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Grazing alters insect visitation networks and plant mating systems

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Summary

1. Many flowering plant species have a facultative or obligate dependence on insect pollination for reproductive success. Anthropogenic disturbance may alter these species interactions, but the extent to which structural changes to plant-pollinator networks affect plant species mating systems is not well understood.

2. We used long-term livestock grazing of a birch wood ecosystem to test whether disturbance of this semi-natural habitat altered floral resources, the structure of plant–insect visitation networks and the mating system of a focal plant species, *Cirsium palustre*.

3. Grazed habitat had a higher species richness of floral resources for pollinators. Visitation networks in grazed habitats were larger, more diverse, with an increase in the number of pollinators per plant species. Controlling for sampling effects, however, showed networks in grazed habitats were less nested and revealed a positive correlation between network connectance and floral species richness.

4. Network connectance was negatively related to *C. palustre* outcrossing rate within grazed and ungrazed sites. However, on average, the effects of grazing, including greater mean connectance, produced higher overall outcrossing rates and more pollen donors compared with ungrazed habitat. The number of different pollen donors, spatial genetic structure and mating among close relatives were all correlated with greater extent of suitable *C. palustre* habitat in the landscape, consistent with the effects of increasing plant population size but limited seed dispersal.

5. Pre-adaptation of *C. palustre* to disturbance coupled with a preponderance of highly dispersive flies attracted to the greater food resources in grazed habitat is a likely mechanism underpinning this increased pollen transport.

6. Habitat modification by long-term mammalian grazing fundamentally shifted visitation network structure and the state of a plant mating system, indicating how ecosystem disturbance can cascade across levels of biological organization through altered interspecific interactions. *Cirsium palustre* retains flexibility to bias reproduction towards selfing where pollen donor diversity is limited; such reproductive flexibility may be an important mechanism structuring plant populations in human-modified landscapes.

Key-words: bipartite networks, *Cirsium palustre*, landscape structure, land-use, outcrossing, pollinators, reproductive ecology, weighted model averaging

Introduction

Analysis of ecological interactions is critical to understand how environmental changes affect biodiversity (Fortuna & Bascompte 2006). One globally common anthropogenic disturbance to semi-natural habitat is livestock grazing. Ungulate grazers through consumption of plant biomass, trampling and excreta can modify ecosystems and alter species' interactions, plant phenological development and reproductive strategies (Grant *et al.* 1996; Jones, Lawton & Shachak 1997; Pykala 2004). Such plant community changes can subsequently affect abundance and diversity at higher trophic levels (Vulliamy, Potts & Willmer 2006; Winfree *et al.* 2009; Woodcock *et al.* 2009) and alter

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mutualistic and antagonistic interspecific interactions (Vazquez & Simberloff 2003; Vanbergen *et al.* 2006; Yoshihara *et al.* 2008). The effects of grazing on plant–pollinator interactions are likely to depend on habitat type, herbivore species or grazing intensity. For instance, cattle introduced to Patagonian forests altered the structure of plant–pollinator networks by extirpating a few abundant, generalist species that were highly connected with many less abundant species in the network (Vazquez & Simberloff 2003). In contrast, intensive cattle grazing of steppe vegetation served to strengthen plant–pollinator interactions by concentrating visitation to the few remaining grazing-tolerant ruderal plants (Yoshihara *et al.* 2008).

As most pollinators are mobile, the structure of the wider landscape can have a direct effect on their abundance, diversity (Sjodin, Bengtsson & Ekbom 2008; Carvell *et al.* 2011) and foraging interactions (Parsche, Frund & Tscharntke 2011). Pollinator responses to landscape structure tend to reflect differences in foraging or dispersal ranges between taxa or groups of species sharing particular traits (Sjodin, Bengtsson & Ekbom 2008; Williams *et al.* 2010; Parsche, Frund & Tscharntke 2011; Rader *et al.* 2011). As central location foragers, social bees can be sensitive to the distribution of nesting and forage habitats in the landscape surrounding the nest, although this depends on species identity and the extent of landscape simplification (Williams *et al.* 2010; Carvell *et al.* 2012; Kennedy *et al.* 2013). Non-social insects with free-living progeny (e.g. Diptera) are affected less by distance between resource patches (Jauker *et al.* 2009; Parsche, Frund & Tscharntke 2011). In either case, pollen can be transported by insect vectors from local to landscape scales (Dick, Etchelecu & Austerlitz 2003; Rader *et al.* 2011).

Many flowering plant species have a facultative or obligate dependence on insect pollination for reproduction and ultimately population persistence (Ollerton, Winfree & Tarrant 2011). Disruption of plant–pollinator interactions may affect plant species' ability to achieve outcrossed mating, increasing biparental inbreeding (i.e. mating among close relatives) or self-fertilization (Eckert *et al.* 2010). This increases the risk of inbreeding depression which – as outcrossing plant species often carry high loads of potentially deleterious alleles – can negatively impact plant fitness and may drive population evolutionary change (Ward *et al.* 2005; Eckert *et al.* 2010). Variation in conspecific plant densities or dispersion, such as arising from habitat fragmentation, may affect plant reproductive success by changing the pollinator-mediated connectivity between individuals in a plant population at fine (e.g. metres, Kunin 1997) and coarse (e.g. kilometres, Dick, Etchelecu & Austerlitz 2003) spatial scales. Habitat disturbance by grazing mammals may also directly (e.g. trampling, consumption) or indirectly (e.g. altered pollinator foraging) affect pollen deposition and seed set by altering the densities, dispersion and connectivity of conspecific plants (Vazquez & Simberloff 2004). It is not known, however, to what extent modification of interspecific interactions at a plant community level can cascade down to affect the mating systems (e.g. proportion of outcrossed to self-mated individuals, differences in paternity) of plant species.

We used long-term livestock grazing of a birch wood ecosystem to test whether disturbance of this semi-natural habitat modified community-level visitation networks and a plant mating system. *Cirsium palustre* (L.) Scop. (Asteraceae) was chosen as the focal species for the mating system analysis because it is an important pollinator forage plant common to many European habitats, it is dependent on insect pollinators for outcrossing, it is diploid making it amenable to quantitative analysis of plant mating systems and it occurred in both grazed and ungrazed birch habitat (Vanleeuwen 1981; Carvell *et al.* 2006). Previous work has shown that grazed birch woods support a greater diversity and abundance of grazing-tolerant flowering species than ungrazed woods (Vanbergen *et al.* 2006). We predicted that this elevation of floral resources would increase the diversity, connectivity and generalization of interactions in plant–insect visitation networks. In turn, we expected such changes to visitation network structure and – given pollinator mobility – the extent of *C. palustre* habitat in the landscape (as a proxy for its abundance) would both be related to variation in this plant species' mating system.

