# Vegetation composition and structure are important predictors of oviposition site selection in an alpine butterfly, the Mountain Ringlet *Erebia epiphron*.

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## Abstract

Knowledge of species' ecological requirements is key for designing effective conservation management. In butterflies, the needs of larval stages are often the most specialised part of the life cycle, but for many species information on this is lacking. The Mountain Ringlet *Erebia epiphron* is a cold-adapted butterfly found in alpine grasslands in mountainous regions of Europe. Efforts to devise conservation strategies for this climate change-threatened species are hampered due to its basic ecology being poorly understood. Here, we describe a study on the autecology of Mountain Ringlets across its British distribution, focusing on the habitat preferences of egg-laying females as a proxy for larval preferences. Female Mountain Ringlets placed their eggs predominantly on *Nardus stricta* and *Festuca ovina*, but also on several other host plant species, suggesting larvae may be more broadly polyphagous than previously realised. Sites chosen for eggs had higher abundance of larval host plants, intermediate leaf litter cover, and lower cover of grass tussocks than random locations, as well as a shorter and sparser grass sward. Although the main host plant is ubiquitous in upland areas of Britain, our findings suggest that this butterfly's egg and larval stages have specialised ecological requirements, requiring specific microhabitat features characterised by a narrow range of vegetation composition and structural characteristics. Many habitat associations are liable to be explicable as adaptations to ensure placement of eggs and larvae in sites within optimal (warm or buffered) microclimates. We tentatively suggest that the distribution of Mountain Ringlets in the landscape is thermally-constrained.

**Keywords**: conservation; habitat preferences; uplands; sheep grazing.

## Introduction

Recent decades have witnessed marked declines in the abundance and range of several butterfly species in the UK (Asher et al. 2001, Fox et al. 2015) and elsewhere in Europe (Van Swaay et al. 2009, van Strien et al. 2019). A common pattern of change has been disproportionate losses of butterflies that are habitat specialists or sedentary, leading to increasingly depauperate, homogeneous butterfly communities dominated by widespread, mobile generalists (Warren et al. 2001, Mattila et al. 2011). For habitat specialists in the UK, the dominant driver of population declines has been deterioration of habitat quality caused by substitution of traditional agricultural and forestry practices with intensive management (Thomas et al. 1986, Warren 1991), coupled at the landscape scale with elevated risks of local extinction following habitat loss and fragmentation (Hanski 1998).

Designing effective conservation strategies to halt and reverse the declines of threatened butterflies demands detailed knowledge of their ecological requirements (Thomas et al. 2011b). The complex nature of the butterfly life-cycle means that specific requirements are often needed by each of the separate development stages (e.g. eggs, larvae, pupae, adults; Dennis et al. 2003), but those of the larvae can be particularly limiting. Detailed autecological studies often show that the larvae are dependent on ephemeral, narrow niches within their biotope characterised by larval hostplants in preferred growth forms or microhabitats (Thomas et al. 2009, Thomas et al. 2011b). For example, adult Silver-spotted Skippers *Hesperia comma*, a rare butterfly on calcareous grasslands in southern Britain, demonstrated unspecific requirements generally, but the process of oviposition was highly selective, with females only laying eggs on hostplants in very short (< 3 cm) vegetation swards with plenty of surrounding bare ground (Thomas et al. 1986). Knowledge of these specific ecological requirements and provisioning them via appropriate conservation management has been pivotal in countering the decline of *H. comma* (Davies et al. 2005) and other endangered habitat specialist butterflies (e.g. Heath Fritillary *Melitaea athalia*, Warren 1991).

While our understanding of the needs of the larval stages of many butterflies has improved in recent decades, there are still species for which even basic ecological requirements are poorly documented (García-Barros and Fartmann 2009). One such species is the Mountain Ringlet *Erebia epiphron* (Asher et al. 2001). The *Erebia* genus contains a large number of specialist cold-adapted species found at high latitudes and altitudes in the Holarctic region, with Europe representing a centre of speciation (Schmitt et al. 2016). The Mountain Ringlet is the only high-altitude representative of the *Erebia* genus present in Britain, where it occurs in upland grasslands at elevations between 350-900m in two disjunct populations in the Lake District in north-west England and the Central Highlands of Scotland (Asher et al. (2001). Matt Grass *Nardus stricta* is widely considered the main larval host plant in Britain (Asher et al. 2001), but observations have also been made of a small number of eggs being laid on Sheep's Fescue *Festuca ovina* (Shannon 1995), raising the possibility that larval stages may not be strictly monophagous.

Relatively few studies of Mountain Ringlets have been undertaken either in Britain (Boyd-Wallis 1994, Bayfield et al. 1995, Shannon 1995) or more widely across Europe (but see recent studies for the *silesiana* subspecies e.g. Kuras et al. 2003, Konvicka et al. 2016), and none of these focus on detailing the habitat preferences of ovipositioning females (which we assume to be informative of larval preferences). The paucity of knowledge on this species may stem from the remote, inaccessible locations it occupies, along with its preference for only flying in warm, sunny conditions. These weather conditions are infrequent in the regions that the species occupies in Britain, limiting detection. More widely, it also appears to be a general feature of satyrid butterflies whose larvae feed on grasses, are rather inconspicuous and emerge to feed at night that the preferences of these species are poorly documented (García-Barros and Fartmann 2009).

The conservation status of the Mountain Ringlet in the UK is uncertain (Asher et al. 2001), as it is not sufficiently well sampled by the standard butterfly monitoring schemes and the extent of its distribution is almost certainly under-recorded (Masterman 2008). Nonetheless, evidence has emerged suggesting that Mountain Ringlets may be vulnerable to the impacts of climate change, with a programme of repeat surveys of previously occupied sites discovering disproportionate losses of low-lying Mountain Ringlet colonies in recent decades (Franco et al. 2006; A. Suggitt, pers. comm.), a finding repeated in other related *Erebia* congeners in continental Europe (Scalercio et al. 2014, Stuhldreher and Fartmann 2014). The Mountain Ringlet has also been classified as the butterfly species most vulnerable to climate change-mediated decline within its current range in the UK (Thomas et al. 2011a). Thus, conservation strategies that facilitate adaptation to the worst effects of climate change impacts via appropriate habitat management are potentially needed for this species, but currently our ability to design and implement such measures is hindered by an absence of knowledge of its ecological requirements.

In this study, we describe a four-year investigation working across multiple Mountain Ringlet colonies in the English Lake District and the Central Scottish Highlands to improve our general understanding of the ecological requirements of Mountain Ringlets, with a particular focus on determining the habitat preferences of ovipositioning females as a proxy for the requirements of larval stages. We have two primary objectives. Firstly, we seek to update and expand our current understanding of the range of hostplants and microhabitats that female Mountain Ringlets seek out for their eggs (and subsequently their larvae). Secondly, exploiting a use-availability experimental design and a generalised linear mixed modelling framework, we aim to identify key ecological conditions with respect to vegetation composition and structure, topography and soil moisture characteristics that define more precisely the ecological requirements of Mountain Ringlet larval stages.

