist	High	33995
Standfussiana lucernea	1.06	1.05	1.05	3.17	4.21	2.06		Low	100

Taxon	Optimum	Upper Optimum	Lower Optimum	Tolerance	Tolerance Upper	Tolerance Lower	Model Tolerance	Model Optimum	Presences
Stilbia anomala	1.05	1.05	1.05	4.17	4.48	3.62	Generalist	Low	573
Syngrapha interrogationis	1.05	1.05	1.05	3.88	4.32	3.24	Generalist	Low	447
Thalpophila matura	5.47	5.65	3.7	3.71	4.11	3.42	Generalist	High	9203
Tholera cespitis	1.08	1.05	1.05	3.81	4.21	3.38	Generalist	Low	1604
Tholera decimalis	3.99	4.42	1.05	4.4	4.56	4.09	Generalist	Intermediate	4243
Timandra comae	4.1	4.29	3.91	3.69	4.03	3.41	Generalist	Intermediate	21339
Triodia sylvina	5.65	5.65	5.65	4.52	4.56	4.47	Generalist	High	10621
Tyria jacobaeae	2.68	3.2	1.05	4.48	4.53	4.39	Generalist	Intermediate	18611
Xanthia togata	1.05	1.05	1.05	4.5	4.54	4.47	Generalist	Low	8511
Xanthorhoe decoloraria	1.05	1.05	1.05	2.76	3.26	2.26		Low	361
Xanthorhoe ferrugata	3.41	3.8	3.08	3.9	4.28	3.57	Generalist	Intermediate	6040
Xanthorhoe fluctuata	5.65	5.65	5.65	2.81	2.94	2.68		High	32613
Xanthorhoe montanata	1.05	1.05	1.05	4.47	4.5	4.44	Generalist	Low	27860
Xestia agathina	1.05	1.05	1.05	3.51	3.96	2.99	Generalist	Low	905
Xestia baja	1.12	1.05	1.05	4.58	4.59	4.56	Generalist	Low	9509
Xestia c-nigrum	5.43	5.65	3.98	3.72	3.95	3.5	Generalist	High	34053
Xestia castanea	1.05	1.05	1.05	4.27	4.49	3.91	Generalist	Low	760
Xestia ditrapezium	3.83	4.31	1.05	4.55	4.58	4.49	Generalist	Intermediate	3567
Xestia sexstrigata	3.21	3.71	1.05	4.55	4.57	4.51	Generalist	Intermediate	13764
Xestia triangulum	4.37	4.5	4.26	4.45	4.51	4.39	Generalist	High	32390
Xestia xanthographa	5.64	5.65	5.65	4.52	4.55	4.49	Generalist	High	34089
Xylena solidaginis	1.05	1.05	1.05	3.35	4.03	2.62	Generalist	Low	204
Xylena vetusta	2.22	3.57	1.05	3.49	4.02	2.55	Generalist	Low	1828
Zygaena filipendulae	3.42	3.8	3.1	4.31	4.47	4.12	Generalist	Intermediate	5558
Zygaena trifolii	1.05	1.05	1.05	2.2	2.85	1.65		Low	584