Materials and methods

STUDY SYSTEM AND HABITAT MODIFICATION

In Northern Scotland, birch woods (*Betula* spp.) are a widely distributed, semi-natural habitat that have had a long history of use for forage and shelter habitat for cattle (Armstrong *et al.* 2003). Twenty birch-dominated woods were selected from a pool of 3161 deciduous woodland fragments in the River Dee catchment of Aberdeenshire, Scotland, according to the presence (n = 10) or absence (n = 10) of livestock grazing (Fig. 1, Appendix S1: Table S1, Supporting information). Grazing was light to moderate (e.g. 2007: mean = 8·4 cattle ha^{-1}) and long term (median = 30 years, Appendix S1: Table S1 for more details, Supporting information). Landowners confirmed sites where livestock were absent had not been grazed by livestock for at least 70–100 years. Roe deer (*Capreolus capreolus* Linnaeus, 1758) herbivory occurred at all sites, but was lighter relative to the effects of cattle herds that were shut into woodlands for extended periods. The grazing treatment was not confounded by any other systematic management (e.g. logging). To assess cattle impact on the habitat, the community composition and structure of the herbaceous plant understorey were surveyed in each site (July 2007 & 2008) in a series of six quadrats (1 m^2) placed randomly on the nodes of a 4 × 4 grid (5 m spacing) situated 50 m from woodland edges (see Appendix S1 for details and analysis, Supporting information).

LANDSCAPE COVER OF SEMI-NATURAL HABITAT

Woodland area and the combined percentage cover of semi-natural habitats were derived from the CEH Land Cover Map (LCM 2007) of the UK, a computer-classified land cover map derived from satellite-based multispectral scanners. Semi-natural habitat was defined here as the sum percentage cover of the broad habitat classes: bog, marsh, acid grassland, rough low-productivity grassland, heather grassland, heather and dwarf shrub, deciduous

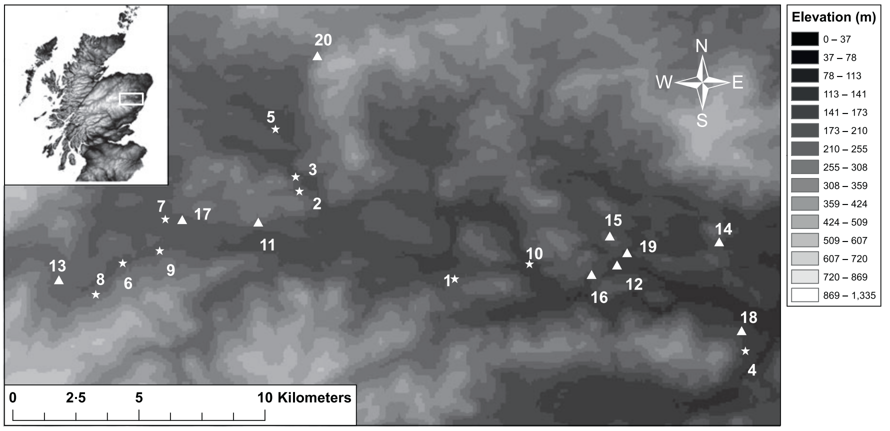

Fig. 1. Digital elevation map (SRTM 50 × 50 m) of Scotland with study area in the River Dee catchment denoted by the white and enlarged rectangles. Increasing elevation (m a.s.l.) indicated by lighter shading. Grazed woods are denoted by stars, ungrazed woods by triangles, numbers correspond to the woodland site description in Appendix S1: Table S1 (Supporting information).

woodland, all of which are utilized by a range of insect pollinators for forage, nesting, overwintering and mating habitat. Although this estimate of semi-natural habitat was not ground-truthed in this study, LCM2007 accuracy – the probability that a polygon of a particular LCM2007 habitat class accords with that on the ground – is calibrated by correspondence with the ground-based national Countryside Survey (CS2007) data, which recorded broad habitat cover across 591 1 × 1 km squares in Great Britain (Morton *et al.* 2011). Using correspondence data in Morton *et al.* (2011), the LCM2007 estimate (ha) of semi-natural habitat cover used in this paper had high (83%) correspondence with the CS2007 ground survey data. Semi-natural habitat cover was quantified using ArcGIS™ (version 9.3.1, ESRI®, Redlands, California 92373-8100, USA) in buffers (500, 1000, 1500, 2000, 2500, 3000 m radii) around the perimeter of each focal woodland encompassing known pollinator dispersal and foraging distances (Osborne *et al.* 2008; Rader *et al.* 2011; Carvell *et al.* 2012). Subsequently, woodland area and semi-natural habitat cover were accounted for when testing the prediction that habitat modification would affect visitation network structure.

INSECT VISITATION AND FLORAL RESOURCES

Of the 20 woodlands within which habitat modification was assessed only a subset (5 grazed, 5 ungrazed) were surveyed for insect visitation and floral resources (see Appendix S1: Table S1, Supporting information). This was done due to the trade-off between site replication and the logistical effort required in quantifying plant–pollinator interactions. Insect visitation was surveyed using two standardized transects (50 × 2 m; 15 m apart) randomly situated prior to the onset of flowering in the centre of each wood and at least 50 m from the woodland edge. Transects were walked (~10 m min^{-1}) in dry weather between 10:00 and 17:00 when the ambient temperature was at least 14 °C. Twenty visits were carried out between 20 May and 27 August 2009 with the order that sites were visited randomized on each date to avoid introducing systematic bias due to time of sampling. The species identity and frequency of all insect–flower interactions (physical contact with plant reproductive organs, nectar or pollen feeding) were recorded. Apart from species identifiable without killing (e.g. *Rhingia campestris,* Meigen, 1822, Syrphidae), all flower visitors were collected and subsequently identified to binomial species or recognizable taxonomic unit (RTU) for taxonomically difficult groups (Appendix S2, Supporting information). RTUs have been shown to be an effective approach to allocating species equivalent identifications where binomial species identification proves impractical (Oliver & Beattie 1996). The abundance and species richness of floral resources per woodland were quantified by counting the number of inflorescences (defined as a single flower or an umbel, spike or capitulum on multi-flowered stems) per plant species per transect across the season (May, June, July, August 2009).

To assess the sampling completeness of the visitation survey, we computed accumulation curves to determine how the insect species richness and the number of links (connectance) in the visitation network accrued with increased sampling effort (Chacoff *et al.* 2011). Sampling effort was defined as the number of censuses over the field season. Separate accumulation curves were assessed for ungrazed or grazed sites pooling the site-level data in each category, but splitting it into the 20 census dates. For each accumulation curve, these network data by census date were randomly selected and added together (e.g. census $x_1 + x_2$) after which species richness and number of links were recalculated. This step was repeated (e.g. census $x_1 + x_2 + x_3$…) until the network comprised all the separate census webs. This overall process was repeated a total of 500 times.

NETWORK INDICES

A matrix of the sum of recorded visits for every pair-wise insect–plant interaction was created for each wood. Network architecture parameters were derived using the 'bipartite' package in the R statistical environment (Dormann *et al.* 2009) to test the prediction that grazing would enhance the diversity, generalization and connectivity of these networks. The chosen indices were as follows: (i) nestedness (NODF) which reflects the tendency for specialist species to interact with generalists in mutualistic networks; (ii) connectance (*C*), the number of realized links in the network; (iii) vulnerability, the weighted mean number of insect visitors per plant species (*Vqw*), an increase in this metric indicates a more robust network; (iv) linkage density (*Lq*), the mean number of links per species weighted by the frequency of interactions; and (v) the interaction diversity (*H*′) of the web (Tylianakis, Tscharntke & Lewis 2007; Dormann *et al.* 2009; Ulrich, Almeida-Neto & Gotelli 2009).