## Methods

### *Study species*

The Mountain Ringlet is a satyrine butterfly restricted to mountainous regions of Europe (Kudrna et al. 2011). Across the two disjunct populations in Britain, the species is univoltine, with the main flight period in June or July. Mountain Ringlets are found in upland grassland (Asher et al. 2001), where they feed on nectar from Tormentil *Potentilla erecta*, Thyme *Thymus polytrichus*, Heath Bedstraw *Galium saxatile*, and Meadow Buttercup *Ranunculus acris*, amongst other species (Boyd-Wallis 1994, Shannon 1995, Masterman 2008). The principle larval host plant is assumed to be Matt Grass *N. stricta* (Asher et al. 2001), but other species may also be important (Shannon 1995). After hatching, caterpillars feed until September, and then overwinter as a third instar at the base of grass tussocks, emerging from hibernation in April (Asher et al. 2001). Other *Erebia* species favour sites with well-developed litter layers to buffer overwintering larvae from extremes of temperature (Slamova et al. 2013, Stuhldreher and Fartmann 2014), but it is uncertain whether leaf litter is also important for *E. epiphron*. A biennial life-cycle has been demonstrated for Mountain Ringlets in captivity (Wheeler 1982), but this has not yet been confirmed in the wild in Britain.

### *Study colonies*

We studied Mountain Ringlets at several colonies in the Lake District (2015-17) and at one location in the Central Scottish Highlands (2017-18). Following on from a pilot study in 2015 at RSPB's Haweswater reserve (54.51º, -2.84º), the study expanded to include colonies across the species' distribution in the Lake District. To select areas for sampling, we obtained Mountain Ringlet records between 1980-2014 from the Cumbria Biological Records Centre, defining the current distribution of the species in the Lake District as all 1-km squares with sightings since 2000. This yielded a total of 81 occupied squares. From these, we selected a stratified sample of 24 squares, comprising four from each combination of three altitude (< 400 m, 400-600 m, > 600 m) and two aspect (north-facing, south-facing) categories.

To target search effort at areas where the species was liable to be present within selected 1-km squares, we firstly excluded areas of unsuitable habitat, including woodland, low-lying intensive pasture, cropped land and lakes. Secondly, we also disregarded areas within squares that were below 400m. The adoption of this lower boundary was informed by an assessment of the altitudinal distribution of Mountain Ringlet records since 2000, which demonstrated that, with the exception of one colony (see below), the butterfly was rarely recorded below this threshold. For the colony at Irton Fell-Whin Rigg (54.40º, -3.34º), a lower altitudinal boundary of 150 m was used, as the species is known to occur at far lower elevations here. Lastly, we also excluded areas of difficult terrain (e.g. scree banks, cliff faces), where surveys would have been unsafe.

Squares selected for sampling in the Lake District reflected a range of altitudes (range: 200-800m), aspects and latitudes (range: 54.40ºN – 56.53ºN). Furthermore, although the dominant land management at most colonies was sheep grazing, the intensity of grazing regimes varied, with some areas also characterised by low intensity mixed grazing regimes. We expect therefore that our colonies reflected many of the key gradients in environmental variability that Mountain Ringlets encounter in the UK, including topography, microclimate and habitat. In total, with information from the pilot year (data collected across four 1-km squares), we surveyed Mountain Ringlets in 28 1-km squares in the Lake District between 2015-17, recording ringlets in 21 of these, with information on the habitat preferences of egg-laying females collected from 15 squares. Weather conditions were not conducive to egg-laying behaviour on those dates that the remaining six squares were visited, and these remain unsampled.

Mountain Ringlets were also examined at Ben Lawers National Nature Reserve (NNR) in Scotland (56.53º, -4.25º). This area hosts a rich arctic-alpine flora, due to the underlying Dalradian calcareous mica schist bedrock giving rise to basic soils (Trivedi et al. 2008). As part of a programme of management to protect and restore rare upland and montane plant communities, large grazing exclosures were established at Ben Lawers, which provided a unique opportunity to study Mountain Ringlets in the presence and absence of grazing pressure, and thus extends the range of environmental conditions under which the butterfly is investigated here. We collected information on microhabitat preferences of egg-laying females in two 1 km squares inside and two 1 km squares outside of grazing exclosures (established in 1991 and 2000) at altitudes between 520-760 m. Including sites in both England and Scotland, egg-laying behaviour was documented in 19 1 km squares (total = 184, mean = 9.7 eggs, range = 1-18 eggs) during this study.

*Sampling design*

Mountain Ringlets larvae are difficult to locate during uninformed ‘cold’ searches due to their inconspicuous nature and nocturnal habits, and thus we opted to study the microhabitat preferences of egg-laying females as a proxy for larval requirements. On warm days (> 14 ºC) during the flight period (June and July), we intensively surveyed suitable habitat within selected 1 km squares for female Mountain Ringlets. During these surveys, efforts were made to ensure that sampling intensity was equitably distributed across the area of suitable habitat to avoid inadvertent sampling biases. When a female was located, we followed that individual until an egg was laid, although on a small number of occasions we abandoned females if they demonstrated no obvious signs of egg-laying behaviour in otherwise suitable weather conditions after 30-60 minutes. Female Mountain Ringlets generally lay eggs singly, so we typically recorded a single egg-laying event per individual. However, we experienced several occasions (see Results for more details) when an individual female laid multiple eggs in quick succession and within close proximity (e.g. within a couple of centimetres of each other) and these were treated as a single egg-laying event. Only on a handful of occasions did we observe the same female laying eggs in two separate sites, which we recorded separately.

At each site where eggs were laid, we recorded information on several variables related to host plant, habitat, soil moisture and topographic characteristics. We identified the host plant species on which an egg was laid, as well as measuring the height of the egg above the ground. We used a 50 cm quadrat oriented on a N-S axis with the egg at the centre to quantify vegetation composition and structure around the egg site. The quadrat was partitioned into nine equally-sized subdivisions, and we separately quantified vegetation characteristics at two spatial scales: in the central 17 cm subdivision, and across the entire 50 cm quadrat. However, initial exploratory analyses showed that egg-laying Mountain Ringlets responded far more strongly to microhabitat characteristics in the immediate vicinity of the egg site (e.g. 17 cm central quadrat subdivision) rather than further away (e.g. across the 50 cm quadrat). Therefore, we only present and analyse information here collected at the smaller spatial scale. For each quadrat, we estimated the percentage cover of each plant species with greater than 5% cover. As *Nardus stricta* and *Festuca ovina* are considered the key host plants for Mountain Ringlets (Asher et al. 2001), we summed the percentage cover estimates for both species to derive a single measure of *host plant availability*. *Leaf litter cover* and *tussock cover* within quadrats were also measured.

We recorded information on two variables reflecting vegetation structure at egg sites. Vegetation height in quadrats was assessed using a variant of the 'direct' approach, which is known to be the most appropriate method for measuring short turf (Stewart et al. 2001). This involved placing an upright garden cane marked with 1 cm colour gradations at the sampling point and estimating an average sward height in the area surrounding the cane, excluding emergent flowering vegetation. In addition, we quantified vegetation density within quadrats by placing a horizontal 11 cm garden cane marked with thin (2 mm thick) coloured bands at 1 cm intervals at each sampling point, and then counted the number of visible (> 50%) bands that could be seen at a distance of 1 m. Lower values indicated a denser sward.