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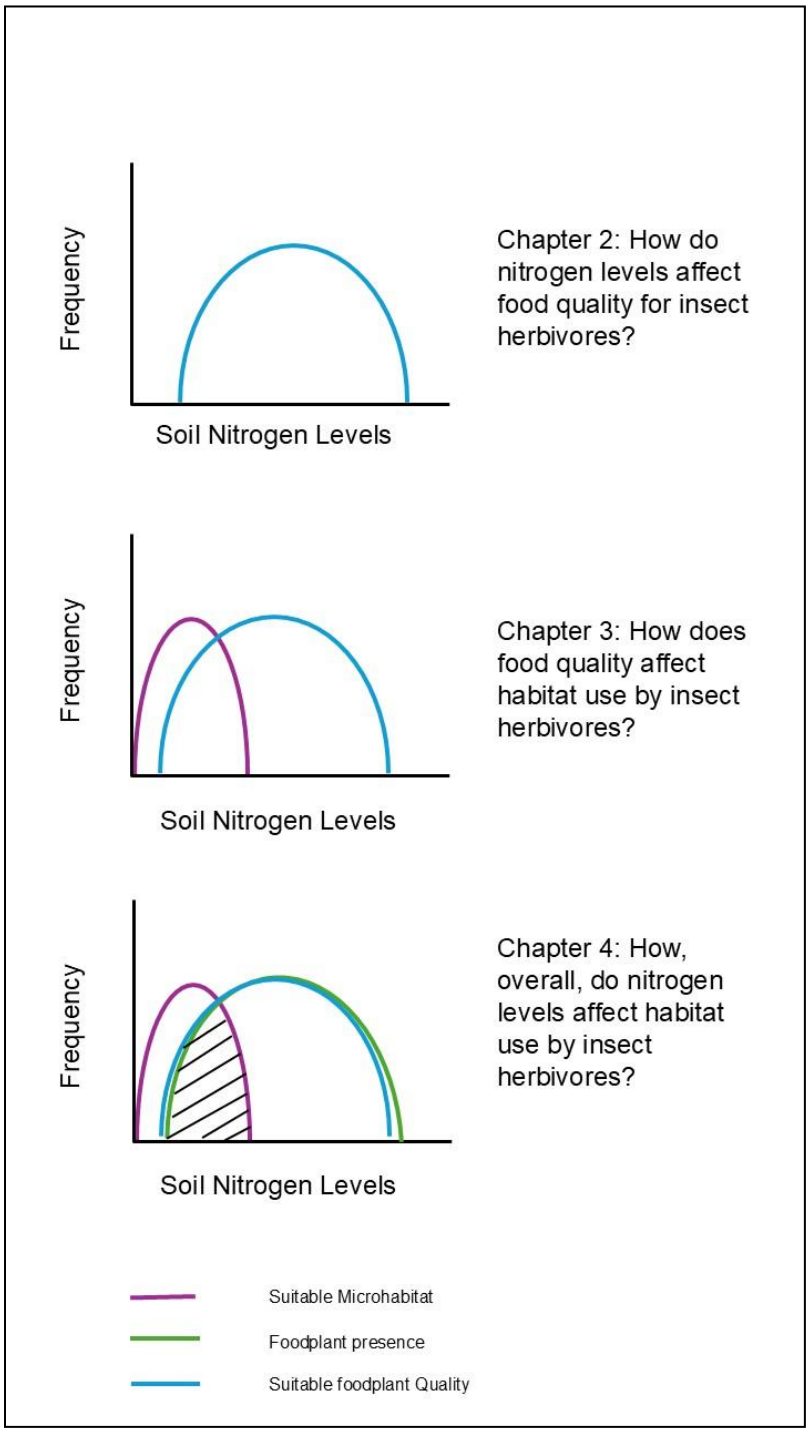
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Chapter 5: General Discussion

The effects of rising levels of nitrogen pollution on a given insect species ultimately depend on how the availability of its habitat is constrained by levels of soil nitrogen. These constraints arise via the effects of soil nitrogen levels on multiple aspects of habitat quality – the availability of foodplants (via the impacts of soil nitrogen on the outcome of competition structuring plant communities), the availability of sufficiently high quality foodplants (via the impacts of soil nitrogen on plant nitrogen content), and the availability of suitably warm microclimates (via the impacts of soil nitrogen on plant productivity and vegetation structure). In this thesis I aimed to develop understanding of how different insect species are affected by nitrogen pollution by contributing to the understanding of the structure of and interaction between these constraints (Figure 1).



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3123 **Figure 1:** *Schematic visualising how the data chapters of this thesis address questions relating to how*

3124 *nitrogen levels affect habitat quality for insects.*

How do nitrogen levels affect food quality for insect herbivores?