We also performed these analyses after controlling for the varying number of individuals sampled across different sites using rarefaction to standardize network size (Gotelli & Colwell 2001). This was achieved by random deletion of insect species from each site-level network and subsequent recalculation of network metrics (connectance, *Vqw, Lq, H'*). We repeated this 1000 times, and a line of best fit through the mean of these values setting network size to that of the smallest meaningful network (Site 16 = 57 individuals) was used to derive an overall standardized value for each index. While nestedness is more robust to sampling effort, it also tends to increase with the number of interactions in a network (Bascompte *et al.* 2003; Nielsen & Bascompte 2007). Thus, the absolute values of nestedness (NODF) generated for each site were compared to a null model (as detailed in Vázquez *et al.* 2007) of the network (1000 randomizations) to enable the calculation of a standardized z-score ($Z = [x-\mu]/\sigma$). This z-score allowed comparison of nestedness across networks of different size and complexity (Almeida-Neto *et al.* 2008; Ulrich, Almeida-Neto & Gotelli 2009). The abundance of potential insect visitors to *C. palustre* was also calculated assuming that (i) an insect species recorded visiting other *Cirsium* spp. (*C. vulgare, C. arvense*) would also visit *C. palustre* and (ii) an insect species recorded visiting *Cirsium* spp. at a given site was likely to have visited *Cirsium* spp. at other sites, even if only physically recorded at these other sites on alternative plant species.

PLANT MATING SYSTEM

Cirsium palustre is a common herbaceous plant across Europe, is found in all our study woodlands and is an important pollinator forage plant (Carvell *et al.* 2006). It is diploid, predominantly outcrossing, but is self-compatible and may have the capacity for autonomous self-pollination (Mogford 1974; Vanleeuwen 1981). At the end of flowering (September 2009), multiple seed heads were collected from 3 to 4 mother plants per wood used in the study of insect visitation ($n = 9$, very heavy cattle trampling immediately prior to sampling destroyed the available thistle standing crop in the woodland at Site 1). Seed was germinated in glasshouses, and DNA was extracted from 15 to 20 seedlings per mother plant depending on availability. Each seedling was genotyped using a set of four nuclear microsatellite loci [Caca01, Caca05, Caca10, Caca22 (Jump *et al.* 2002)]. Basic statistics and tests for presence of null alleles were conducted using Genepop 4.1.1 (Rousset 2008). Using the mixed-mating model approach (Ritland & Jain 1981) and the software mltr (Ritland 2002), the following mating system parameters were derived: single (t_s) and multilocus (t_m) outcrossing rate, single (rp_s) and multilocus (rp_m) correlation of paternity, selfing due to biparental inbreeding, that is, matings among related individuals ($t_m - t_s$) and the degree of relatedness among pollen donors ($rp_m - rp_s$). See Appendix S3 (Supporting information) for more detail.

LANDSCAPE EXTENT OF *C. PALUSTRE* HABITAT

Pollinators such as bees and flies forage at spatial scales from metres to kilometres (Rader *et al.* 2011; Carvell *et al.* 2012), and so variation in *C. palustre* mating systems might also be due to pollinator foraging between plants in the wider landscape surrounding the woodland fragment (Dick, Etchelecu & Austerlitz 2003). Resource constraints precluded a landscape survey of *C. palustre* populations, so we developed an indicator of the extent of habitat suitable for *C. palustre*, effectively an index of its potential niche space, in a landscape buffer surrounding each birch wood. First, we used the National Vegetation Classification (Rodwell 1991) to identify the plant communities for each LCM habitat class and, according to its known vegetation associations, estimated the mean abundance (DOMIN) of *C. palustre* in each associated LCM class (above semi-natural habitats plus coniferous forest). This metric was then weighted according to the percentage cover of these LCM habitat classes in a 500 m radius around each wood to give an index of the possible extent of suitable *C. palustre* habitat (Cp_{habitat}) for each site to be subsequently fitted as a covariate to plant mating system models. As with the estimate of semi-natural habitat cover and following Morton *et al.* (2011), this measure of the extent of suitable *C. palustre* habitat derived from LCM2007 was determined to have a similarly high level of congruence (84%) with CS2007 ground survey data.

STATISTICAL ANALYSIS

Visitation network and plant mating parameters per woodland were analysed with generalized linear models (GLM, SAS 9.01, SAS Institute Inc., Cary, NC 27513-2414, USA) with a Gaussian distribution. Where required data were log- and logit (proportions)-transformed to ensure that model assumptions of residual homogeneity of variance and normality were met. Prior to analysis, we tested for spatial autocorrelation in the explanatory and response variables using Moran's/with an inverse distance weighting.

An information theoretical approach for small sample sizes (AICc) was used to define a subset of models in which there was 95% confidence that the most appropriate model was included (Burnham & Anderson 2002; Whittingham *et al.* 2006). Given the low replication of sites, we limited the candidate list of explanatory variables for our models to five parameters expected to be important based on known pollinator ecology and after initial data exploration. The minimum distance between sampling locations was 1555 m, and the proximity of three sites in particular (Appendix S1, Table S1: sites 15, 16 and 12, Supporting information) meant that for reasons of statistical independence only the percentage cover of semi-natural habitat at 500 m radius could be fitted to subsequent models of insect visitation networks (below). However, data exploration showed this 500 m estimate of semi-natural habitat cover was positively correlated with the estimates at all other buffer scales (Spearman rank correlation coefficient ranged from 0·66 to 0·89; 1000–2000 m all $P < 0{\cdot}01$; 2500 & 3000 m $P < 0{\cdot}05$). This implied that the estimate of semi-natural cover these foraging insects would encounter in the local landscape (500 m buffer) would also be a reasonable indicator of the effects of semi-natural habitat extent in the wider landscape. Thereafter, the five explanatory variables for the visitation network responses were livestock presence, mean floral abundance, floral species richness, woodland area and landscape cover (500 m radius) of semi-natural habitat. Explanatory variables fitted to models of the plant mating system were the (i) livestock presence, (ii) Cp_{habitat}, (iii) the abundance of insects recorded interacting with *Cirsium* spp., (iv) rarefied network connectance and (v) z-score of nestedness (NODF). The choice of the latter two variables was informed by the outcome of the visitation network analysis.

