Fragmentation in calcareous grasslands – species specialization matters

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Abstract

Habitat fragmentation resulting from anthropogenic land-use change may negatively affect both biodiversity and ecosystem structure and function. However, susceptibility to fragmentation varies between species and may be influenced by for instance specialization, functional traits and trophic level. We examined how total and specialist species richness, species composition and functional trait composition at two trophic levels (vascular plants and sap-feeding hoppers) vary with habitat fragmentation (patch size and connectivity) in dry calcareous grasslands in southeast Norway. We found that fragmentation affected plant and hopper species composition both totally and of habitat specialists, but with a net species loss only for the specialists, indicating greater susceptibility of specialized species. Reductions in patch size and increasing isolation negatively affected plant specialists with different sets of traits, effectively reducing the number of species with trait combinations suitable to persist in small *and* isolated patches. Fragmentation influenced trait composition of the total hopper community, but not of habitat specialists. A lesser degree of habitat association could explain why hoppers, despite belonging to a higher tropic level, seemed to be less susceptible to fragmentation than plants. Nonetheless, our study shows that habitat fragmentation affects both species richness, species composition and trait composition of plants and hoppers, indicating that fragmentation leads not only to a loss of species, but also alters dominance hierarchies and the functionality of grassland communities.

Keywords

Calcareous grasslands; Functional traits; Habitat fragmentation; Auchenorrhyncha; Vascular plants

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Introduction

Anthropogenic land-use change is currently the most serious threat to global biodiversity (e.g. Haddad et al. 2015) and can result in fragmentation of natural habitats through a combination of habitat loss and patch isolation (Fahrig 2003). These two aspects of habitat fragmentation may together lead to reductions in population sizes and colonization rates, which increase the risk of local species extinctions (Ewers and Didham 2006 and references therein). Such fragmentation-driven reductions in biodiversity may in turn affect ecosystem structure and functioning (Hooper et al. 2005; Isbell et al. 2011).

However, not all species are equally susceptible to habitat fragmentation. Rare species and species with a high degree of microhabitat specialization are predicted to be more vulnerable compared to common, generalist species (Ewers and Didham 2006; Henle et al. 2004), as specialists are more dependent on the resources in a given habitat type and cannot utilize surrounding areas. A greater sensitivity of specialists has been shown for widely different groups of organisms such as plants (e.g. Adriaens et al. 2006; Dupré and Ehrlén 2002; Kolb and Diekmann 2005), fungi (Nordén et al. 2013), arthropods (e.g. Brückmann et al. 2010; Martinson and Fagan 2014; Sang et al. 2010) and birds (Devictor et al. 2008; Matthews et al. 2014). This distinction between generalists and specialists is important, as the negative impacts of habitat fragmentation on specialist species, which are often most important in a conservation perspective, may be masked by generalist species (Jones et al. 2015; Matthews et al. 2014; Nickel and Achtziger 2005).

Furthermore, how reduced habitat patch size and increased patch isolation affect the species in a community depend on species characteristics such as reproduction potential, dispersal capacity and persistence (e.g. Ewers and Didham 2006; Henle et al. 2004). For instance, species producing few diaspores may be more negatively affected by isolation than

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56 species with a high reproductive output (Dupré and Ehrlén 2002; Kolb and Diekmann 2005).
57 Further, a high degree of patch connectivity could be more important for species with short
58 dispersal distances compared to long-distance dispersers (Higgins et al. 2003; Jones et al.
59 2015). Finally, traits that positively affect population persistence, such as a long life span,
60 may allow for the formation of remnant populations that persist despite unfavourable
61 environmental conditions and low recruitment rates (Eriksson 1996), making the species less
62 sensitive to habitat fragmentation, at least on short to intermediate time-scales. However, such
63 remnant populations may also be an indication of an unpaid extinction debt (Tilman et al.
64 1994, see also Kuussaari et al. 2009) eventually leading to local extinction.

65 Habitat fragmentation effects have also been suggested to vary between trophic levels
66 (Ewers and Didham 2006). Organisms at higher trophic levels are predicted to be more
67 susceptible to fragmentation as they depend not only on the persistence of a particular habitat,
68 but also their host or prey species, which may in turn show reduced abundance or occurrence
69 when patch size or connectivity is reduced (Schnitzler et al. 2011). Although several recent
70 studies have found greater sensitivity to fragmentation at higher trophic levels (e.g. Cagnolo
71 et al. 2009; Komonen et al. 2000; Kruess and Tscharntke 1994; Roslin et al. 2014), this is not
72 always the case (Schnitzler et al. 2011). Apparently, more knowledge is needed about whether
73 species from different trophic levels living in the same habitat respond similarly to
74 fragmentation. Furthermore, how functional traits determine sensitivity to fragmentation
75 within a landscape for species at different trophic levels is not well investigated (but see
76 Barbaro and van Halder 2009).

77 Although studies of effects of habitat fragmentation have traditionally had a strong
78 focus on species richness or occurrence, rather than species composition (Tscharntke et al.
79 2012), it is increasingly accepted that species composition is a more sensitive indicator of

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ecological change at local scales than species richness (e.g. Vellend et al. 2013, Stork et al. 2016). The use of species abundance measures rather than simple presence-absence allows for detection of more fine-scale population declines long before local extinction takes place. For instance, Rösch et al. (2015) found that although species richness did not differ between large and small grassland fragments, some of the rarest species were found only in large habitat patches. Identification of changes in species abundances, and thus species composition, in response to habitat patch size and connectivity, may therefore be of high value for conservation purposes. Relating variation in species composition to functional traits variation along gradients in patch size and connectivity can aid in identifying traits characterizing species that are particularly sensitive to fragmentation (Barbaro and van Halder 2009).

Here, we examine how species and functional trait composition at two trophic levels, vascular plants and sap-feeding hoppers (Hemiptera, Auchenorrhyncha), vary with the degree of habitat fragmentation in dry calcareous grasslands in southeast Norway. Dry calcareous grasslands are biodiversity hotspots (Habel et al. 2013), but are declining throughout Europe (LIFE 2008), including Norway (Lindgaard and Henriksen 2011), mainly due to land-use change. Fragmentation of grassland habitats has been shown to decrease species richness and abundance of both plant (Adriaens et al. 2006; Bruun 2000; Evju et al. 2015) and insect species (Brückmann et al. 2010, 2011; Rösch et al. 2013; Öckinger and Smith 2006). Hoppers have low mobility and are thus expected to be more affected by habitat fragmentation than larger, more mobile insect taxa (Rösch et al. 2015). Moreover, whereas little is known about the effect of fragmentation on insect trait distribution in these habitats (although see Öckinger & Smith 2006), sensitivity to fragmentation of grassland plants has been shown to vary among species, with a higher sensitivity particularly for species with a short lifespan or lack of clonal reproduction, traits that are related to low persistence (Bruun 2000; Maurer et al.