My first data chapter investigated how nitrogen levels constrain food quality for insects. It showed that the impact of rising plant nitrogen content on insect performance is unimodal, with nitrogen limitation under normal conditions alleviated by rising plant nitrogen content up to a point, after which performance declines, likely because further nitrogen additions induce or exacerbate limitations of another nutrient. My results provide some evidence that this other nutrient is phosphorus. The foodplants of two herbivore species (*C. pamphilus* and *L. phlaeas*) showed declining N:P ratios with increasing nitrogen addition, and when tested separately, these nitrogen levels induced phosphorus deficiency in one of these species (*L. phlaeas*). On the other hand, the nitrogen-fixing *L. corniculatus* showed a declining nitrogen content and a decreasing N:P ratio with increased N addition (in agreement with previous work), and it may be that the reverse trend was driving the response of *P. icarus*.

I also aimed to test the hypothesis that these unimodal responses are driven by species-specific requirements for nitrogen and phosphorus, by comparing the chemistry of plants on which the three tested herbivore species' performance peaked, and that these requirements arise from the interaction between adult and foodplant stoichiometry by comparing the chemistry of these plants to that of the foodplants in general, and the adult butterflies. Unfortunately, I was not able to do the latter, as analysis of adult chemistry was not completed in time, and while I did find differences in the chemistry of the plants on which species' performance was maximised, my performance curves are probably not well-resolved enough to suggest that these represent differences in species' requirements, in spite of the large sample of larvae in my experiments. Thus, overall, this chapter provides support for the application of insect nutritional ecology to the problems of changing food quality under nitrogen pollution, and that the effects of nitrogen pollution will be dose-dependent, and context-dependent (dependent on the levels of other nutrients, and how foodplants respond to increased soil nitrogen

levels). This adds to the growing body of work showing the effects of nitrogen pollution on food quality for insect herbivores are as much dependent on the levels of other nutrients as on levels of nitrogen and emphasises the importance of considering these nutrients in future work.

While I did not find strong support for species-dependent effects, a promising approach for resolving these in the future may come from my new method for analysing differences in frass and foodplant chemistry. This provides an intuitive way of understanding the relative deficiency of different pairs of nutrients in food for herbivores. I was able to use this to resolve optimal food C:N and N:P ratios for *L. phlaeas* that were a close match with theory using a relatively small number of frass samples, and this approach could be used for almost any pair of nutrients present in detectable levels in frass, to build a species' 'nutritional niche'. This could therefore provide a powerful method for adding to understanding of interspecific variation in insect nutritional requirements, which remains relatively poor, and will underpin any re-structuring of insect communities via changes in host nutritional quality under nitrogen pollution. Pairing this approach with successful independent and combined manipulation of nitrogen and phosphorus would enable future studies to resolve whether phosphorus limitation drives declines in insect performance at high nitrogen levels, enabling work assessing how foodplant nitrogen and phosphorus affect insect performance for the first time to measure the nutrient deficiencies that drive the relationship between host quality and performance.

The decline in insect performance at high levels of plant nitrogen content in my experiments is a striking result but was only achieved at very high levels of nitrogen addition. My nitrogen doses were however only applied for a short period and studies along gradients of chronic deposition show that much lower doses can lead to similar increases in plant nitrogen content. Over my gradient of nitrogen addition (0 to 350kg N ha^{-1} yr^{-1}), nitrogen content increased 1.63 times for *P. pratensis* and 2.41 times for *R. acetosa*, declining 0.82 times for *L. corniculatus*. By comparison, Hicks et al. (2000) found increases in nitrogen content of between 1.4 and 2.3 times for upland plants along a gradient of 4 to 14 kg N ha-1 yr-1 of atmospheric nitrogen deposition in the UK, and Roth et al. (2021) and Pitcairn et al.

(1995) report similar shifts along gradients of between 14 and 22 kg N ha^{-1} yr^{-1} and 5 to 35 kg N ha^{-1} yr^{-1}, respectively.