Finally, we also collected information on a further four covariates describing soil moisture content and topography at egg sites. *Soil moisture* was estimated by calculating a mean value of measurements taken at three of the four corners of the quadrat using a ML3 ThetaProbe soil moisture meter. We derived estimates of a*ltitude*, *aspect*, and *slope* for each egg site using the OS Terrain 50 Digital Terrain Model (DTM; Ordnance Ordnance Survey, 2017) in ArcGIS (ESRI 2017) using 10-figure grid references obtained in the field with a GPS. *Aspect* was defined as a categorical variable, with egg sites classified as north-facing (270°- 89°) or south-facing (90°- 269°).

Characteristics at egg sites were contrasted with those at random points within suitable habitat in the same 1 km square. This approach equates to a use-availability sampling design for estimating resource selection functions, which is frequently used for studying wildlife-habitat relationships (e.g. Johnson et al. 2006). The random points were generated using ArcGIS (ESRI 2017). We sampled an equivalent number of random points per 1 km square (total = 184, mean per square = 9.2, range per square = 1-17) as egg sites and recorded the same suite of vegetation composition and structure, topographic and soil moisture characteristics at each of them using identical approaches to those outlined above. We note, however, that due to the failure of our soil moisture meter in 2015 approximately 20% of the random points lacked soil moisture measurements, which impacts the manner in which this variable was subsequently evaluated in statistical models (see below).

### *Statistical analyses*

We started by calculating summary statistics for the Mountain Ringlet egg dataset, including the proportion of eggs laid on different host plants, the proportion on dead and live plant tissue and the mean height of eggs above the ground. We next compared plant species composition at egg sites and random points in two ways. Firstly, percent cover of individual plant species was compared between egg sites and random points using nonparametric Wilcoxon's rank-sum tests. Separate tests were carried out for each plant species that occurred in more than 5% of quadrats, calculating both standard *p*-values and *p*-values corrected for multiple-testing (Holm 1979).

Secondly, patterns of plant species composition at egg sites and random locations were interpreted using detrended correspondence analysis (DCA). DCA is an unconstrained ordination technique that extracts dominant axes of variation from complex, multivariate community composition data, with species exhibiting unimodal response curves in relation to these axes (Hill and Gauch 1980, Ter Braak 1986). We derived a site-by-species matrix of percent cover data from all vegetation quadrats and for all species (n = 40) present in more than 1 % of quadrats. We ran the DCA on these data using the *decorana* function in the R vegan package (Oksanen et al. 2010) and then plotted the species centroids on a two-dimensional ordination plot. Next, we used the *envfit* function (also in the *vegan* package) to calculate centroids reflecting the average plant species composition at both egg sites and random points for 13 different 1 km squares. We did not calculate centroids for six squares due to small sample sizes of eggs (n < 4) and merged these data in with that of neighbouring squares. We then connected the random point and egg site centroids from each 1 km square by a vector to better depict differences in average species composition between the two types of site within individual squares (e.g. Britton et al. 2009) Specifically, the length of the vector quantifies the extent to which average plant species composition at each 1 km square (reflected by the random points, tail of the vector) differs from that selected by Mountain Ringlets for egg-laying (represented by the arrowhead of the vector), and the direction of the vector provides insights into whether Mountain Ringlets seek out consistent or divergent ecological niches for their eggs across different 1 km squares. Vectors that converge at a similar spot in ordination space indicate selection of similar plant species compositions by butterflies across the different study squares, whereas diverging vectors reflect spatial differences in the plant species composition of sites preferred for egg-laying.

We conducted a more detailed analysis of the microhabitat preferences of egg-laying Mountain Ringlets by modelling use-availability data using binomial generalised linear mixed models (GLMM). In this model, the response variable was coded as ‘1’ for egg sites and ‘0’ for random points. We started by deciding on the most appropriate random effects specification for the binomial GLMM. To do this, we fitted several GLMMs with different combinations of random terms (but no fixed effects). Variables fitted as random effects included *colony ID* (where Mountain Ringlet colonies overlapped several 1-km squares), *square ID*, and *female ID* (to account for nonindependence of eggs laid by the same female). Models specifying *colony ID* and *female ID* often struggled to calculate variance estimates for these terms, with variances tending to collapse to zero. In contrast, the variance for *square ID* was consistently estimable and thus we retained this random effect specification in all subsequent models.

We then constructed a global model comprising all vegetation composition, vegetation structure, and topographic explanatory variables (Table 1; note *soil moisture* was not included in the global model due to a high proportion of missing data, explained above). Prior to using this model as the basis of multi-model inference (see below), we firstly conducted exploratory analyses to test for collinearity amongst the explanatory variables, and nonlinear relationships between response and explanatory variables (Zuur et al. 2010). We calculated variance inflation factors (VIFs) for the global model to assess whether collinearity may bias parameter estimates. As none of the VIFs exceeded values beyond which collinearity is typically regarded to be problematic (e.g. VIF > 10, Dormann et al. 2013), all explanatory variables were retained. Moreover, exploratory analyses demonstrated that *leaf litter cover* was nonlinearly related to Mountain Ringlet habitat use, and thus as well as the main effect, we also fitted models specifying a quadratic term for this covariate.

We examined quantile residuals from the global model for evidence of lack-of-fit or violations of distributional assumptions using the DHARMa package in R (Hartig 2018). There were no indications that the residuals displayed heteroscedascity, deviated from uniformity (Kolmogorov-Smirnov test: $p = 0.837$), or were strongly spatially autocorrelated (Moran's I = 0.001, $p < 0.833$).

Binomial GLMMs comprising all-subsets combinations of the explanatory variables were fitted to the Mountain Ringlet use-availability data, and Akaike's information criteria (AIC) and associated weights ($w_i$) were used to rank each model according to their relative support (Burnham & Anderson 2002). Smaller AICc values and larger weights imply greater relative support for a model (Burnham & Anderson 2002). Inference regarding the importance of individual explanatory variables was not based on the best-supported model but was derived across all fitted models. To this end, we calculated model-averaged parameter estimates and associated confidence intervals (Galipaud et al. 2017), and considered individual explanatory variables to be key predictors of Mountain Ringlet habitat use if confidence intervals did not encompass zero (Burnham and Anderson 2002). All GLMMs were fitted using the glmmTMB package (Brooks et al. 2017), and multi-model inference was provided by the MuMin package (Barton 2018).

## Results

We observed 138 female Mountain Ringlets laying a total of 184 eggs during this study. Most females (82%) were seen to lay single eggs, but a non-trivial proportion of females (18%) laid multiple eggs (maximum = 7) in close proximity (e.g. within a couple of cms). The most commonly recorded host plants were *Nardus stricta* (n = 68, 37%) and *Festuca ovina* (n = 54, 29%), but a variety of other species were used less frequently (*Anthoxanthum odoratum*, 2%; *Agrostis sp.*, 5%; *Carex dioica*, < 1%; *Carex nigra*, < 1%; *Carex panicea*, 2%; *Carex pilulifera*, < 1%; *Carex pulicaris*, < 1%; *Deschampsia cespitosa*, < 1%; *Deschampsia flexuosa*, 3%; *Festuca rubra*, < 1%; *Juncus squarrosus*, 3%; *Molinea caerulea*, < 1%; Moss spp., 4%; *Potentilla erecta*, 1%; *Sphagnum* sp., < 1 %; unknown, 7%). Most eggs were laid on the underside of dead grass leaves (75% vs 25% on live plant tissue) close to the ground (mean height = 3.2 cm, s.d. = 2.3 cm).