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104 2003; Piqueray et al. 2011), and for species with low reproductive potential (Evju et al. 2015;
105 Saar et al. 2012). These findings are, however, based on species occurrence data only.
106 In the current study, we examine both species richness and species composition and
107 functional trait composition of vascular plants and hoppers in habitat patches of varying size
108 and connectivity, aiming to expand our understanding of how sensitivity to fragmentation,
109 and the relationship between habitat specificity, traits and sensitivity, vary across trophic
110 levels. More specifically, we investigate 1) the role of habitat configuration (patch size and
111 connectivity) on plant and hopper species richness and community composition, 2) the
112 importance of species traits for moderating the relationship between species composition and
113 habitat configuration, and 3) whether including all species can mask important patterns of
114 habitat specialists.
115 We predict that 1) species richness of habitat specialists decreases with reduced patch
116 size and connectivity, but that this pattern will be weaker when including generalist species,
117 and 2) that species composition of habitat specialists, but not of the full community, varies
118 along gradients in patch size and connectivity. Moreover, we expect 3) gradients in specialist
119 species composition to be reflected in functional trait composition, with change towards high
120 persistence and high dispersal capacity in small and isolated habitat patches. Finally, we
121 expect 4) patterns to be more pronounced for hoppers than for plants, as hoppers belong to a
122 higher trophic level and have limited dispersal capacity.
123
124 **Materials and methods**
125 ***Study area and design***
126 The study was conducted close to the city of Oslo in the inner parts of the Oslo fjord in
127 southeast Norway (see Fig. 1 in Evju et al. 2015) in 2012−2014. Oslo has a mean annual

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temperature and rainfall of 5.7 °C and 763 mm, respectively (Norwegian Meteorological Institute 2015). The study sites were located in dry calcareous grasslands on Cambro-Silurian bedrock on islands or on the mainland. The grasslands occur naturally in a narrow zone between the sea and the forested inlands. They are sun and wind exposed, and the vegetation is dominated by drought-tolerant forbs and graminoids. The habitat is naturally fragmented due to the partial location on islands and the dependency on bedrock qualities, exposure and a warm and dry climate, but habitat loss due to anthropogenic land-use change has led to further fragmentation (Evju and Stange 2016). The grasslands are surrounded by forests and urban and suburban settlements. Yet, these grasslands are biodiversity hotspots for many groups of organisms, including vascular plants and invertebrates, and have a high occurrence of nationally red-listed species confined to this habitat (Henriksen and Hilmo 2015).

We mapped fragments of dry calcareous grasslands near Oslo by superimposing a grid system of 500 × 500 m grid cells onto the landscape and randomly selecting 50 grid cells for field survey. Calcareous grassland was found in 22 of these 50 grid cells, totalling 93 habitat patches. We determined patch size for all patches and used a habitat distribution model to estimate patch connectivity as the sum of all neighbour patches within a radius of 1 km of the focal patch, weighted by distance to and size of neighbour patches (Hanski 1994, see Evju et al. 2015 for further details). We used median values of patch size of the 93 patches to define "small" and "large" patches (below and above median size, respectively) and correspondingly median values of patch connectivity to define "high" and "low" connectivity, and categorized all patches as either 1) small with low connectivity, 2) large with low connectivity, 3) small with high connectivity, or 4) large with high connectivity. A subset of 20 habitat patches was sampled in a random, stratified manner, ensuring the inclusion of five patches from each of the four categories, to be included in this study. The size of the 20 habitat patches ranged from

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50 to 2300 m^2 (mean ± standard deviation: 762 ± 584 m^2) and patch connectivity from 0.062 to 3.92 (1.60 ± 1.42).

Sampling of species and environmental variables

Plants

In each of the 20 habitat patches, we recorded the occurrence of all vascular plant species (patch-level species richness). Within each patch, plot-level vascular plant species richness and composition was recorded in 0.5 × 0.5 m sample plots. We randomly placed the sample plots by dividing each patch into grid cells of 1 × 1 m, numbering them and randomly sampling from the pool of potential plots. The number of plots per patch (n) was weighted with patch area so that $n = 0.15 \times \log_2(\text{patch area})$, rounded off to the nearest integer, to avoid oversampling of small patches. As a result, between 7 and 13 plots per patch were sampled, giving a total of 201 plots over the 20 patches. Each plot was divided into 16 subplots (0.125 × 0.125 m), and the presence of all vascular plant species was recorded in each subplot, giving species abundance as sub-plot frequency. One plot had been mowed and was therefore excluded from the data set, giving a total of 200 plots for analysis. The field work was carried out in August 2012 (18 habitat patches) and August 2013 (2 habitat patches).

In each plot we also recorded environmental variables relevant for describing habitat quality for grassland plants: the percentage cover of stone and gravel and of shrubs. A high cover of stone and gravel reflects a shallow soil cover, which should be beneficial for the drought-tolerant calcareous grasslands plants, whereas a high shrub cover implies reduced light availability and thereby reduced habitat quality. From the species abundance data, we calculated the plot-level abundance of invasive species, defined by the Norwegian Black list of species (Gederaas et al. 2012) (see **Table A1.1**), as invasive species are frequently reported

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as a serious threat to dry calcareous grassland biodiversity in the study area (County Administrator of Oslo and Akershus 2010).

Hoppers

Due to limited resources, and to ensure sampling in all patches within a short time-period, the hoppers were sampled in a subset of 12 of the 20 habitat patches by randomly selecting three patches from each of the four predefined categories. These 12 patches ranged from 168 to 2300 m^2 (mean ± SD: 714 ± 644 m^2) with patch connectivity ranging from 0.11 to 3.86 (1.76 ± 1.53), i.e. covering the range of patch size and connectivity in the full dataset. In each patch we recorded hopper species richness and composition by collecting hoppers using a G-vac suction sampler (Stewart and Wright 1995) with a fixed square net of 0.5 × 0.5 m mounted on the nozzle. We carried out sampling in August 2012, June 2013 and July 2014, with three samples per habitat patch per year, giving a total of nine samples per patch across the study period. One sample consisted of three consecutive 0.25 m^2 plots, each with a suction time of 30 sec. The plot location within the habitat patch was based on randomly selected cells within the 1 × 1 m grid used for vascular plants as described above, but sample plots for hoppers and vascular plants were not identical.