Thus, shifts in nitrogen content of the kind I observed appear entirely possible under relatively low levels of atmospheric deposition, and they may lead to more severe negative impacts on herbivore performance in systems where plant nitrogen content increases more and phosphorus levels are lower than in our treatments. For example, on weakly-buffered heathland soils, stronger increases in plant nitrogen content under simulated deposition appear to be the norm (Phoenix et al., 2012) and acidification caused by nitrogen deposition can also result in the release of metal ions that immobilize phosphorus, thus simultaneously increasing nitrogen availability and decreasing phosphorus availability (Vogels et al., 2017).

My experiment necessarily restricted larval food choice, and in the field larvae may be able to avoid or buffer potential impacts of increased plant nitrogen content by feeding on different plant individuals, host plants or plant parts, although selective feeding to maintain phosphorus intake has rarely been reported insects (Harrison et al., 2014), but has also rarely been examined. Similarly, oviposition by female butterflies could take into account host quality and avoid plants where larval performance will be lower. Equally, because my results suggest that under normal conditions insects will often be nitrogen-limited (at least those not feeding on nitrogen-fixing hostplants), it seems unlikely there will have been much selection against laying on high-nitrogen host plants, indeed the opposite may be more likely, raising the intriguing question of whether nitrogen-rich hosts under pollution could represent an ecological trap for insect herbivores. Further work exploring how female egg-laying choices, and larval food choices are able to buffer the impacts of excessive nitrogen would therefore be interesting.

When considering the effects of changes in host quality under pollution in practice, perhaps the most important thing to consider is the other changes to habitat quality that nitrogen pollution causes

alongside changes in plant chemistry, simultaneously increasing vegetation density and altering plant competition in open habitats, potentially leading to the loss of warm microhabitats around hostplants, or the hostplants themselves (Nijssen et al., 2017; WallisDeVries & van Swaay, 2006; Yang et al., 2017). There is evidence that in northern and western Europe where nitrogen pollution is highest, two of my three study species require warm, open micro-habitats around hostplants for successful larval development - *L. phlaeas* (Streitberger et al., 2014) and *P. icarus* (Habel et al., 2016). Furthermore, the forbs that they feed on may be out-competed by faster-growing species under nitrogen pollution, particularly the nitrogen-fixing *L. corniculatus* which may be expected to lose its competitive advantage under eutrophication (eg. Helsen et al., 2014).

In practice then, although my experiments suggest that both species could experience negative impacts of high levels of nitrogen pollution via host quality, it may be that populations are already lost due to changes in vegetation structure or host plant abundance before such impacts occur. Better understanding of the likely impacts of nitrogen pollution would come from work assessing which of microhabitat and host quality exercise the strongest constraints on herbivore habitat availability, and their relationship with each other and nitrogen levels in the environment.

The importance of host nutritional quality in practice

It was these considerations that motivated the second chapter of my thesis, exploring the relative importance of host nutritional quality and the microhabitat around hostplants in explaining habitat use by one of the herbivore species studied in my first chapter, *L. phlaeas*. I found that egg-laying was dictated mostly by microclimate, and no evidence of a role for host quality, even in spring when temperatures were higher, and therefore *L. phlaeas* might be expected to be less constrained by microclimate around hostplants (because even ‘cool’ microclimates will be warm in spring).

This chapter does not explicitly attempt to answer the question: ‘will thermophilous herbivores benefit from improved host quality under nitrogen pollution or be negatively impacted by loss of suitable

microclimates?'. An experimental design such as the one used by Yang et al. (2017) would be more appropriate for this. Rather, the focus was more fundamental, on testing whether the trade-off between high quality host plants, and suitable microclimates existed, and how this constrained herbivore habitat use. I hypothesised this trade-off on the basis of the reported positive relationship between soil nitrogen and the productivity, density and height of grassland vegetation, and plant nitrogen content, alongside the negative relationship between vegetation height and density and temperature at ground level. Thus, I expected the trade-off to be driven by an underlying gradient of soil nitrogen, but not having measured soil nitrogen levels, I cannot be sure this is the mechanism driving the observed trade-off. Further work that relates plant nitrogen content, soil nitrogen content and vegetation structure in different contexts would be valuable to confirm the mechanism behind this trade-off and whether it is a general challenge for insect herbivores in open habitats. It seems likely that other factors that affect microclimate around host-plants independently of vegetation structure, for example aspect, would moderate this trade-off and it would be valuable to understand how these also interact with host nutritional quality and microhabitat to shape habitat use.