Percent cover for 9 of 18 common plant species differed significantly between egg sites and random locations (Fig. 1), but this declined to only five species after controlling for multiple testing. The amount of *Nardus stricta* and *Festuca ovina* present at sites chosen for oviposition greatly exceeded that at random locations (*N. stricta*: W = 21061, $p < 0.001$, *F. ovina*: $W = 20607$, $p > 0.001$), suggesting that Mountain Ringlets females strongly select egg sites where these plant species are abundant. There was also an indication that ovipositing female butterflies positively favoured sites with a greater abundance of two *Carex* species (*C. panicea*: $W = 18462$, $p = 0.009$; *C. pilulifera*: $W = 18253$, $p = 0.014$), although the preference was not as strong as for the putative host plant species and these *Carex* species were generally less abundant in the sward. Evidence of strong avoidance was only apparent for one plant species after accounting for multiple testing, specifically *Juncus squarrosus* ($W = 14252$, $p = 0.031$).

The detrended correspondence analysis showed that the majority of vectors for different 1 km squares clustered in the same area of ordination space and were approximately of equal length and parallel direction (Fig. 2). This implies that differences in average species compositions between the random points and egg sites were relatively consistent across the dispersed 1 km squares. Typically, these vectors suggested that random points (tail end of the vector) were characterised by a dominance of *Agrostis spp.*, *Deschampsia flexuosa* or *Juncus squarrosus*, while egg sites (arrowhead of the vector) were constituted to a greater degree by *Nardus stricta* and *Festuca ovina*. There were a few clear outlying vectors that varied in their starting points and directionality. One of the most pronounced outliers was

NN5938 within the grazing exclosure at Ben Lawers, suggesting that the removal of grazing has had a demonstrable impact on the plant species composition at that site (Fig.2). However, even these outlying vectors tended to converge on the same region of ordination space (Fig. 2b). Thus, regardless of broader differences in average plant species composition across sites due to disparate management or grazing regimes, female Mountain Ringlets appear consistent in their selection preferences, seeking sites for egg laying that are characterised by similar plant species compositions dominated by *Nardus stricta* and *Festuca ovina*.

In the binomial GLMMs, vegetation composition was an important determinant of Mountain Ringlet egg site selection, with several covariates represented in highly ranked models (Table 1a). Female Mountain Ringlets demonstrated a strong preference for laying eggs at sites with a high abundance of the two key host plants (Fig. 3a), *Nardus stricta* and *Festuca ovina*, indicated by the fact that the model-averaged parameter estimates for the *host plant availability* covariate did not encompass zero (Table 1b). There was also a curvilinear relationship between probability of use and *leaf litter cover* (Fig. 3b), with females laying their eggs disproportionately at sites with intermediate levels of leaf litter. *Tussock cover* was negatively related to the probability of a site being selected (Fig. 3c), implying that females may actively avoid areas where tussock-forming grasses predominate.

Two vegetation structure covariates were also found to be strongly associated with the selection of egg sites in Mountain Ringlets. Sites characterised by lower vegetation height (Fig. 3d) and less dense grass sward were preferentially selected by females over locations with tall, rank vegetation. In contrast, evidence suggesting an important role of topographic characteristics in selection of egg sites by Mountain Ringlets was limited, with only *slope*, but not *altitude or aspect*, shown to have a strong association with probability of use (Table 1b). Together, the vegetation composition and structure characteristics accounted for a substantial proportion of the total variation, with marginal and conditional $R^2$ values for the best-fit model of 0.599 and 0.783 respectively. Finally, *soil moisture* was not significant ($\beta$ = -0.212, s.e. = 0.254, $p = 0.403$) when added to the best-fit model.

## Discussion

This study provides the first comprehensive assessment of oviposition habitat preferences for Mountain Ringlets. We provide novel information on the host plants species selected by egg-laying females, showing that *Festuca ovina* may be a more important food plant than previously realised for larval Mountain Ringlets (at least in Britain). More importantly, we find evidence that Mountain Ringlets are highly specialised in their microhabitat requirements, with females seeking out sites for their eggs and larvae characterised by a narrow range of vegetation composition and structural characteristics. Many of these preferences are likely to be adaptations to their harsh alpine environment, evolved to ensure that eggs and larvae are placed in sites with buffered microclimates.

### *Host plant species and sites selected for oviposition*

Mountain Ringlet females laid eggs predominantly on *Nardus stricta* and *Festuca ovina*, and strongly selected sites for egg-laying with a greater abundance of these plant species than at random points. Oviposition on and selection for sites with abundant cover of *Nardus stricta* is documented (Boyd-Wallis 1994) and is consistent with the long-held view that this grass is a key larval food plant for Mountain Ringlets in Britain (Asher et al. 2001). Selection of *Festuca ovina* for egg-laying, in contrast, is a relatively novel finding for the species in Britain, having only been indicated by one prior small-scale study of Mountain Ringlets in the Lake District (Shannon 1995), although we note that *F. ovina* and related fescues are important host plants of other Mountain Ringlet subspecies and congeneric *Erebia* species in continental Europe (Kuras et al. 2001, Stuhldreher and Fartmann 2015). The preference for egg sites dominated by *Nardus stricta* and *Festuca ovina* was a consistent feature across all studied Mountain Ringlet colonies (see Fig. 2), irrespective of between-site heterogeneity in plant communities due to differences, for example, in grazing management.

As well as potentially being an important host plant, evidence implies that *Festuca ovina* may be the favoured Mountain Ringlet host plant. Experimental feeding trials on larvae of two separate Mountain Ringlet subspecies (*E. e. mnemon* and *E. e. silesiana*) found that when larvae were offered a choice of several fine-leaved grasses, a notable preference for *Festuca* spp. (*F. ovina* and *F. supina* respectively) was demonstrated, whereas *Nardus stricta* was rejected (Kuras et al. 2001; R. Menendez, unpubl. data). In addition, Mountain Ringlet larvae provisioned with a diet solely of *Nardus stricta* exhibited lower growth rates and higher mortality compared with other dietary treatments providing either *Festuca ovina* or a mixture of the two host plants (Bayfield et al. 1995). Differences in the quality and nutritional value of the two host plant species may underpin the apparent preference for *Festuca ovina*. For example, *Nardus stricta* tissues harbour higher concentrations of silicates than most other upland grass species (Massey et al. 2006), which is associated with less efficient conversion of ingested food matter to body mass (reducing growth rates) and reduced feeding efficiency (due to elevated rates of mandible wear) in folivorous lepidopteran larvae (Massey and Hartley 2009).