For network data, the candidate set of models was restricted to the null model (intercept-only), individual univariate effects of each parameter and all possible pair-wise combinations of parameters. For mating system data, multivariate models always included grazing in addition to all possible pair-wise combinations of the remaining four parameters. This was because we had an *a priori* expectation that grazing would affect both the mating system and network parameters, and so we wanted to ensure the analysis examined network effects only after direct grazing effects had been accounted for. For both network and mating system analyses, there were thus a total of 16 competing models (Whittingham *et al.* 2006). For each of these models, an AICc difference (Δ_i) was calculated to estimate the relative support for each model:

$$\Delta_i = \mathrm{AICc}_i - \mathrm{AICc}_{\min}$$

where $\mathrm{AICc}_{\min}$ is the lowest recorded value for any model and AICc_i is the model-specific AICc value. From this, Akaike weights

(w_i) (Burnham & Anderson 2002) were subsequently calculated as:

$$w_i = \frac{\exp[1 - \frac{1}{2}\Delta_i]}{\sum_{r=1}^{R} \exp\left[-\frac{1}{2}\Delta_r\right]}$$

where w_i represents the probability that model i would be selected as the best fitting model if the data were collected again under identical conditions. As the w_i of all R models sums to 1, this provided the basis for selecting a subset of models where there is a 95% confidence that this subset contains the most appropriate model of those considered for the data. This approach to variable selection allows consideration of all models in the candidate set, while weighting them for their plausibility. Where multiple competing models exist (i.e. the models within the 95% confidence set), an approach is needed to provide estimates for the parameters (β) identified. To achieve this, parameter estimates for the

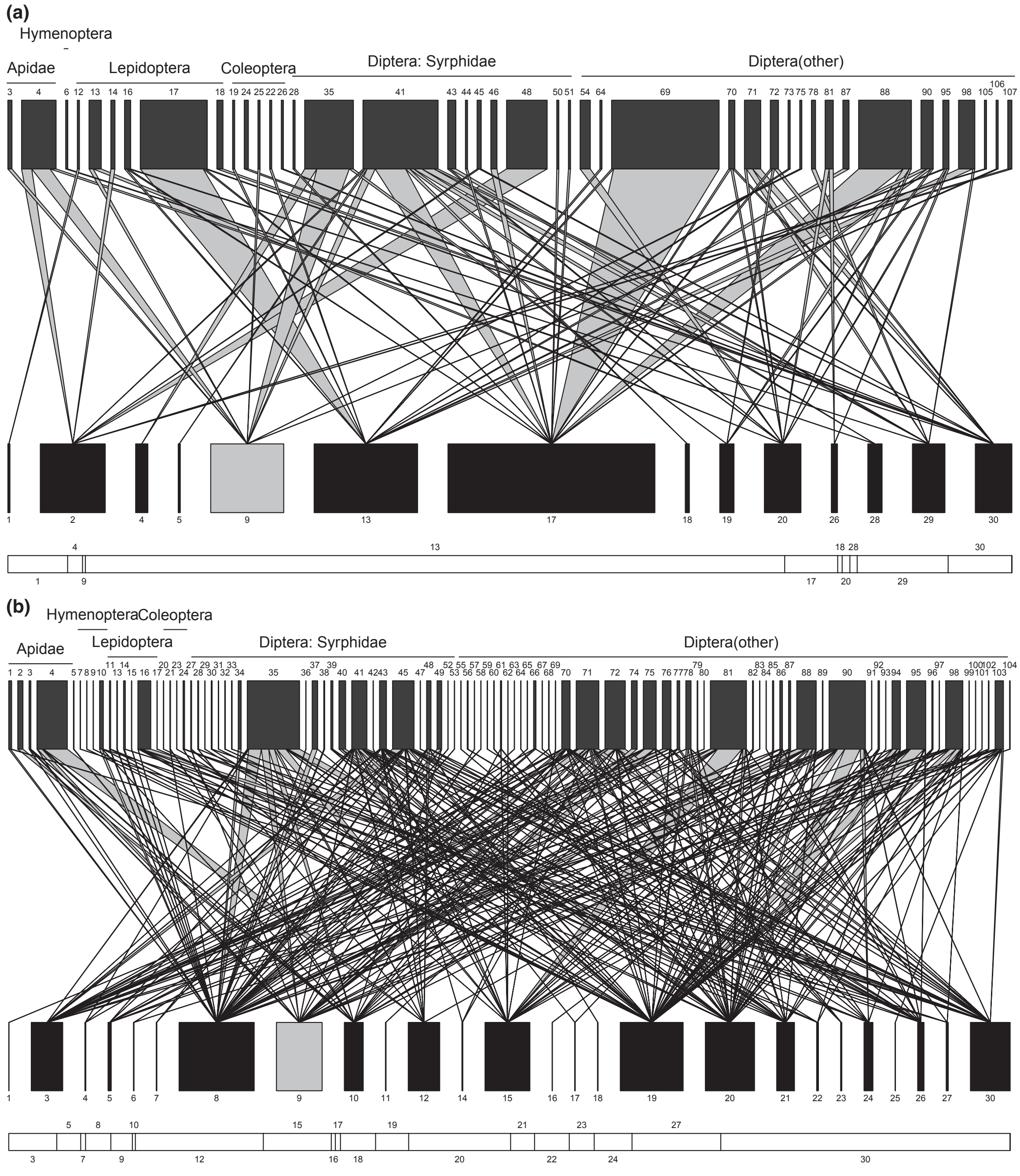

Fig. 2. Bipartite insect–plant visitation networks summed across (a) four ungrazed and (b) five grazed birch woodlands. The upper and lower levels of the networks comprise the insects and plants, respectively. The thickness of individual bars (i.e. a single species) is proportional to the summed visitation frequency for that species in the network. The lines between the two levels of the network represent the interactions between plant and insect species pairs with line thickness proportional to increasing frequency. Floral abundance (only species with >20 floral units), independent of recorded interactions, of the plant species present in the networks is displayed in a composite column graph beneath each network. See Appendix S2 (Supporting information) for species/RTU names relating to plant (P) and insect (I) codes, note grey bar is the focal plant *Cirsium palustre*.

explanatory variables were weighted by their Akaike weight and then averaged across all models (Burnham & Anderson 2002). This was done using:

$$\beta_j = \sum_{i=1}^{R} w_i \beta_{ji}^{+}$$

where w_i is the Akaike weight for model i, and β_{ji}^{+} represents the estimate of β_i if parameter j is included in the model. Where parameter j is not included in the model, β_i is set at zero.

Results

HABITAT MODIFICATION

The initial vegetation survey (2007–2008) showed that dicotyledonous herb and grass species dominated grazed habitats whereas ungrazed assemblages were typified by plants providing few foraging resources for pollinators such as pteridophytes, sedges, bryophytes and grasses intolerant of grazing (Appendix S1, Supporting information, also see Vanbergen *et al.* 2006). The survey of pollinator floral resources (2009) revealed greater species richness of inflorescences in the grazed compared with ungrazed habitat (means ± SEM: grazed = 14·8 ± 2·6, ungrazed = 7·0 ± 1·2; GLM $F_{1,8}$ = 8·29, P = 0. 02; Figs 2 and 3a). In contrast, inflorescence abundance did not differ between ungrazed and grazed habitat (ungrazed: 3887·8 ± 1942·9, grazed: 2628·2 ± 847·59; GLM $F_{1,8}$ = 0·00 P = 0·99). Although a superabundance of *Galium saxatile* L. flowers contributed much to the total floral abundance in ungrazed sites (Fig. 2a, Appendix S1: Table S1 – sites 12 & 15, Supporting information), they were comparatively poorly visited by insects. Elimination of *G. saxatile* from the data set did not alter the lack of a statistically significant difference in floral abundance between grazed and ungrazed habitat (ungrazed: 1189·2 ± 477·9, grazed: 2587·2 ± 854·83, GLM $F_{1,8}$ = 2·42, P = 0·16).