Species feeding on host plants with higher nitrogen content tend to have faster and more flexible development (Poyry et al., 2017), implying that if this trade-off is a general phenomenon, it may have far-reaching consequences for the evolution of butterfly life-histories. In the context of nitrogen pollution, this trade-off and the preference of *L. phlaeas* for warmer hosts over high quality hosts implies that this and similar herbivore species are more likely to be affected by the loss of suitable microclimates under nitrogen pollution than by changes in host quality, providing important context for the experiments in my first chapter. This aligns with previous work by Klop et al. (2015), who performed a similar study with the butterfly *Lasiommata megera,* assessing the microhabitat requirements of larvae in the field, and the response to changes in plant nitrogen content under laboratory conditions. They found only positive effects of fertilisation, and a tendency to use warmer microhabitats, suggesting that the negative effects of atmospheric nitrogen deposition on *L. megera*

population trends were likely to reflect losses of suitable microhabitats offsetting any improvement in host quality. Similarly, Yang et al. (2017) tested the response of the larvae of the moth *Gynaephora alpherakjj* to nitrogen fertilisation of foodplants in alpine grassland, and found that growth rate and feeding rate declined with fertilisation in the field, in spite of positive impacts in the laboratory, attributing this difference to the lower temperatures at ground level in fertilised plots.

Interestingly, I found that molehills produced warm micro-climates under high quality host plants, disrupting the trade-off between host quality and microclimate. This highlights the potential for soil disturbance to contribute to the maintenance of early-successional insect species in the more productive grasslands resulting from nitrogen pollution, mirroring similar findings for less competitive, light-demanding plants in more eutrophic grasslands (Seifan et al., 2010). Such disturbance could be provided at larger scales by grazing animals, or mechanical disturbance of soil by human activity.

In the context of nitrogen pollution, the results of my second chapter suggest that it will be important to consider insect responses to nitrogen pollution in a multi-dimensional way. Nitrogen pollution affects the availability of multiple components of insect niches simultaneously – microhabitats, high quality foodplants, and even the foodplants themselves. Focusing on changes in the availability of one of these niche components risks under-estimating insect sensitivity to pollution if this does not overlap with other niche components.

Integrating insect responses to nitrogen levels

These insights led me to my third chapter, which attempted to take a broader view, and model the relationship between insect occurrence and nitrogen levels in the landscape. This relationship should integrate all the ways in which nitrogen levels constrain habitat availability for a given insect species, to provide a more robust measure of insect sensitivity to nitrogen pollution, on the basis that species whose requirements are met at high nitrogen levels are likely to be beneficiaries of pollution, and vice versa.

My methods mainly only resolved weak signals of species' preferences from their distributions. In many cases, this likely reflects the reality that many common generalist species are largely indifferent to nutrient levels, but in others, probably insufficient sampling and the coarse scale of my analysis. This meant that in many cases the analysis probably did not resolve the true shape of species' responses to nitrogen levels (preventing it from providing accurate measures of their tolerance). However, it did at least seem to be effective at classifying their tendency to occupy higher or lower nutrient habitats. The optima I derived for each species (reflecting this tendency) showed a high degree of internal consistency (species mostly occurred alongside species with similar scores, suggesting that the optima were capturing an environmental gradient), and were positively correlated with similar optima derived for Dutch butterflies (Oostermeijer & van Swaay, 1998), and with expert assessment of species' requirements.