Thirty percent of Mountain Ringlet eggs were placed on host plants other than *Nardus stricta* and *Festuca ovina*, with *Agrostis* sp., *Juncus squarrosus*, *Deschampsia flexuosa*, *Carex* *panicea* and mosses species where oviposition was recorded repeatedly. Together with the observation that females laid eggs at sites (c. 10%) where *Nardus stricta* and *Festuca ovina* were absent, these findings argue that Mountain Ringlet larvae may be more broadly polyphagous than generally acknowledged, potentially utilising a range of graminoids as food plants. Experimental feeding trials documented that *E. e. silesiana* larvae also readily accepted *Deschampsia flexuosa* as an alternative host food plant to *Festuca supina* (Kuras et al. 2001). *Deschampsia flexuosa* was also present at many of the egg sites in this study lacking *Nardus stricta* and *Festuca ovina*, where it may have been used as an alternative larval food resource. Further direct observations of larval stages feeding on hostplants in the wild are needed to confirm experimental observations that larvae are more broadly polyphagous than realised (e.g. Kuras et al. 2001).

Female Mountain Ringlets typically laid their eggs close to the ground on the underside of dead, prostrate host plant leaves. Several butterfly species also deposit their eggs close to ground level, including *Hesperia comma* (Thomas et al. 1986), *Melitaea aurelia* (Eichel and Fartmann 2008), and *Coenonympha tullia* (Čelik and Vreš 2018), although laying eggs on dead plant material appears to be less commonplace (but see Wiklund 1984, Čelik and Vreš 2018). We suggest that female butterflies are seeking out warmer microclimates for their eggs and larvae. Ambient temperatures tend to be warmer closer to ground level than further away due to radiative heat (e.g. Thomas et al. 1986). Furthermore, Wallis De Vries (2006) showed that the surface temperature of dead plant matter increased substantially above ambient air temperature, whereas green plant tissue remained similar to ambient due to the effects of evaporation. Alternatively, incidental predation of butterfly eggs by grazing animals can lead to high rates of mortality in intensively grazed systems (Van Noordwijk et al. 2012). As many of the upland areas where Mountain Ringlets occur in Britain are grazed intensively by sheep, the placement of eggs low in the sward, attached to dead plant material that is presumably less attractive forage, may minimize the potential risks of grazing mortality.

### *Effects of surrounding vegetation composition and structure on oviposition site selection*

In the more detailed assessment of habitat preferences of egg-laying Mountain Ringlets using GLMMs, we found evidence that several vegetation composition and structural characteristics were associated with egg site selection. Firstly, sites chosen for egg-laying had significantly greater quantities of host plants than random points. It is generally expected that an important criteria of egg-site selection is ensuring adequate food resources are available at close proximity for larval growth and survival. Where resources are inadequate, larvae can experience food shortages and suffer high rates of mortality due to starvation, especially in early instars (Kuussaari et al. 2004). Selection pressures to lay at sites with abundant host plant availability are liable to be particularly strong in butterflies that lay clutches of eggs at the same site, where there may be food competition amongst offspring (Fartmann and Hermann 2006), but strong links with host plant availability have also been demonstrated by other species that deposit eggs singly (e.g. Large Heath *Coenonympha tullia*, Weking et al. 2013, Čelik and Vreš 2018).

Another driver for selecting sites with abundant host plant resources may be larval mobility, with those butterflies characterised by highly sedentary larvae being more pressured to locate egg sites with greater host plant densities than mobile species, as they need to complete their life-cycle within a confined area. Interestingly, Curtis *et al.* (2015) showed that adult abundances and host plant densities for 27 butterfly species in the UK were more strongly correlated where species were less mobile. Previous authors have commented on the ‘sluggish’ or ‘passive’ nature of *Erebia* caterpillars (*E. epiphron*, Frohawk 1924, *E. sudetica*, Kuras et al. 2001), which may be indicative of a sedentary character that could underpin the need for high densities of food plants immediately adjacent to egg sites. Alternatively, the association documented here between host plant availability and egg site selection may not demonstrate active selection for areas with abundant host plants, but could simply be due to the fact that such areas may be more detectable to searching females (Fartmann and Hermann 2006).

As well as host plant availability, the quantity of leaf litter also influenced egg site selection in Mountain Ringlets, with females preferring sites with intermediate amounts of litter (30-60%). In the congeneric species *Erebia medusa*, Stuhldreher & Fartmann (2014) demonstrated that patch-level occupancy was positively related to the amount of litter in a patch. The thick accumulations of leaf litter functioned as a microclimatic buffer, which reduced the extremes of temperature to which overwintering larvae were subjected. We propose that the preference exhibited here by Mountain Ringlets may also indicate a requirement for threshold levels of accumulated leaf litter to buffer against strong fluctuations in winter temperatures. However, favouring intermediate levels of leaf litter may imply the existence of trade-off, whereby sufficient litter is required to safeguard against low winter temperatures, but the litter layer is not so well-developed as to potentially obstruct the germination or growth of host plants. Finally, it is also noteworthy that sites dominated by *Nardus stricta* tended to have the most well-developed litter layers, perhaps suggesting that females selecting sites with a high abundance of this plant may not be doing so not to secure food resource for their larvae, but instead for the dense litter layer provided.

Other vegetation characteristics associated with egg site selection of Mountain Ringlets were vegetation height and density, and the cover of tussocks. Sites selected for egg-laying had shorter and sparser vegetation (also see Stuhldreher and Fartmann 2015). Short, sparse grass swards permit ovipositioning females easy access to the surface layer to deposit their eggs on suitable low-growing hostplants, and they also ensure a warm microclimate by minimising the potential shading effects of taller vegetation (e.g. Thomas et al. 1986). Konvička *et al.* (2002) also reported that adult Mountain Ringlets were more strongly associated with short grassy vegetation for activities such as mating, basking and nectar-feeding. Furthermore, egg sites had lower cover of tussocks than random locations. We originally anticipated that tussock cover might be positively associated with the probability of site being used for egg-laying, as these presumably also provide microclimatic buffering from extreme winter temperatures, but this expectation was not supported. Key tussock-forming graminoids (e.g. *Molinia caerulea* and *Eriophorum vaginatum*) in Mountain Ringlet upland habitats are often quite dominant, with low presence of larval hostplants in the sward, and the availability of warm microclimates is liable to be in short supply, as even the pockets of low growing vegetation in between tussocks are overshadowed by surrounding vegetation. Boyd-Wallis (1994) also showed that Mountain Ringlets avoided egg sites with a high cover of *Molinea caerulea* at one colony in Scotland.

Few topographical variables emerged from this study as important predictors of egg site selection. The sole exception was *slope*, which was negatively associated with probability of use, but this may represent an artefact of our sampling method. Following egg-laying females on even moderate slopes is difficult, and we excluded very steep slopes from consideration due to safety concerns, so we may have undersampled egg laying attempts in such areas. Mountain Ringlets might actually be expected to be positively associated with steeper slopes, as the gradient favours low-growing, sparse vegetation communities often with host plants present, which would provide appropriate microclimates. The absence of associations with altitude and aspect may also be more to do with the spatial scale at which the study was conducted rather than reflecting the lack of importance of these variables. Topographic factors are liable to be more crucial for governing the distribution of Mountain Ringlet colonies across a landscape, rather than dictating finer-scale microhabitat associations within individual patches. Indeed, Bayfield et al. (1995) found that the majority of Mountain Ringlet colonies in Scotland were on south, south-west or south-east facing slopes, with none recorded on north or north-west facing slopes.