The identification to species level of female specimens of several leafhopper genera (*Alebra, Anaceratagallia, Arboridia, Balclutha, Chlorita, Cicadula, Elymana, Empoasca, Euscelis, Jassargus, Javesella, Kelisia, Rhopalopyx, Ribautodelphax, Streptanus*) was not possible (Biedermann and Niedringhaus 2004). Females were therefore allocated to species based on proportion of males in the same sample (251 individuals, 10% of the total sample). If only one species of a genus was represented in the dataset, all female specimens were allocated to that species. If male specimens were not present, females were only identified to

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genus level (389 individuals, 16%) (see Rösch et al. 2015). Fragmented specimens or specimens that could not be identified to genus level were excluded from the analysis (83 specimens in total, 3.5 % of the total sample).

Denno & Roderick (1991) emphasize the role of the vegetation in structuring hopper communities. We therefore recorded several environmental variables relevant for describing habitat quality for the hoppers: patch-level plant species richness, both totally and of habitat specialists, and shrub cover. In addition, plant species composition, derived as mean patch ordination scores from the analyses of the plant species-plot matrices of all plants and habitat specialists (see ***Statistical analyses***) was used.

Species traits

A list of habitat specialists of vascular plants (Evju et al. 2015) was used to characterize the plant species as either habitat generalists or specialists (**Table A1.1**), that is, species which are strongly confined to dry calcareous grasslands within the study area. For all plant species, information on functional traits important for reproductive potential, dispersal and persistence (**Table 1a**) were collected from trait databases (Fitter and Peat 1994; Kleyer et al. 2008; Kühn et al. 2004) and the local flora (Lid and Lid 2005). Median trait values were used when several records were available in the databases. To characterize reproductive potential, we used the number of seeds produced per ramet. Dispersal capacity was represented by seed mass and dispersal agent (wind, animal or unassisted, cf. Jones et al. 2015). Plant persistence was represented by lifespan, clonality and three traits characterizing competitive ability. Species were classified as short-lived (annual or biennial) or long-lived, being either non-clonal, clonal with shortly creeping clonal offspring, or clonal with far-creeping clonal offspring (cf. Fitter and Peat 1994). Finally, canopy height, specific leaf area (SLA) and leaf

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size were selected to represent competitive ability, with tall, fast-growing (high SLA) plants with large leaves predicted to have a higher competitive ability (Weiher et al. 1999) and thus higher persistence.

The hopper species were also classified into habitat generalists and specialists (**Table A1.2**). We used two criteria for the classification: habitat preference and expert opinion (H. Nickel, A. Endrestøl). Some species that are not considered specialists on calcareous grasslands elsewhere in Europe are in Norway restricted to the calcareous grasslands in the inner Oslo fjord due to favourable climate and the associated vegetation communities found here. Functional traits of hoppers were aggregated mainly from Biedermann and Niedringhaus (2004), Nickel (2003), Ossiannilsson (1978, 1981, 1983) and Söderman (2007) (**Table 1b**). Reproductive potential was represented by the number of generations per year. As short-winged (brachypterous) morphs of dimorphic hopper species have low dispersal capacity, but higher fecundity than long-winged morphs (Denno and Roderick 1991), we used the proportion of brachypterous individuals sampled to represent both dispersal capacity and reproductive potential. The proportion of brachypterous individuals could be expected to vary among patches according to available resources and population density (della Giustina 2002; Denno and Roderick 1991), but in our dataset the abundance of most species was too low to calculate patch-specific proportions. Thus, the data were pooled over all patches. Dispersal capacity was also represented by body length, as larger species may be better dispersers (Denno and Roderick 1991). We used the mean body size across both sexes for species with sexually dimorphic body size. Overwintering style is a trait that should be related to population persistence, as more exposed overwintering modes result in higher winter mortality (Denno and Roderick 1991). Overwintering style (egg, nymph or adult) was therefore used as a proxy for population persistence. Finally, diet width has been hypothesized

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248 to influence population persistence as polyphagous species utilizing a range of host plants
249 may have a higher probability of survival (MacLean 1983) in a fragmented landscape. Thus,
250 we included diet width (monophagy, oligophagy, polyphagy) as a persistence trait.
251
252 ***Statistical analyses***
253 To examine whether measures of habitat quality varied systematically along gradients in
254 patch size or connectivity, we used linear mixed-effect models with the habitat quality
255 variable as response and either patch size or connectivity as predictor. Plots nested in patch
256 were used as random factors to account for the spatial autocorrelation of sample plots. For
257 plants, cover of stone and gravel, cover of shrubs and abundance of invasive species were
258 used as response variables. For hoppers the relevant environmental variables showed a large
259 degree of collinearity. We calculated variance inflation factors (VIFs) (Zuur et al. 2007) and
260 used these to simplify our set of environmental variables. We retained cover of shrubs, total
261 plant species richness, habitat specialist plant species richness, and mean patch score along
262 GNMDS axis 2 of the total plant species composition.
263 Further, we used generalized mixed-effect models to examine whether species
264 richness of plants and hoppers at the sample-plot level varied between patches according to
265 patch size and connectivity. Because of low species richness and abundance of hoppers, we
266 pooled data across samples for each year, giving three replicates for each habitat patch. We
267 analysed total species richness and richness of habitat specialists separately, and plots nested
268 in patch were used as random factors. Predictor variables were patch size, patch connectivity
269 and their interaction. Likelihood ratio tests were used for model simplification and to assess
270 variable significances. Models were run with poisson-distributed error terms.

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We used multivariate ordination techniques (global non-metric multidimensional scaling; GNMDS) to derive the first and second ordination axis of community composition for both plants and hoppers. Separate ordinations were run for total species composition and species composition of habitat specialists. The two-dimensional GNMDSs were run with Bray-Curtis dissimilarity measure, 100 initial configurations, maximum 200 iterations and stress tolerance 10^{-7} and axes were scaled in half-change units. We calculated the correlation coefficient between GNMDS ordination axis scores and environmental variables using the non-parametric Kendall's τ.

To directly test the importance of patch size and connectivity for species composition, we used the constrained ordination technique canonical correspondence analysis (CCA). We applied a step-wise procedure where we first separately tested the importance of patch size, and of patch connectivity. The variable that alone explained most of the variation in species composition was then used as a conditioning variable. By using the other variable as a constraining variable, we tested whether it contributed to explaining additional variation in species composition. Finally, we tested the significance of the interaction between patch size and connectivity by using both patch size and connectivity as conditioning variables. We used Monte Carlo permutation tests with 999 permutations to test whether the explanatory variables explained more variation in the species composition than a random variable.

To examine how trait composition of the habitat specialists varied along gradients of patch size and connectivity, we calculated community-weighted mean traits per sample plot as the mean plot trait value weighted by the abundance of the species in the plot. Community-weighted means thus reflect the traits of the most abundant species in the community. Due to the widely different scales of the traits, the community weighted mean traits were centred and scaled before applying a constrained ordination technique, redundancy analysis (RDA), to test

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the importance of patch size and connectivity for explaining variation in community trait composition. The test procedure followed the description given for the CCA.