If some of the difficulties of resolving species' responses can be addressed, this approach therefore has the potential to provide an objective way of classifying insect sensitivity to nitrogen pollution. Existing work that attempts to do this and relate this to species' trends of changes in community structure has primarily focused on lepidoptera, with some work extending to Orthoptera and Hymenoptera too. This in part reflects the likely high sensitivity of these taxa to nitrogen pollution, but also an existing bias – these taxa are well-studied, and there is a reasonable degree of understanding of species' thermal, forage and nutritional requirements and therefore their likely sensitivity to nitrogen pollution.

This existing knowledge enabled me to develop some tests our indices using previous work by Oostermeijer & van Swaay (1998) and expert assessment of species' requirements. Of course, these approaches do not provide a true test of my indices – the analysis of Oostermeijer & van Swaay (1998) is not necessarily any more correct than mine (although I would suggest that the smaller spatial scale of their work is a significant advantage), and expert scores are inherently subjective (although the high consensus among experts is encouraging). Thus, there is a degree of circularity in deriving 'objective' scores from data and testing them against these standards – if expert scoring is a robust standard, what

need for data-derived scores? I would argue that these scores provide a good sense-check for my indices, and that the high agreement between indices and validation scores suggests the approach used in this chapter works. The novel insight is thus in part the scores themselves, but also this validation of the approach, and the suggestion that it could be applied to less well-studied insect taxa where there are no existing indices, and the paucity of experts and detailed ecological study means that expert scoring would not be possible. It could therefore provide new insights into the extent to which other insect taxa are constrained by nitrogen levels, potentially highlighting the sensitivity of other taxa that have not featured in work on nitrogen pollution, or even previously un-known or under-appreciated mechanisms via which these less well-studied insect taxa could be affected by nitrogen pollution.

My indices also offer the opportunity to assess changes in community structure due to nitrogen pollution by calculating community nitrogen indices in a similar fashion to WallisDeVries & van Swaay (2017). The usefulness of community temperature indices for assessing responses of a wide variety of taxa to climate change, and the power of Ellenberg indicator values for studies of plant communities suggests these could be a powerful way of assessing the impacts of nitrogen pollution on insect communities. WallisDeVries & van Swaay (2017) calculated these for Dutch butterfly communities using counts from standardised monitoring, and recent methodological advances present opportunities for their improvement and wider use. Methods used to calculate measures of functional diversity in data with imperfect detection for example, may enable the calculation of these indices from unstandardised occurrence records (Jarzyna & Jetz, 2016; Palacio et al., 2020; T. Roth et al., 2018). This could enable assessment of community change due to nitrogen pollution using occurrence records across the wide variety of insect taxa that currently lack standardised monitoring. WallisDeVries & van Swaay (2017) also do not integrate the tolerance scores of Oostermeijer & van Swaay (1998) into their indices, but Carroll et al. (2018) were able to do this in an assessment of changes in plant community indices over time by weighting species' scores by the inverse of their tolerance. Their multi-level modelling approach also provides a promising framework for a more

nuanced understanding of the different scales at which communities are changing over time that could also be applied to community nitrogen indices.

Indices also provide the possibility of testing more subtle patterns of change than just a shift towards more nitrophilic species over time. Indices derived from non-spatially under-sampled data in this analysis suggested that more species were associated with low-nutrient habitats than high-nutrient habitats. This result fits with the indices derived for Dutch butterflies by Oostermeijer & van Swaay (1998) who found a similar pattern. WallisDeVries (2014) posits that this pattern, and the low dispersal capacity of many species of low-nutrient habitats reflects the dominance of low-nutrient habitats prior to human activity. WallisDeVries (2014) therefore suggests that historically these species of low-nutrient habitats now regarded as habitat specialists were in fact habitat generalists, and vice versa, a re-framing that our results add further support to. The higher frequency of species of low-nutrient habitats also implies that continuing nitrogen pollution should lead to a gradual replacement of a large variety of species of low-nutrient habitats by a smaller variety of species of high-nutrient habitats. This hypothesis of 'nitrogen homogeneity' was advanced for plants by Gilliam (2006) and recent work has found patterns consistent with it in plant communities across Europe (Staude et al., 2020). Although homogenisation is already apparent in insect communities in Europe (Carvalheiro et al., 2013), the role of nitrogen pollution in driving this change has not been assessed, and this would be a further valuable use of our indices, for example by testing how beta diversity varies across insect communities with different community nitrogen indices.