### *Management implications*

Sheep grazing is a key land use of many of the upland areas that Mountain Ringlets inhabit in Britain. Our findings imply that sheep grazing creates habitat types characterised by vegetation composition and structural characteristics that are suitable for Mountain Ringlets. Firstly, *Nardus stricta* is relatively unpalatable grass species that is frequently avoided by selective grazers such as sheep, and high stocking rates in upland areas can increase the prevalence and dominance of *Nardus stricta* in the sward by effectively grazing out all of the more palatable grass species (Grant et al. 1996). Grazing appears, therefore, to maintain a general plant species composition across the landscape that is broadly suitable for Mountain Ringlets. Secondly, sheep grazing also creates areas with short, sparse swards, which allow egg-laying females ready access to low-growing host plants and warm microclimates for developing eggs and larvae.

There are reasons to expect, however, that very intensive sheep grazing regimes, which are characteristic of much of the Lake District, may not be optimal for Mountain Ringlets. For example, high stocking rates tend to lead to a less well-developed litter layer, potentially rendering overwintering larvae more vulnerable to mortality due to low temperatures, particularly at sites not further buffered by continuous snow lie.  Patches of longer vegetation (e.g. Carex flushes) are also needed to provide shelter for adult Mountain Ringlets during periods of inclement weather (S. Ewing pers. obs.), but these might be a rarer resource under intensive grazing regimes.  On the other hand, complete stock removal is not liable to be beneficial in the long-term for Mountain Ringlets either, as this would allow forbs or dwarf shrubs to dominate (Pakeman et al. 2003), reducing the availability of larval host plants and denying the butterfly access to warm microclimates.  This is illustrated, for example, by the low availability of Mountain Ringlet host plants in the Ben Lawers grazing exclosures relative to other grazed sites (exclosure: random site mean *N. stricta* cover – 4.7%, *F. ovina* cover – 5.5%; grazed sites: random site mean *N. stricta* cover – 20.6%, *F. ovina* cover – 14.5%)

Rather, we hypothesise that intermediate grazing might prove be a more suitable management regime for Mountain Ringlets, to create a *Nardus*-, *Festuca*- and flower-rich, but structurally-heterogeneous grass sward that caters for the full spectrum of microhabitat and microclimatic features required by all life-cycle stages of Mountain Ringlets.  Designing an appropriate grazing prescription that gives rise to such a sward would benefit from experimental grazing trials (Dennis et al. 2008).  Moreover, such an intermediate intensity grazing regime may have wider environmental benefits for a broader array of upland biodiversity (Evans et al. 2015).

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## Compliance with ethical standards

### *Competing interests*

The authors declare that there are no conflicting or competing interests.

### *Research involving human participants*

This research did not involve human participants requiring ethics approval.

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## Tables and Figures

Table 1. a) AIC modelling results from the analysis investigating environmental predictors of oviposition preferences of a mountain butterfly *Erebia ephiphron*. The top ten best-supported models (i.e. lowest AIC values) are presented, along with associated measures of model fit and evidential support. '$K$' reflects the number of parameters fitted in a model, '$\Delta$AIC' is the number of AIC points between a model and the most highly ranked model, and '$W_i$' a model's AIC weight. b) Model-averaged parameter estimates, standard errors and 95% confidence intervals for covariates included in the analysis.

Figure 1. Mean percent cover of different plant species at egg sites (light grey) and random points (dark grey). Asterisks denote that percent cover of species was significantly different between egg sites and random points based on a nonparametric Wilcoxon's rank-sums test. '*' – $p < 0.05$, '**' – $p < 0.01$, '***' – $p < 0.001$, '****' – $p < 0.0001$. $p$-values only remained significant for *Nardus stricta*, *Festuca ovina*, *Carex panicea, Carex pilulifera and Juncus squarrosus* after correcting for multiple testing (see Results for details). The superscript '†' placed in front of the species names on the y-axis denotes those species on which egg-laying was recorded.

Figure 2. Ordination plot from the detrended correspondence analysis. The labels identify the location of plant species centroids in ordination space. The vectors illustrate the difference in average plant species composition between the egg sites and random points for Mountain Ringlet in 13 separate 1 km squares. The tail of each vector is plotted in ordination space according to the average plant species composition at the random points at that site, whereas the arrowhead reflects the average plant species composition at sites at that square where eggs were laid. The length of the vector is proportional to the difference in species composition between the egg sites and random points, and the direction of the vector provides insights into whether Mountain Ringlets seek out a consistent or divergent ecological niches for their eggs across different 1 km squares.

Figure 3. Model based predictions showing effects of vegetation composition and structure on egg site selection of Mountain Ringlets in Britain. Derived from the best-fit binomial GLMM, these depict (a) the positive association between probability of a site being selected for egg-laying and percentage cover of host plants; (b) the quadratic relationship between probability of site selection and leaf litter cover; c) the negative relationship between probability of site selection and percentage cover of tussocks; and d) the negative association between probability of site selection and vegetation height. The solid line in each of the graphs represents the fitted values, and the shaded areas show the 95% confidence intervals. Marginal histograms represent the distribution of each explanatory variable at egg sites (top histogram shaded light grey) and random points (bottom histogram shaded dark grey).