All analyses were performed in R version 3.2.2 (R Core Team 2015) using RStudio version 099.489 (RStudio, Inc., Boston, Massachusetts, USA). We used the packages vegan (Oksanen et al. 2015) and MASS (Venables and Ripley 2002) for the multivariate analyses, and lme4 (Bates et al. 2015) for the mixed-effects models.

Results

Environmental variables

For the 20 habitat patches sampled for plants there was a tendency for larger patches to have a higher plot-level cover of shrubs ($\beta = 0.238$, SE = 0.116, $p = 0.055$), while invasive species were more abundant in small patches ($\beta = -0.515$, SE = 0.134, $p = 0.001$) and isolated patches ($\beta = -0.280$, SE = 0.131, $p = 0.047$).

For the subset of 12 habitat patches sampled for hoppers patch-level shrub cover increased with patch size ($\beta = 0.248$, SE = 0.049, $p < 0.001$). In addition, patch-level plant species richness increased slightly with patch size ($\beta = 0.141$, SE = 0.057, $p = 0.033$), whereas the number of habitat specialist plants increased with both patch size ($\beta = 0.376$, SE = 0.066, $p < 0.001$) and patch connectivity ($\beta = 0.137$, SE = 0.058, $p = 0.040$).

Plant species richness

We recorded 158 taxa of vascular plants, between 27 and 59 species per patch. A total of 27 species (17%) were classified as habitat specialists (**Table A1.1**). The mean number of plant species per plot was 13.7 (range: 4−29), of which habitat specialists on average constituted 33% (mean: 4.5, range: 0−12). The plot-level species richness was not related to patch size or

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connectivity (**Table 2a**). In contrast, the plot-level habitat specialist species richness increased with increasing patch size, and with a significant patch size × connectivity interaction, suggesting that the positive effect of patch size increased with increasing connectivity.

Plant species composition

Species turnover of plants between plots was low, as indicated by relatively short ordination axes (**Fig. 1**, **Fig. A2.1**). The longest axis was about 1 half-change unit, corresponding to a 50% species turnover. Species composition, both totally and of habitat specialists only, varied along gradients in soil depth (cover of stone and gravel), cover of shrubs and cover of invasive species (**Fig. 1, Fig. A2.1, Table A2.1**). Additionally, both patch size and patch connectivity correlated with the ordination axes. Thus, although other environmental variables were important in structuring species composition, there seemed to be a shift in species composition also from small to large and isolated to well-connected patches.

The constrained ordinations showed that patch size and connectivity were significant predictors of variation in species composition of both the total plant community and of the habitat specialists (**Table 3a, Table A2.2a**). For the specialists, patch connectivity alone explained 3.7% of the variation in the species-plot-matrix. Both patch size and the interaction between connectivity and size contributed to explaining additional variation, with all variables combined explaining 6.2% of the variation. Similarly, all variables combined explained 5.0% of the variation in total species composition.

Plant trait composition

Patch connectivity, patch size and their interaction in combination explained significantly more of the variation in plant trait composition than expected at random (total plant

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community: 8.2%, habitat specialists: 5.7%; **Table 3b, Table A2.2b**). For the specialists, plots in isolated patches were dominated by non-clonal species with many wind-dispersed seeds, whereas short-lived species and species with few seeds without any specific dispersal mechanism were more abundant in well-connected patches **(Fig. 2)**. The abundance of tall, long-lived species with large, thin leaves (high SLA) and large seeds increased with increasing patch size (**Fig. 2**). The pattern was somewhat different for trait composition of the total plant community: Short-lived species were more abundant in isolated patches, whereas long-lived species dominated in well-connected patches (**Fig. A2.2**). Species with wind-dispersed seeds were more common in large patches, while species without a specific dispersal mechanism were typical of small patches. For both the total plant community and the habitat specialists the interaction between patch size and connectivity had the same direction as the main effect of patch connectivity, suggesting that a large patch size enhanced the effect of connectivity.

Hopper species richness

For hoppers, 2397 individuals were determined to 74 taxa, of which 68 (2364 specimens) were determined to species level and 6 (33 specimens) to genus level (**Table A1.2**), with species richness ranging from 14 to 30 per patch. In total, 13 species (18%) were classified as habitat specialists. Plot-level species richness was 11.8 (range 4−22), including on average 3.2 (27%) habitat specialists (range: 0−6). Plot-level species richness of hoppers was not related to patch size or connectivity (**Table 2b**). Specialist hopper richness was, however, positively related to patch connectivity.

Hopper species composition

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Species turnover between plots was slightly higher for hoppers than for plants, as indicated by the somewhat longer ordination axes (**Fig. 3**, **Fig. A2.3**). Total species composition of hoppers varied with plant species composition and plant specialist species richness (**Fig. 3, Table A2.3**). Additionally, both patch size and patch connectivity correlated with the ordination axes. Specialist species composition shifted along gradients in plant species richness and composition, but not patch size or connectivity (**Fig. A2.3, Table A2.3**).

As both richness, composition and structure of plant communities were partly influenced by habitat configuration, however, we wanted to directly test the effects of patch size and connectivity also on hopper species composition. Patch connectivity alone explained 5.8 and 8.5% of the variation in the species-plot-matrix for the total hopper community and habitat specialists, respectively (**Table 3c, Table A2.4a**). Patch size did not to contribute to explaining additional variation in the matrix.

Hopper trait composition

The constrained ordination showed that patch size explained 8.4% of the variation in trait composition for the total hopper community (**Table A2.4b**), significantly more than expected at random. Plots in large patches were dominated by species which overwinter as adults and have several generations per year, whereas plots in small patches were dominated by species with lower reproductive potential (**Fig. A2.4**). There was no additional variation in trait composition related to patch connectivity. Trait composition of the specialist hopper community was not influenced by habitat configuration (**Table 3d**).

Discussion

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Most aspects of the calcareous grassland plant and hopper communities in the study area were affected by habitat fragmentation. This is in line with previous studies showing that increasing isolation and decreasing patch size influence plants and invertebrates in grasslands throughout Europe (e.g. Brückmann et al. 2010; Krauss et al. 2010; Rösch et al. 2015). Whereas most fragmentation studies focus on one community property, mainly species richness, our study shows that fragmentation also affects species composition and trait composition, implying that habitat fragmentation leads not only to local species extinctions, but also alters species dominance hierarchies and the functionality of these communities.