VISITATION NETWORKS

Networks were constructed for the nine surveyed sites (excluded site 14: Fig. 1 & Appendix S1: Table S1, Supporting information) that yielded sufficient data to calculate raw network indices and the z-score of NODF. Another ungrazed network (site 16, Fig. 1 & Appendix S1: Table S1, Supporting information) was eliminated following rarefaction due to insufficient data, thereby reducing sample size to eight sites in the analysis of standardized network indices: *C*, *Vqw*, *Lq*, *H'*. Accumulation curves showed that the number of sampled links (connectance) in the network quickly reached a plateau between 5 and s10 censuses (Appendix S4, Supporting information). Whereas the total of 20 censuses was insufficient to completely sample the species pool, the differences between grazed and ungrazed networks were nevertheless substantial (Appendix S4, Supporting information).

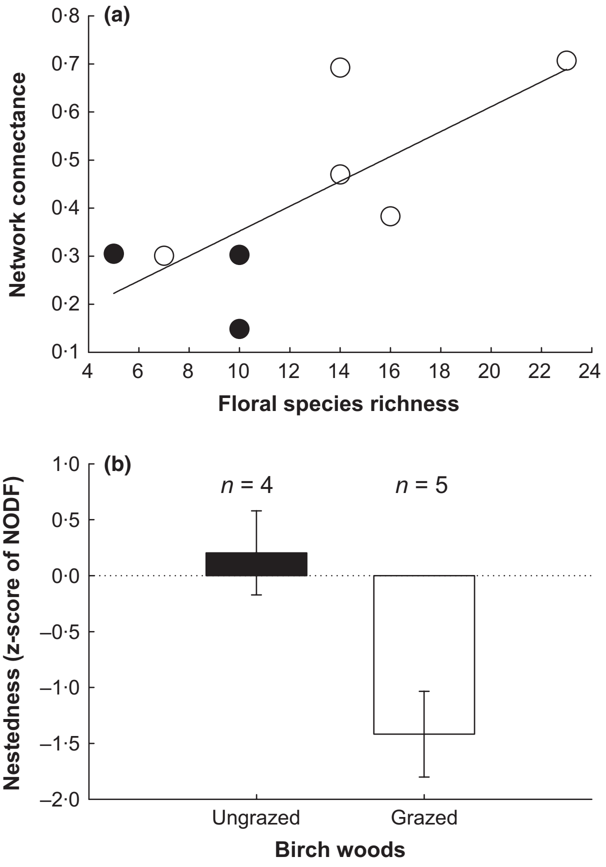

Fig. 3. The effect on visitation network (a) connectance and (b) nestedness (standardized by z-transformation of the NODF index) of increased floral species richness (associated with grazing) and cattle presence, respectively. Black symbols/bars = ungrazed woods, open symbols/bars = grazed woods. Data in (b) are means ± SEM, where the negative departure from zero indicates grazed sites were less nested than ungrazed sites. Data limitations mean that n = 8 sites for (a) and 9 sites for (b) – see results for details.

Both grazed and ungrazed networks were dominated by flies (Syrphidae, Muscidae, Scathophagidae), with bees (Apidae) and other insects (Coleoptera, Lepidoptera), a minor component of the community (Fig. 2). Grazed woods supported a greater number of plant and insect visitor species than ungrazed woods (Fig. 2). Summed selection probabilities (w_i) from GLMs showed that the presence of cattle increased the interaction diversity of the visitation networks (Mean ± SE grazed: 3·65 ± 0·13; ungrazed: 2·28 ± 0·37, Table 1). Moreover, the linkage density and the mean number of insect visitors per plant species (vulnerability *Vqw*) were positively related to the greater floral species richness of grazed sites (Table 1). Once network size (number of individuals sampled) was standardized by rarefaction, the effects of cattle grazing on interaction diversity and floral richness on linkage density and vulnerability were, however, either dramatically reduced or eliminated (Table 1).

However, network connectance was positively related to the greater floral richness of grazed woods after rarefaction (Fig. 3a, Table 1). Moreover, while cattle grazing

Table 1. The best set of general linear models (95% confidence set) explaining the effects of woodland management, floral abundance and richness, woodland area and landscape semi-natural habitat cover on visitation network properties. The model summed selection probability (w_i) – summed across all models where a predictor was present – and parameter estimates (β) are presented. Parameter estimates were generated by averaging across all models, using the selection probabilities to weight this process. Semi-natural habitat cover was estimated in a buffer of 500 m radius around each site. The count and percentage of total (n = 16) candidate models contained in 95% subset are in parentheses for each network parameter, and those with $w_i > 0{\cdot}40$ are highlighted in bold

Network index	Management (Grazed/Ungrazed)	Floral abundance (mean count)	Floral richness (sum)	Area (ha^{-1})	Landscape structure (% Semi-natural)
Nestedness (NODF) (8, 50%)					
w_i	0·37	0·09	0·13	0·03	0·08
β	2·03/3·53	$4{\cdot}50 \times 10^{-04}$	0·05	0·00	−0·01
Z-score of NODF (8, 50%)					
w_i	**0·58**	0·08	0·03	0·12	0·02
β	**−0·92/0·10**	$1{\cdot}49 \times 10^{-04}$	$-2{\cdot}02 \times 10^{-03}$	0·00	$-2{\cdot}25 \times 10^{-03}$
Linkage density (4, 25% rarefaction: 5, 31%)					
w_i	0·33	0·00	**0·87**	0·11	0·00
β	0·07/0·08	0·00	**0·03**	$3{\cdot}65 \times 10^{-03}$	0·00
Rarefaction w_i	0·05	0·04	0·04	0·05	0·00
Rarefaction β	−0·01/0·09	$4{\cdot}18 \times 10^{-06}$	$1{\cdot}62 \times 10^{-04}$	0·00	0·00
Connectance (8, 50%; rarefaction: 6, 37%)					
w_i	0·22	0·02	**0·60**	0·23	0·00
β	−0·01/0·06	0·00	$\mathbf{-2{\cdot}97 \times 10^{-03}}$	0·00	0·00
Rarefaction w_i	0·13	0·29	**0·43**	0·02	0·00
Rarefaction β	0·03/0·03	$-1{\cdot}00 \times 10^{-04}$	**0·01**	0·00	0·00
Vulnerability (both: 5, 31%)					
w_i	0·18	0·04	**0·90**	0·19	0·00
β	0·07/0·05	$-1{\cdot}56 \times 10^{-06}$	**0·03**	0·00	0·00
Rarefaction w_i	0·04	0·00	0·05	0·05	0·05
Rarefaction β	−0·02/0·11	0·00	$1{\cdot}73 \times 10^{-03}$	0·00	$5{\cdot}91 \times 10^{-04}$
Interaction diversity (both: 5, 31%)					
w_i	**0·88**	0·15	0·03	0·00	0·25
β	**1·02/2·29**	0·00	$1{\cdot}91 \times 10^{-03}$	0·00	$-3{\cdot}75 \times 10^{-05}$
Rarefaction w_i	0·14	0·05	0·12	0·00	0·04
Rarefaction β	−0·11/0·30	$3{\cdot}21 \times 10^{-05}$	$-8{\cdot}91 \times 10^{-03}$	0·00	$-1{\cdot}93 \times 10^{-04}$

increased the absolute nestedness of the networks (NODF Mean ± SE grazed: 14·91 ± 0·92; ungrazed: 9·87 ± 2·38, Table 1), once variation in network size was controlled for (z-score of NODF), it was revealed that network nestedness was reduced by grazing (Fig. 3b, Table 1).