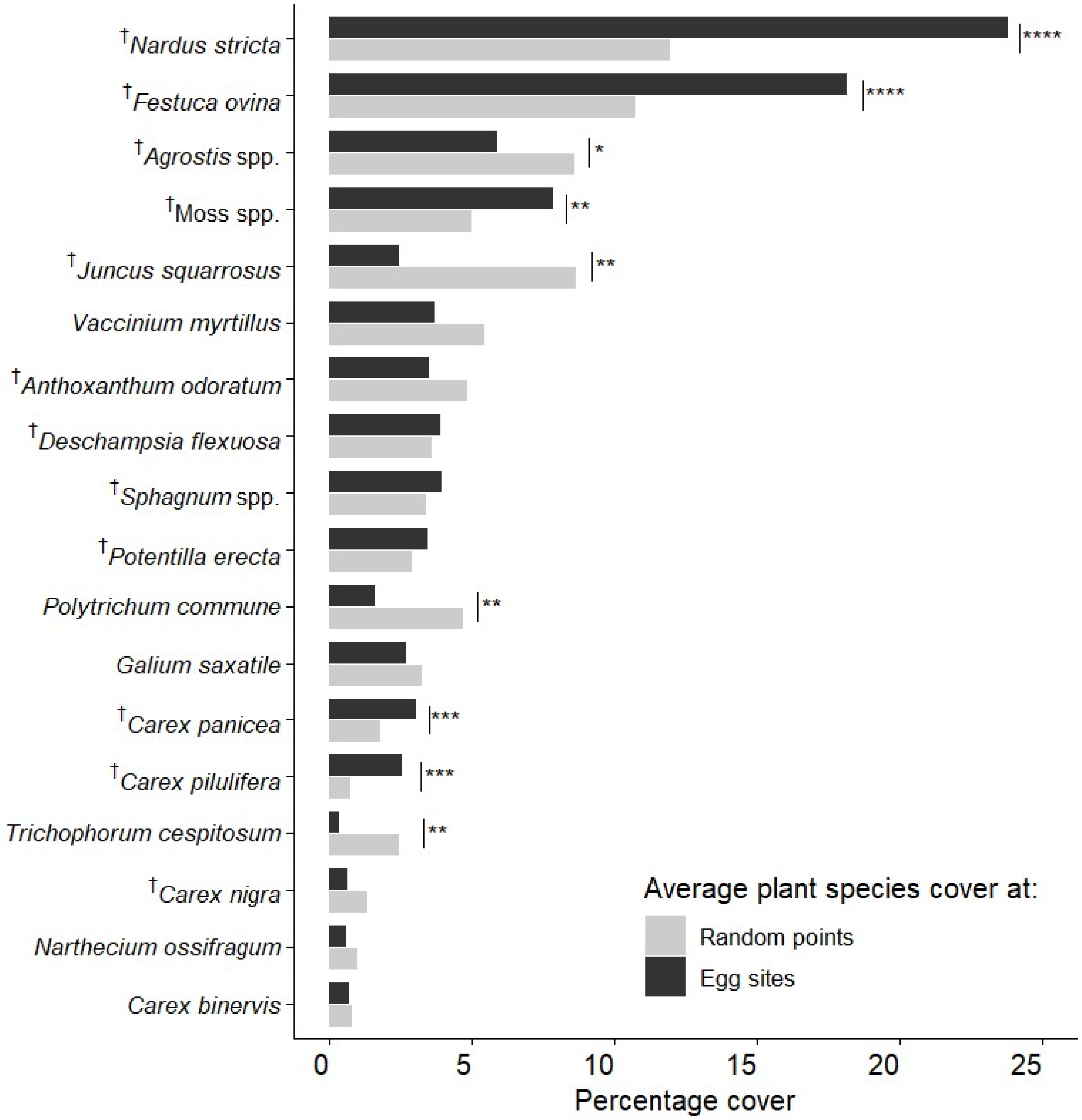
**Table 1a**

| no. | Model description | $K$ | AIC | ΔAIC | $W_i$ |
|---|---|---|---|---|---|
| **1** | **hostplants + lflitter + lflitter$^2$ + tussocks + veght + vegden + slp** | **9** | **314.25** | **0.00** | **0.339** |
| 2 | hostplants + lflitter + lflitter$^2$ + tussocks + veght + vegden + slp + asp | 10 | 315.06 | 0.81 | 0.226 |
| 3 | hostplants + lflitter + lflitter$^2$ + tussocks + veght + vegden + slp + alt | 10 | 315.09 | 0.84 | 0.222 |
| 4 | hostplants + lflitter + lflitter$^2$ + tussocks + veght + vegden + slp + alt + asp | 11 | 316.21 | 1.96 | 0.128 |
| 5 | hostplants + lflitter + lflitter$^2$ + tussocks + veght + slp + alt | 9 | 320.12 | 5.88 | 0.018 |
| 6 | hostplants + lflitter + lflitter$^2$ + tussocks + veght + slp | 8 | 320.15 | 5.90 | 0.018 |
| 7 | hostplants + lflitter + lflitter$^2$ + tussocks + veght + slp + asp | 9 | 321.78 | 6.84 | 0.011 |
| 8 | hostplants + lflitter + lflitter$^2$ + tussocks + veght + slp + alt + asp | 10 | 321.44 | 7.19 | 0.009 |
| 9 | hostplants + lflitter + lflitter$^2$ + veght + vegden + slp | 8 | 322.06 | 7.81 | 0.007 |
| 10 | hostplants + lflitter + lflitter$^2$ + veght + vegden + slp + alt | 9 | 323.21 | 8.96 | 0.004 |

**Table 1b**

| model term | estimate | std. error | model-averaged CIs |
|---|---|---|---|
| *host plant availability (hostplants)* | 1.418 | 0.228 | (0.955, 1.882) |
| *leaf litter (lflitter)* | 1.168 | 0.230 | (0.716, 1.619) |
| *leaf litter$^2$* | -0.524 | 0.169 | (-0.855, -0.193) |
| *tussocks cover (tussocks)* | -0.700 | 0.259 | (-1.206, -0.193) |
| *vegetation height (veght)* | -0.890 | 0.269 | (-1.418, -0.363) |
| *vegetation density (vegden)* | 0.604 | 0.228 | (0.158, 1.050) |
| *slope (slp)* | -0.785 | 0.234 | (-1.243, -0.327) |
| *altitude (alt)* | 0.371 | 0.369 | (-0.352, 1.094) |
| *aspect (asp)* | 0.426 | 0.420 | (-0.397, 1.249) |

834 **Figure 1**


†Nardus stricta
†Festuca ovina
†Agrostis spp.
†Moss spp.
†Juncus squarrosus
Vaccinium myrtillus
†Anthoxanthum odoratum
†Deschampsia flexuosa
†Sphagnum spp.
†Potentilla erecta
Polytrichum commune
Galium saxatile
†Carex panicea
†Carex pilulifera
Trichophorum cespitosum
†Carex nigra
Narthecium ossifragum
Carex binervis
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**
Average plant species cover at:
Random points
Egg sites
0
5
10
15
20
25
Percentage cover