Species richness and composition

As hypothesized, we found lower plot-level species richness of both specialist plants and hoppers when habitat patches were increasingly isolated, of plants also when patch size decreased. Several studies show that connectivity is important for species richness of various insect taxa in fragmented landscapes (Kormann et al. 2015; Öckinger et al. 2010; Öckinger and Smith 2006), and our study suggests that habitat configuration is indeed important for hoppers confined to dry and warm grasslands (see also Biedermann 2002 and references therein; Rösch et al. 2013; 2015). The richness of grassland plants has been shown to vary with both patch size and landscape connectivity (e.g. Adriaens et al. 2006; Bruun 2000; Brückmann et al. 2010), also in our study area, where we have previously demonstrated a higher patch-level specialist species richness in larger and more well-connected patches (Evju et al. 2015, Evju & Sverdrup-Thygeson 2016). The higher plot-level species richness suggests that this pattern is not only due to higher microhabitat diversity in large fragments, but also to colonization-extinction dynamics (Giladi et al. 2014; Rosenzweig 1995).

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In contrast to our findings for the habitat specialists, total species richness of plant and hoppers were not affected by habitat fragmentation. This higher sensitivity of habitat specialists confirms previous results from the same study area (Evju et al. 2015) and other studies from calcareous grasslands (e.g. Adriaens et al. 2006), as well as other grassland habitats (Henderson 2010). Together, our findings emphasize the need to focus on habitat specialists when assessing the negative impacts of habitat fragmentation on species richness, as reduced connectivity and patch size may not affect richness of species with broad habitat requirements, which can persist also in matrix habitats (Jones et al. 2015; Matthews et al. 2014).

Whereas fragmentation effects on plant and hopper specialist were obscured by including generalists when examining species richness, this was not the case when considering species composition. Contrary to our expectation, both plant and hopper generalist and specialist species composition were affected by habitat fragmentation. Similarly, Rösch et al. (2015) found that species composition of plant and invertebrate specialists and generalists varied with patch size. By simultaneously examining effects of fragmentation on species richness and community composition, we show that species abundances and/or species identity of both the total and the specialist plant and hopper communities are influenced by fragmentation, but with a net species loss only for the specialists.

Functional traits

The shift in species composition was accompanied by a corresponding shift in the composition of functional traits of the total and specialist plant community, as well as the total hopper community, indicating that the abundant species in the community are functionally

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437 different in small and isolated compared to large and well-connected patches. Further, our
438 results suggest that different suites of traits determined species sensitivities to different
439 aspects of fragmentation. For plant specialists, reproduction and dispersal-related traits were
440 more strongly affected by isolation, as suggested by Lindborg et al. (2012), implying that as
441 the distance between remaining fragments increases, the abundance of specialist plants with a
442 relatively short life-cycle and low reproduction and dispersal capacity declines (Evju et al.
443 2015; Jones et al. 2015). On the other hand, there was a tendency for traits associated with
444 population persistence, such as a long life span, to be positively related to patch size. This
445 may seem counterintuitive, as long-lived species could be expected to persist as remnant
446 populations (sensu Eriksson 1996) despite suboptimal habitat conditions in small habitat
447 patches (see Maurer et al. 2003; Piqueray et al. 2011). However, our findings are in
448 accordance with Lindborg et al. (2012), who suggest that persistent species may be more
449 sensitive to habitat loss because of their limited capacity for re-colonization once locally
450 extinct. Although reductions in patch size and increasing isolation occur simultaneously as a
451 habitat is fragmented, our results emphasize that the two processes favour species with
452 different sets of traits, effectively reducing the number of species with a trait combination
453 suitable to persist in small *and* isolated patches.

454 Contrary to the plants, the shift in hopper specialist species composition was not
455 accompanied by a corresponding shift in trait composition. This was unexpected, as trait
456 composition of the total hopper community was influenced by patch size (although not
457 connectivity), with higher abundance of traits corresponding to low reproductive output in
458 small patches. Several factors may contribute to this lack of pattern for the specialists. First,
459 the definition of habitat specialists may be less straightforward for hoppers than for plants.
460 Hoppers depend on their host plants, but few of the species in our study were monophagous

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461 on plants restricted to dry calcareous grasslands. Rather, they were confined to dry and warm
462 habitats, which could also prevail outside the defined patches. Second, our definition of
463 specialists included only 13 species, many of which have low abundances, which limits the
464 statistical power. However, the proportion of specialist species was almost identical for
465 hoppers (18%) and plants (17%), indicating no disproportional undersampling of hopper
466 specialists. Third, we have less knowledge of the link between traits and function for
467 invertebrates, including hoppers, than for plants. Thus, we cannot be certain that the traits we
468 have included are relevant descriptors of the variation in function we hypothesize is important
469 for the fragmentation response. Fourth, the traits are compiled mainly from central European
470 literature and may be less relevant for our study system (e.g. Söderman 2007, Endrestøl
471 2014). Finally, a relatively small sample size (in terms of patches) may have restricted our
472 possibilities to find significant patterns.

473 Nonetheless, across organism groups the effect of habitat fragmentation on trait
474 composition varied depending on whether all species or only habitat specialists were included
475 in the analyses. For specialist plants reproduction and dispersal-related traits were affected by
476 isolation, whereas persistence traits were mainly associated with patch size. For the total plant
477 community, the pattern was opposite, with persistence traits related to isolation and dispersal
478 traits to patch size. Together with our findings for the hoppers, where trait composition of the
479 total community, but not specialists, were affected by fragmentation, this indicates again that
480 including generalist species in the analyses may obscure fragmentation effects on habitat
481 specialists, which are the main targets for conservation.

482

483 ***Trophic levels***

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Whereas several other studies show greater sensitivity to fragmentation at higher trophic levels (e.g. Roslin et al. 2014 for a study including both plants and insects), this did not seem to be the case in our study. Specialist hoppers seemed to be less vulnerable to habitat fragmentation than specialist plants. For instance, the main axes of plant habitat specialist composition were related to both patch size and connectivity in the unconstrained ordination. For the specialist hoppers, variables derived from the plant community data explained most of the variation in species composition, with no additional contribution from patch size or connectivity, suggesting that fragmentation influences hopper specialists primarily through the plant species they feed on (Schaffers et al. 2008). As discussed above, none of the specialist hoppers were monophagous on specialist plants species, indicating that the composition of the hopper community was mainly determined by other plant community properties than the occurrence of specialist plants. For instance, total plant species richness, which was not affected by habitat fragmentation in our study, can be an important predictor of hopper species richness (Rösch et al. 2013), as species rich and structurally diverse plant communities provide a larger heterogeneity of resources for the herbivores (Nickel and Hildebrandt 2003; Scherber et al. 2010). Thus, the degree of specialization seems more important for sensitivity to fragmentation than trophic level *per se*.