Floral abundance, woodland area and landscape structure were not important in explaining variation in either the raw or standardized visitation network parameters (Table 1).

PLANT MATING SYSTEM

Despite substantial variation among plant families (Appendix S3: Table S1, Supporting information) and woodland sites (Appendix S3: Table S2, Supporting information), the *C. palustre* mating system was strongly related to cattle grazing, the connectance of visitation networks and the landscape extent of *C. palustre* habitat, a possible proxy for the abundance of this wild plant.

The multilocus outcrossing rate within both grazed and ungrazed contexts tended to decrease with increasing network connectance (Fig. 4a, Table 2). However, grazing was associated with a mean increase in the multilocus outcrossing rate of *C. palustre* from 0·47 in ungrazed sites to 0·74 (note: 1·0 = fully outcrossed) in grazed sites (Table 2), partly reflecting the increased mean network connectivity (Fig. 4a) associated with the greater floral richness of grazed woods (Fig. 3a). There was also evidence that higher numbers of pollen donors were contributing to fertilization in *C. palustre* families in grazed woods as both the single- and multi-locus correlation of paternity were lower in grazed compared to ungrazed habitat (Fig. 4b, Table 2).

Data on either per cent cover or floral densities of *C. palustre* in each site were too sparse to fit to subsequent models. However, the index of landscape-scale habitat suitability for *C. palustre* (Cp_{habitat}), a possible indicator for the abundance of this plant species, was strongly correlated with variation in mating system parameters. Correlation of paternity (Fig. 5a, Table 2) decreased with the increasing extent of suitable *C. palustre* habitat in the local landscape. Spatial genetic structure ($rp_m - rp_s$) increased in response to greater extent of *C. palustre* habitat (Fig. 5c, Table 2). The extent of biparental inbreeding ($t_m - t_s$) was

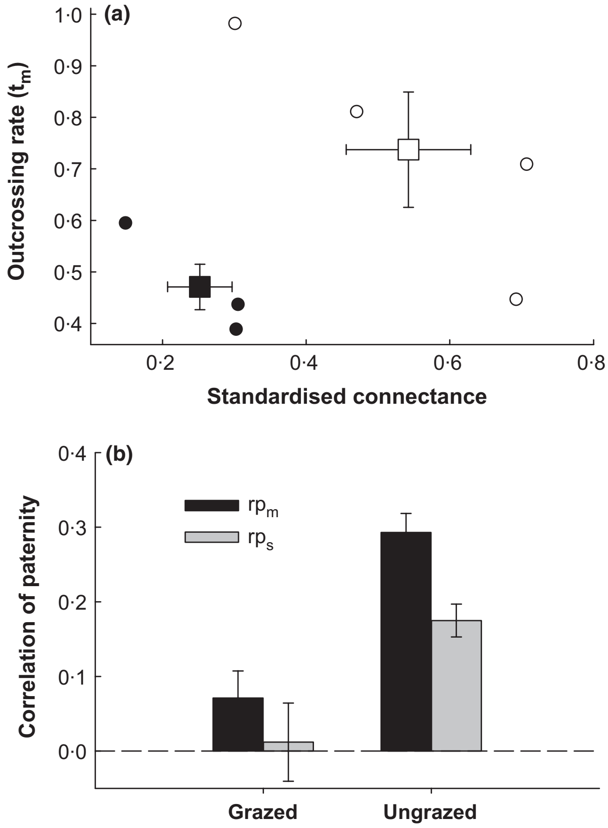

Fig. 4. The effect of (a) network connectance and (b) cattle grazing on (a) the outcrossing rate (multi-locus: t_{m}) and (b) the correlation of paternity in *Cirsium palustre* progeny. Black and open symbols in (a) are ungrazed and grazed sites, respectively, with square symbols the means ± SEM. Black and grey bars in (b) are mean multilocus ($\mathrm{rp_m}$) and single-locus ($\mathrm{rp_s}$) correlation of paternity ± SEM in ungrazed and grazed sites.

positively related to the landscape extent of *C. palustre* habitat, indicating an increase in mating events among closely related plants (Fig. 5b, Table 2). While there appeared a slight tendency towards a greater extent of *C. palustre* habitat in the local landscape of grazed habitat (Fig. 5), this was not statistically significant (GLM $F_{1,7} = 4·31$, $P = 0·08$).

The abundance of insects recorded visiting flowers of *Cirsium* spp. (*C. palustre*, *C. vulgare*, *C. arvense*) was not a major predictor of variability in the plant mating system once the effects of grazing, network connectance and landscape extent of *C. palustre* habitat were taken into account (Table 2).

SPATIAL AUTOCORRELATION

Spatial autocorrelation in all the explanatory variables used in models of visitation networks and plant mating systems was low (all Moran's $I < 0·216$), although significant clustering was detected in semi-natural habitat cover ($P = 0·041$) and mean floral abundance ($P = 0·008$). Autocorrelation in the distribution of cattle grazing only approached statistical significance ($P = 0·052$). Spatial autocorrelation was also low among the ten response variables (Table 1) for visitation network ($I < 0·173$ and $P > 0·05$ in all cases) and six response variables (Table 2) for plant mating system ($I < 0·150$ and $P > 0·05$ in all cases) analyses. Therefore, spatial dependence was unlikely to have been a serious issue for the analyses in this paper.

Discussion

GRAZING-DRIVEN CHANGES TO VISITATION NETWORKS

Consistent with earlier studies in this (Vanbergen *et al.* 2006) and other ecosystems (Grant *et al.* 1996; Pykala 2004), the grazed plant assemblages were typified by a greater number of species that offer floral resources to visiting insects. This difference in plant community structure had a direct impact on visitation network structure between grazed and ungrazed sites. In grazed habitats, networks were larger, more diverse with an increase in the number of pollinators per plant species (*Vqw*), all properties with the potential to confer robustness to environmental stressors through greater species redundancy (Memmott, Waser & Price 2004). However, these differences in linkage density (*Lq*), interaction diversity (*H'*) and vulnerability (*Vqw*) identified between networks in the two habitat types were contingent on network size, that is, the number of pollinator individuals recorded during sampling (Gotelli & Colwell 2001; Ulrich, Almeida-Neto & Gotelli 2009). The observed changes between grazed and ungrazed habitats in these raw indices therefore reflect abundances of foraging insect species as opposed to any fundamental shift in network structure.