**Figure 2**

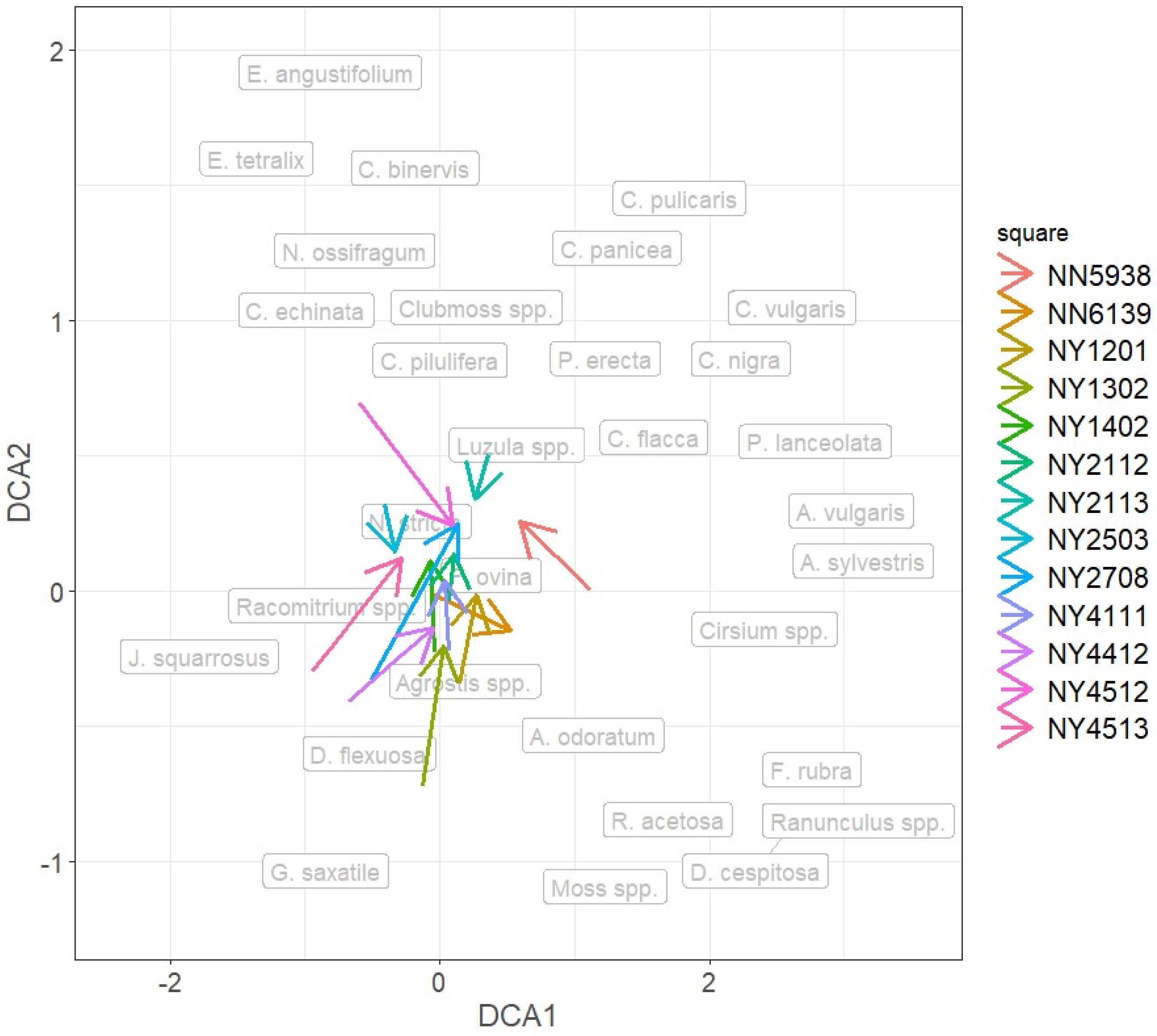
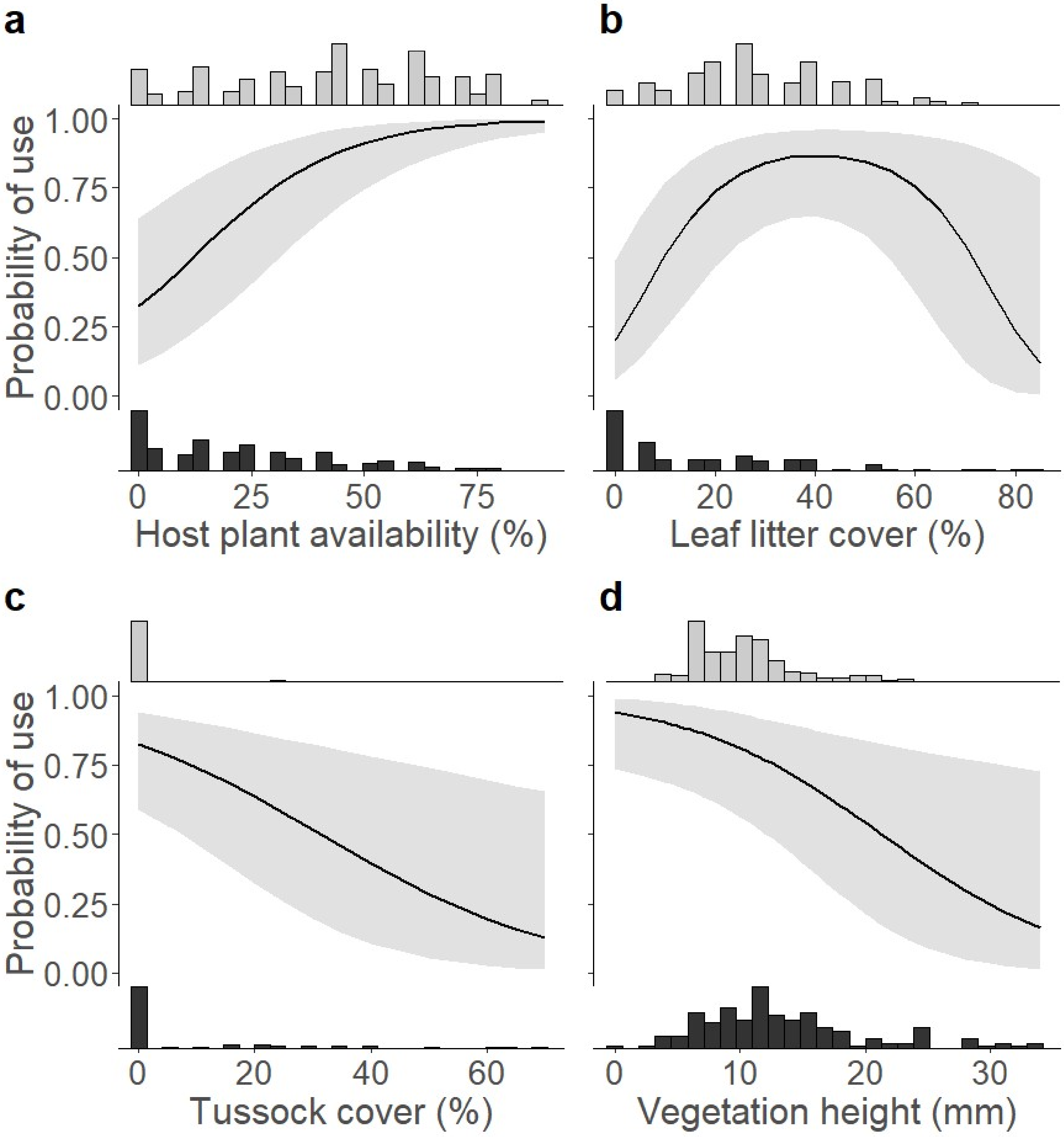

E. angustifolium
E. tetralix
C. binervis
C. pulicaris
N. ossifragum
C. panicea
C. echinata
Clubmoss spp.
C. vulgaris
C. pilulifera
P. erecta
C. nigra
Luzula spp.
C. flacca
P. lanceolata
N. stricta
A. vulgaris
A. sylvestris
F. ovina
Racomitrium spp.
Cirsium spp.
J. squarrosus
Agrostis spp.
A. odoratum
D. flexuosa
F. rubra
R. acetosa
Ranunculus spp.
G. saxatile
Moss spp.
D. cespitosa
2
1
0
-1
-2
0
2
DCA2
DCA1
square
NN5938
NN6139
NY1201
NY1302
NY1402
NY2112
NY2113
NY2503
NY2708
NY4111
NY4412
NY4512
NY4513

**Figure 3**


a
b
c
d
Probability of use
1.00
0.75
0.50
0.25
0.00
0
25
50
75
Host plant availability (%)
0
20
40
60
80
Leaf litter cover (%)
0
20
40
60
Tussock cover (%)
0
10
20
30
Vegetation height (mm)