Environmental variables and invasive species

Local environmental variables can be more important in explaining species occurrences than habitat configuration (Adriaens et al. 2006; Dupré and Ehrlén 2002), and if habitat loss leads to subsequent degradation of remaining habitat, the effects of fragmentation can be reinforced. Whereas hopper species composition was mainly determined by properties of the plant community, plant species composition was, in addition to patch size and connectivity,

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structured mainly by variation in soil depth, cover of shrubs (i.e. light availability) and the cover of invasive species. Invasive species were more abundant in small and isolated patches in our study area, which could partly explain the shift in total plant species and trait composition with habitat fragmentation. Some invasive species, such as *Phedimus spurius*, form dense mats and may displace native species, thereby contributing to the lower species richness of habitat specialists (M. Evju, unpublished results) in small and isolated patches. Restoring habitat quality is therefore important to reduce the negative effects of habitat fragmentation on biodiversity.

It is hard to disentangle the effects of systematic differences in environmental variables between patches (e.g. shrub cover and the cover of invasives) and the effects of habitat fragmentation *per se*. However, the higher cover of invasive species in small and isolated patches in our study system is most likely a direct effect of habitat fragmentation, as invasive species colonize fragmented habitats more easily (Vilà and Ibáñez 2011). Thus, changes in environmental variables and thereby habitat quality is one of the many aspects of habitat fragmentation and needs to be taken into account in management of fragmented landscapes.

Conclusions

Species richness of specialist hoppers increased with connectivity. Large, well-connected patches also contained more specialist plants both at the patch level (Evju et al. 2015) and the plot level (this manuscript). Conservation of large, well-connected patches thus ensures the diversity of habitat specialist of both plants and hoppers. Reduced patch size had the greatest negative effect on plant specialists with traits enhancing population persistence, whereas increased isolation negatively affected short-lived species and species with limited

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recruitment and dispersal capacity. Conservation of large, well-connected patches will therefore also sustain larger populations of habitat specialists that exhibit traits which make them especially vulnerable to fragmentation. Finally, our study shows that ensuring habitat quality in remaining patches is an important aspect of habitat conservation.

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Conflict of interest

The authors declare that they have no conflict of interest.

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734 **TABLES**

735 **Table 1.** Overview of the traits used in the analyses for a) vascular plants and b) hoppers. The traits represent reproductive potential, dispersal
736 capacity and persistence and are hypothesized to predispose species for vulnerability to fragmentation. For each trait is given: a description of the
737 trait, scale, min, max and mean values (continuous traits) or percentage of species with given trait category (ordinal traits) for habitat specialists
738 and generalist species, as well as the data source. Modified from Evju et al. (2015) and extended with hopper data.

739 a)

Characteristic	Predisposing for sensitivity to fragmentation	Trait	Description	Scale	Specialists	Generalists	Source
Habitat requirements							
Habitat requirements	Narrow habitat requirements	Habitat specialist	Species confined to dry calcareous in the study area (1), generalist species (0)	binomial	n = 27	n = 131	Evju et al. 2015
Reproduction							
Reproductive potential	Low reproductive potential	Seed number	No. of seeds per ramet/plant	continuous	Min: 17.0 Max: 13 202.2 Mean: 1 448.7	Min: 5.7 Max: 400 104.8 Mean: 11 539.3	Kleyer et al. 2008
Dispersal							
Dispersal capacity	Low dispersal capacity	Dispersal agent	1. wind-dispersed 2. animal-dispersed 3. no dispersal aid	ordinal	1: 11.1 % 2: 18.5 % 3: 70.4 %	1: 13.7 % 2: 9.9 % 3: 76.3 %	Lid and Lid 2005, Fitter and Peat 1994

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Dispersal capacity	Low dispersal capacity	Seed mass	Weight of germinule (mg)	continuous	Min: 0.02 Max: 26.00 Mean: 3.70	Min: 0.03 Max: 55.63 Mean: 3.09	Kleyer et al. 2008
Persistence							
Lifespan	Short lifespan	Lifespan	1. annual or biennial 2. perennial	nominal	1: 18.5 % 2: 81.5 %	1: 13.7 % 2: 86.3 %	Lid and Lid 2005
Clonal reproduction	No clonal reproduction	Clonality	1. little or no vegetative spread 2. shortly creeping 3. far creeping	ordinal	1: 45.4 % 2: 36.4 % 3: 18.2 %	1: 44.9 % 2: 29.9 % 3: 25.2 %	Lid and Lid 2005, Fitter and Peat 1994
Competitive ability	Low competitive ability	Canopy height	Maximum plant height (cm)	continuous	Min: 15.0 Max: 400.0 Mean: 73.9	Min: 10.0 Max: 4300.0 Mean: 210.3	Lid and Lid 2005
Competitive ability	Low competitive ability	Leaf size	Leaf area (mm^2)	continuous	Min: 4.6 Max: 11428.0 Mean: 1480.8	Min: 9.9 Max: 30968.0 Mean: 1775.9	Kleyer et al. 2008
Competitive ability	Low competitive ability	Specific leaf area	Leaf area per unit leaf mass (mm^2/mg)	continuous	Min: 9.9 Max: 33.2 Mean: 20.5	Min: 3.8 Max: 54.7 Mean: 22.3	Kleyer et al. 2008

740 b)

Characteristic	Predisposing for sensitivity to fragmentation	Trait	Description	Scale	Specialists	Generalists	Source
Habitat requirements							

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Habitat requirements	Narrow habitat requirements	Habitat specialist	Xerothermophilous species mainly found on dry meadow, with a restricted distribution in Norway or Scandinavia	binomial	n = 13	n = 61	Nickel 2003, Ossiannilsson 1978;1981;1983, Söderman 2007, expert judgment
Reproduction							
Reproductive potential	Low reproductive potential	No. of generations	No. of generations per year	continuous	Min: 1.00 Max: 1.50 Mean: 1.04	Min: 1.00 Max: 2.00 Mean: 1.17	Biedermann and Niedringhaus 2004, Söderman 2007
Dispersal							
Dispersal capacity	Low dispersal capacity	Proportion brachypterous	Proportion of short-winged individuals (%)	continuous	Min: 20.0 Max: 100.0 Mean: 65.9	Min: 0.0 Max: 100.0 Mean: 51.6	Field data
Dispersal capacity	Low dispersal capacity	Body length	Mean body length, averaged over males and females (mm)	continuous	Min: 2.15 Max: 4.78 Mean: 3.15	Min: 2.40 Max: 7.50 Mean: 4.11	Biedermann and Niedringhaus 2004
Persistence							
Winter mortality	High winter mortality	Overwintering mode	1. egg 2. nymph 3. adult	categorical	Egg: 61.5 Nymph: 23.1 Adult: 15.4	Egg: 69.5 Nymph: 22.0 Adult: 8.5	Biedermann and Niedringhaus 2004, Nickel 2003
Phagy	Narrow diet width	Diet width	1. monophagy 2. oligophagy 3. polyphagy	categorical	Mono: 46.2% Oligo: 46.2% Poly: 7.7%	Mono: 23.6% Oligo: 38.2% Poly: 38.2%	Nickel 2003