Using indices in this manner is not without its disadvantages however, my indices do not provide any insight into why a given insect species is constrained to a certain nitrogen level, they simply suggest that it is. Thus, using them to replace current trait-based approaches for assessing the effect of nitrogen pollution on insect communities risks losing the mechanistic insights that these provide. One of the suggested advantages of indices over these approaches is that a given trait is likely to affect insect responses to a variety of environmental changes and so changes in communities along this trait axis do

not act as an unambiguous indicator of the effects of nitrogen pollution. The same however is still likely to be true for our indices, WallisDeVries & van Swaay (2017) found that species with lower optima in the analysis of Dutch butterflies by Oostermeijer & van Swaay (1998) had lower mobility and reproductive capacity for example, and our optima were positively correlated with these. Ultimately, the best evidence of the effects of nitrogen pollution on insect populations will come from work linking spatial and temporal variation in nitrogen pollution to changes in vegetation composition and structure, and insect community structure, and the indices developed in this thesis are not a replacement for existing approaches, but could greatly enhance them.

Conclusions

Nitrogen levels can affect habitat availability for insects via their impact on host plant availability, host nutritional quality and microhabitat availability, thereby driving insect responses to nitrogen pollution. I investigated the structure of these constraints, their interaction and their overall effect in this thesis. I found that nitrogen levels had strong effects on host quality for insects that were non-linear and likely driven by limitations of other nutrients induced by high levels of nitrogen, thus being contingent on the availability of other nutrients. This implies that the effects of nitrogen pollution on host nutritional quality will be dose and context-dependent. These strong effects led me to examine how the importance of high host nutritional quality interacts with the requirement for suitable microclimates to dictate habitat use by insect herbivores. I found that host nutritional quality was of secondary importance to micro-climate around host plants, and a trade-off between them, implying that predicting species' responses to nitrogen pollution will require consideration of all the ways in which nitrogen levels constrain habitat availability for them. In an attempt to integrate these interactions, the way in which nitrogen levels constrain insects via multiple mechanisms and shape habitat use, I developed a method for modelling the relationship between the occurrence of British lepidoptera and nitrogen levels in the landscape. This may be a promising approach for understanding species' sensitivity to

nitrogen pollution in the future, particularly for less well-studied insect taxa. Thus, this thesis demonstrates some of the likely complexity of insect responses to nitrogen pollution, while providing the tools for necessary further investigation.

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Appendix

This appendix provides the titles of other work that I have completed during my PhD not directly related to the subject of my thesis in their published form. Some of this is contributions to projects by colleagues, but the majority of it is my own work on the biology and ecology of British Lepidoptera, in recent years particularly leaf-mining microlepidoptera.

1. Christopher D. Terry, J., Langdon, W., & Rossberg, A. G. (2023). Codistribution as an indicator of whole metacommunity response to environmental change. *Ecography*, *4*(4), 527–534. https://doi.org/10.1111/ecog.06605
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5. Langdon, W. B. V. (2023). Dialectica scalariella (Zeller, 1850) (Lep.: Gracillariidae) at Dungeness, Kent: The First British Breeding Record. The Entomologist's Record and Journal of Variation, 135(1), 6–18.

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10. Langdon, W. B. V, Sterling, P. H., Oughton, J., & Hall, P. R. (2024). Aethes francillana (Fabricius, 1794) (Lep.: Tortricidae) feeding on Rock Samphire Crithmum maritimum L. The Entomologist's Record and Journal of Variation, 136, 205–208.