In contrast, cattle grazing and attendant habitat modification was related to a fundamentally increased connectance and decreased nestedness (NODF) of the standardized visitation networks (Almeida-Neto *et al.* 2008; Ulrich, Almeida-Neto & Gotelli 2009). Nested networks are very cohesive because they are centred on a core set of interactions among generalist species. Moreover, another property of nested networks is that specialist species only interact with species in this generalist, and thus less fluctuating, subset of the network (Bascompte *et al.* 2003). This cohesiveness and organizational asymmetry of specialist interactions is thought to lend a degree of stability to the community by allowing rarer specialists to persist (Bascompte *et al.* 2003). The lower relative nestedness of the networks in the grazed habitat implies that this community is potentially less buffered against loss of specialist interactors, which might in the long term have consequences for community stability and pollination functions in the face of further environmental change.

One notable feature of these woodland networks is that they are dominated by flies (Syrphidae, Muscidae, Scathophagidae) and not by bees (Apidae), partly because bee species richness is lower at such high latitudes (Williams & Osborne 2009). It is also likely that

Table 2. The best set of general linear models (95% confidence set) explaining the effects of woodland management (grazed or ungrazed), landscape extent of *Cirsium palustre* habitat, *Cirsium* visitor abundance and visitation network properties (connectance, NODF) on the *C. palustre* mating system. The model selection probability (w_i) summed across all models where a predictor was present and parameter estimates are presented. Parameter estimates (β) were generated by averaging across all models and weighted by the selection probabilities. The count and percentage of total (n = 16) candidate models in 95% subset are in parentheses for each mating system parameter, and those with w_i > 0·40 are highlighted in bold

Mating system parameter	Management (Grazed/Ungrazed)	*Cphabitat* (log)	Cirsium visitor (log abundance)	Connectance (rarefaction)	NODF (Z-score)
Single-locus outcrossing rate, t_s (5, 31%)					
w_i	0·30	0·29	0·00	0·33	0·12
β	0·34/0·14	0·31	0·00	−0·47	−0·01
Multilocus outcrossing rate, t_m (3, 19%)					
w_i	**0·88**	0·07	0·20	**0·88**	0·00
β	**1·62/1·06**	0·11	−0·21	**−2·95**	0·00
Biparental inbreeding $t_m - t_s$ (4, 25%)					
w_i	0·30	**0·51**	0·07	0·00	0·00
β	−0·02/−0·04	**0·07**	$-2{\cdot}34 \times 10^{-03}$	0·00	0·00
Single-locus correlation of paternity, rp_s (5, 31%)					
w_i	**0·61**	0·29	0·04	0·00	0·00
β	**−0·07/−0·52**	−0·04	$-2{\cdot}65 \times 10^{-03}$	0·00	0·00
Multilocus correlation of paternity rp_m (5, 31%)					
w_i	**0·40**	**0·58**	0·02	0·00	0·00
β	**−0·14/−0·24**	**−0·29**	$-5{\cdot}12 \times 10^{-03}$	0·00	0·00
Spatial genetic structure ($rp_s - rp_m$) (4, 25%)					
w_i	0·24	**0·53**	0·04	0·00	0·00
β	0·01/−0·12	**0·07**	$9{\cdot}08 \times 10^{-04}$	0·00	0·00

grazed habitat provided additional niches for flies, both in the form of enhanced floral resources but also cattle dung as a larval habitat and food resource for many of the recorded species (e.g. Muscidae, Scathophagidae) (Laurence 1954). This prevalence of flies may also explain the lack of any effect on network structure of habitat area and landscape extent of semi-natural habitat because flies, unlike social or solitary bees, are not central place foragers with ranges limited by nest location (Jauker *et al.* 2009; Williams *et al.* 2010; Parsche, Frund & Tscharntke 2011; Rader *et al.* 2011).

Unobserved network linkages are a common feature of ecological networks either due to under sampling or actual biological reasons. Accumulation curves showed that sampling effort was sufficient to fully characterize network connectance and, without reaching an asymptote, reveal large differences in species richness between grazed and ungrazed habitat. However, we lack a complementary data set (i.e. insect pollen loads) that would have allowed us to quantify the extent these visitation networks missed particular, probably rarer, species interactions (Olesen *et al.* 2010). Appropriate caution is required in interpretation of these results because network parameters were calculated from interaction matrices summed over a season. Consequently, there is some chance that forbidden links arising from non-overlapping phenophases or physical size constraints (Olesen *et al.* 2010) may have been included in network parameter estimates. However, the focus of this study was comparison of networks from grazed and ungrazed habitat, and we have no reason to suspect the amount of forbidden links would have systematically varied with grazing to confound this analysis.

GRAZING AND LANDSCAPE EFFECTS ON A PLANT MATING SYSTEM

The impact of cattle grazing on the mating system of *C. palustre* was complex, suggesting that multiple processes govern plant outcrossing in this insect-mediated system (Vanleeuwen 1981). First, *C. palustre* outcrossing rates were negatively correlated with increased community connectance within both grazed and ungrazed sites. This suggests that increased connectivity of insect visitation networks may, as a consequence of visitation to a greater diversity of plant species, decrease conspecific pollen transfer either through reduced visitation rates to *C. palustre* or increased deposition of heterospecific pollen onto stigmas. However, despite the negative relationship between outcrossing and network connectance, on average, the effects of grazing livestock on the habitat were related to increased *C. palustre* pollen transfer between unrelated individuals compared to ungrazed contexts. This relative shift in the state of the plant mating system was also seen in the reduction in the correlation of paternity, meaning that higher numbers of different pollen donors were contributing to fertilization in *C. palustre* families in grazed habitat. The state shift in outcrossing and paternity, when the *C. palustre* mating system is embedded in a