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Table 2 Plot-level species richness as a function of patch size and patch connectivity, both totally and of habitat specialists of a) vascular plants and b) hoppers, modelled with generalized mixed-effect models with plot nested in patch as random factor and poisson-distributed error terms. Both patch size and connectivity were $\log_2$-transformed in the analyses. Likelihood ratio tests were used for model selection and to assess variable significances. · p < 0.1, * p < 0.05, ** p < 0.01, *** p < 0.001

a)

	All species		Habitat specialists	
	Estimate	**SE**	**Estimate**	**SE**
Patch size	0.030	0.026	0.068	0.029 *
Patch connectivity	−0.050	0.099	−0.182	0.115
Size × connectivity	0.008	0.011	0.030	0.013 *

b)

	All species		Habitat specialists	
	Estimate	**SE**	**Estimate**	**SE**
Patch size	−0.009	0.040		
Patch connectivity	−0.057	0.214	0.118	0.038 **
Size × connectivity	0.011	0.023		

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753 **Table 3** Constrained ordination results with fraction of variation explained (FVE, in %) of the
754 species/trait composition by the explanatory variables, alone or with conditioning variables,
755 and pseudo-F from permutation tests with 999 permutations. a) CCA of species-plot matrix of
756 habitat specialist plants, b) RDA of trait-plot matrix of habitat specialist plants, c) CCA of
757 species-plot matrix of habitat specialist hoppers, d) RDA of trait-plot matrix of habitat
758 specialist hoppers. The model best explaining the variation in the matrix is shown in bold. · p
759 < 0.1, * p < 0.05, ** p < 0.01, *** p < 0.001

760 a)

Constraining variable	Conditioning variable	FVE	pseudo-F
Size		2.5	4.98 **
Connectivity		3.8	7.67 **
Size	Connectivity	1.7	3.55 **
Size × connectivity	Size + connectivity	0.7	1.51 *
Size + connectivity + size × connectivity		**6.2**	**4.29 ** **

761 b)

Constraining variable	Conditioning variable	FVE	pseudo-F
Size		0.9	1.80
Connectivity		2.0	4.02 **
Size	Connectivity	1.4	2.76 *
Size × connectivity	Size + connectivity	2.3	4.76 **
Size + connectivity + size × connectivity		**5.7**	**3.90 ** **

762 c)

Constraining variable	Conditioning variable	FVE	pseudo-F
Size		4.2	1.39
Connectivity		**8.5**	**2.96 ***
Size	Connectivity	3.8	1.33

763 d)

Constraining variable	Conditioning variable	FVE	pseudo-F
Size		**3.2**	**1.09**
Connectivity		2.3	0.52

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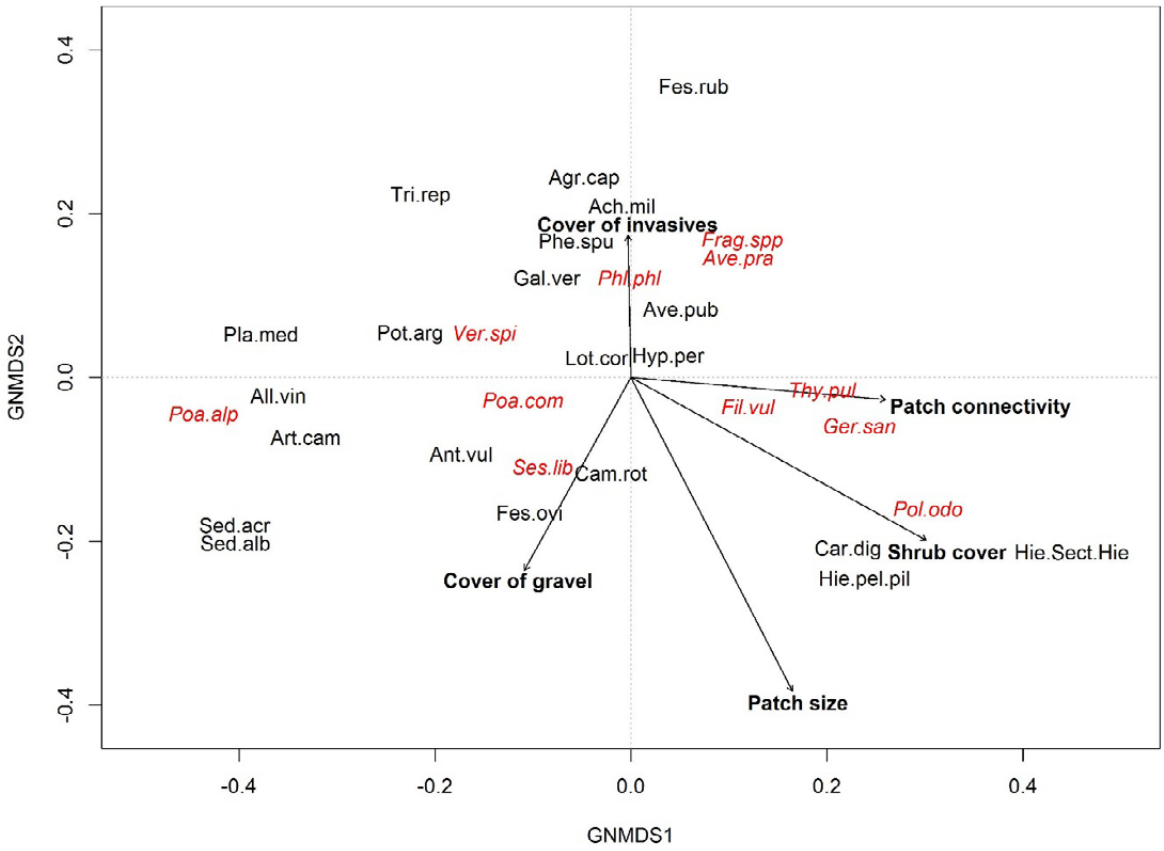
765 **FIGURES**



766

767 **Fig. 1** GNMDS ordination plot of total plant species composition in 200 sample plots in 20

768 habitat patches. Habitat specialists are shown in red italics. Only species with total sub-plot

769 frequency > 100 are shown. Arrows indicate correlations between the ordination and

770 environmental variables, with the length of the arrows proportional to the correlation strength.

771 Some species names were slightly adjusted to avoid overlap. For species abbreviations, see

772 **Table A1.1**.

773

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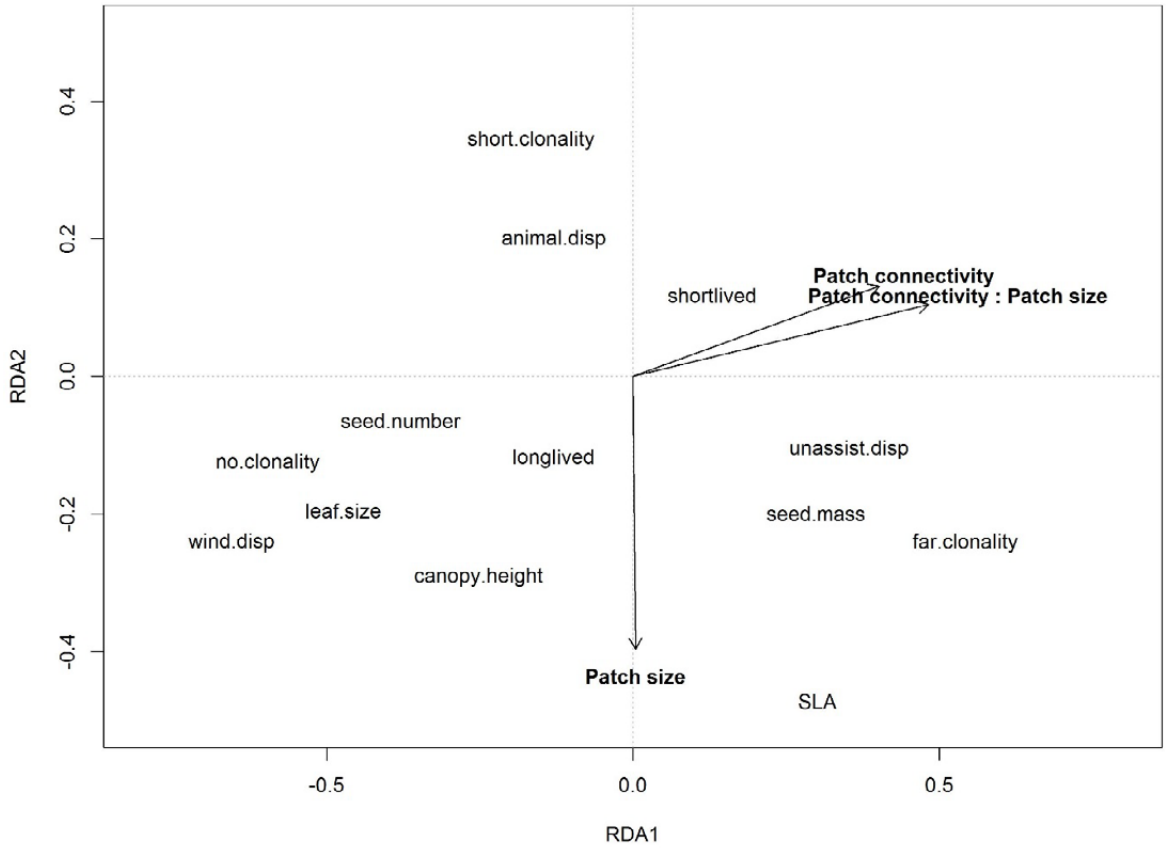

775

776 **Fig. 2** RDA ordination plot of specialist plant community weighted mean trait composition as

777 a response to patch size and connectivity in 200 sample plots in 20 habitat patches. See **Table**

778 **1** for details on traits**.**

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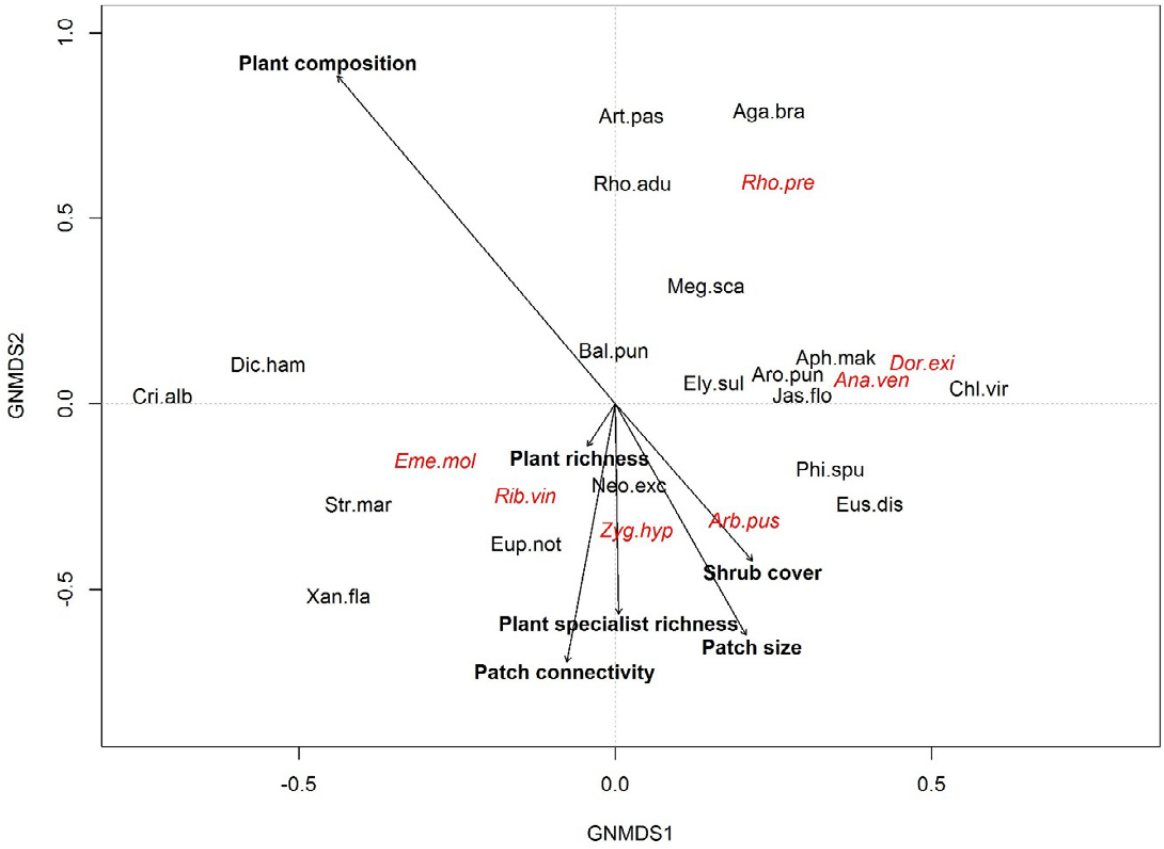

783

784 **Fig. 3** GNMDS ordination plot of total hopper species composition in 36 sample plots in 12

785 habitat patches. Habitat specialists are shown in red italics. Only species with > 15 individuals

786 in the dataset are shown. Arrows indicate correlations between the ordination and

787 environmental variables, with the length of the arrows proportional to the correlation strength.

788 For species abbreviations, see **Table A1.2**.

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