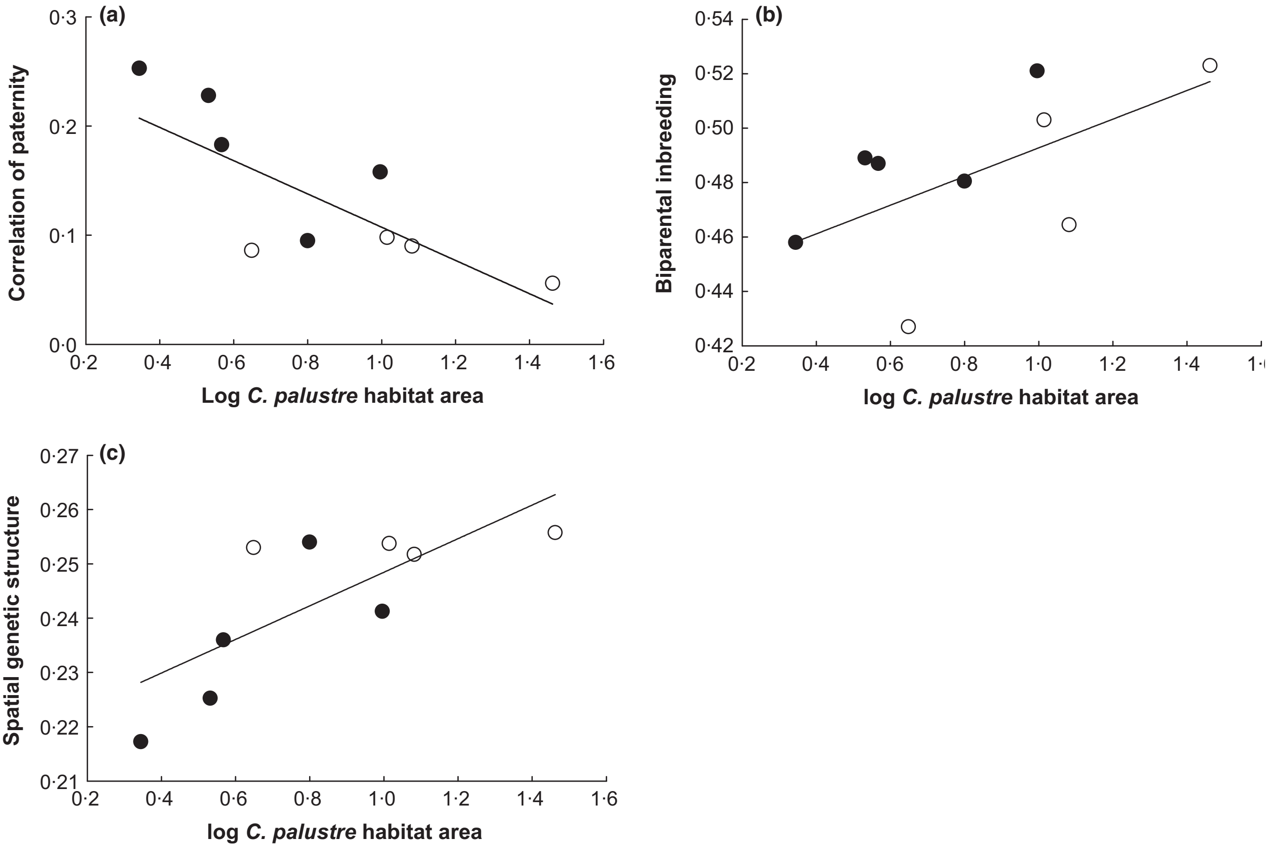

Fig. 5. The effect of the landscape cover of *Cirsium palustre* habitat on (a) the correlation of paternity, (b) biparental inbreeding rate ($t_m - t_s$) and (c) pollen donor spatial genetic structure ($rp_m - rp_s$) in *C. palustre* progeny. Decreasing correlation of paternity equals an increasing number of different conspecific pollen donors. The spatial genetic structure of pollen donors is indicated by the difference between the single- and multilocus paternity estimates ($rp_m - rp_s$). The difference between the estimates of single- and multi-locus outcrossing rates ($t_m - t_s$) indicate the level of biparental inbreeding where mating occurs between close relatives. Black and open symbols in (b) are ungrazed and grazed sites, respectively.

grazed habitat, could be due to either increased insect visitation to *C. palustre,* but no effect of *Cirsium* visitor abundance was found, or greater pollen transport distances, for instance where cattle trampling increases the spatial separation between conspecific plants. Alternatively, a higher density of less related pollen donors in grazed sites might be the explanation (Kunin 1997). Grazing and trampling by herbivores, such as cattle, can reduce the cover of competitively dominant plant species to create germination niches in the sward (Pakeman 2004). Such disturbance processes could facilitate *C. palustre* colonization (Grime, Hodgson & Hunt 2007), thereby increasing the potential for gene flow among unrelated plants and a reduction in spatial genetic structure of the populations within woods (Vazquez & Simberloff 2004; Eckert *et al.* 2010). While this would be consistent with the observed increases in the numbers of less related pollen donors and outcrossing rates in grazed habitat, the available data on *C. palustre* densities at the scale assessed in the plant species and floral density surveys were too sparse to analyse.

However, beyond the effects of grazing on the habitat, there was a strong relationship between the plant mating system and the extent of *C. palustre* habitat in the local landscape, a possible proxy for the population size of this plant species. First, a greater area of *C. palustre* habitat was correlated with a decrease in the correlation of paternity, that is, an increase in the number of different pollen donors in the landscape. Secondly, there was positive relationships between the local extent of *C. palustre* habitat and both biparental inbreeding and spatial genetic structure. Together, this suggests that the greater niche space provided by certain habitats resulted in development of patches of related *C. palustre* individuals and that seed dispersal in this birch habitat must be predominantly local, despite effective wind dispersal in this species.

In this study system, grazing-associated changes to insect-mediated connectivity of conspecific plants together with niche availability and plant spatial organization in the wider landscape were important factors governing the mating system. Across grazed and ungrazed woodlands, both complete outcrossing and mixed-mating strategies were evident. Hence, *C. palustre* maintains a flexibility to adjust mating systems when pollinators or mates are limiting in the local environment (Vanleeuwen 1981; Eckert *et al.* 2010). The greater spatial homogenization of the pollen pool (more pollen donors from a wider area accessible to each mother plant) arising from *C. palustre* being embedded in the more connected networks of grazed habitat raises questions about the interaction between plant reproductive strategies and disturbance. Greater homogeneity of the pollen pool is expected to be beneficial by reducing the likelihood of inbreeding and increasing the probability of mating with superior genotypes (Ward *et al.* 2005; Eckert *et al.* 2010). Anthropogenic disturbance of plant mating systems tends to reduce outcrossing rates (Eckert *et al.* 2010). The increase in outcrossing rate observed here probably reflects the adaptation of *C. palustre* to disturbed environments (Grime, Hodgson & Hunt 2007) and/or increased pollen transport by the preponderance in these networks of highly dispersive flies (Rader *et al.* 2011). Populations of *C. palustre* in the ungrazed habitat may therefore represent a suboptimal situation for the species, in which a flexible mating strategy becomes biased towards selfing to deal with reduced pollen donor diversity. There were no other insect-pollinated plant species in this system that was sufficiently abundant in both the grazed and ungrazed habitat and with the diploid nature that facilitates quantitative analysis of the plant mating system. If in other ecosystems, more candidate plant species are available then it would be interesting to explore whether plant mating system responses to pollinator community changes are contingent on the plant's capacity to tolerance or adaptation to disturbance.

Conclusion

Network analyses are informative methods for assessing the response of ecological communities to anthropogenic environmental change. This study shows how standardization of network metrics to account for sampling effects reveals modifications to fundamental network architecture alongside changes to the abundance and richness of species networks. Assessment of absolute and relative network properties is important to understand fully the consequences of environmental change for interaction biology. Using a novel integration of network analysis, information theoretical model selection and mixed-mating models of plant mating, we also showed that long-term mammalian grazing modified the habitat to a state where visitation network structure and outbreeding success of a predominantly insect-pollinated plant species was fundamentally altered. This finding demonstrates how anthropogenic disturbance of ecosystems can cascade across levels of biological organization through altered interspecific interactions with the potential to affect plant fitness and ultimately adaptation.

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Supporting Information

Additional Supporting information may be found in the online version of this article:

Appendix S1. Plant survey 2007–2008 Methods and Results.
Appendix S2. Species/RTU list from networks.
Appendix S3. Plant mating systems Methods and Results.
Appendix S4. Accumulation curves.