Journal of Environmental Management 94 (2012) 125–131

Contents lists available at ScienceDirect

Journal of Environmental Management

journal homepage: www.elsevier.com/locate/jenvman

Does forest fragmentation affect the same way all growth-forms?

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ARTICLE INFO

Article history:
Received 26 October 2010
Received in revised form
12 April 2011
Accepted 14 June 2011
Available online 15 September 2011

Keywords:
Patch size
Degree of isolation
Species traits
Forest specialist species

ABSTRACT

Fragmentation of natural habitats is one of the main causes of the loss of biodiversity. However, all plants do not respond to habitat fragmentation in the same way due to differences in species traits. We studied the effect of patch size and isolation on the biodiversity of vegetation in the mixed-oak forests in the north of the Iberian Peninsula. The aim was to evaluate whether all the growth-forms of vegetation are equally affected by forest fragmentation in order to improve the management strategies to restore this type of vegetation.

This study has shown that the effect of the area and spatial isolation of the patches was not the same for the different growth-forms. Fragmentation had a mainly negative effect on the richness and diversity of forest specialist species, especially ferns and herbaceous growth-forms. Moreover, the presence and/or cover of woodland herbaceous species (such as *Lamiastrum galeobdolon* and *Helleborus viridis*) and of woodland ferns (namely *Asplenium adiantum-nigrum, Asplenium trichomanes, Polystichum setiferum, Dryopteris affinis*) were negatively affected by patch size, possibly due to the reduction of habitat quality. These species have been replaced by more generalist species (such as *Cardamine pratensis*, *Cirsium* sp., *Pulmonaria longifolia* or *Rumex acetosella*) in small patches. Patch isolation had a negative effect on the presence of forest specialist species (namely, *L. galeobdolon*, *Frangula alnus*, *Hypericum androsaemum*, *A. adiantum-nigrum and Athyrium filix-femina*) and favored colonization by more generalist species such as *Cirsium* sp., *Calluna vulgaris*, *Erica arborea* or *Ulex* sp. Thus, in this region special attention should be paid to the conservation of forest specialist species, especially ferns and herbs. In conservation policy focused on forest specialist species, the most valuable species in forest ecosystems, conservation of large forest areas should be promoted.

1. Introduction

The excessive destruction and fragmentation of natural and semi-natural habitats on the Earth's surface is recognized as one of the principal causes of the loss of wild biodiversity (D'eon and Glenn, 2005; Fischer and Lindenmayer, 2007; Haines-Young, 2009; Harrison and Bruna, 1999; Hobbs, 2000; Meffe and Carroll, 1997; Wilcox and Murphy, 1985; Wood et al., 2000). The effects of habitat fragmentation on biodiversity have been studied for several decades, resulting in a vast literature on this topic. Despite continued debate about the relative importance of habitat fragmentation and habitat loss (Fahrig, 2003; Hanski and Gaggiotti, 2004), it is generally accepted that the size and spatial distribution of habitat remnants alters the patterns of species distribution and abundance within a landscape (Ewers and Didham, 2006).

The processes of reduction, spatial separation and increased isolation of habitats caused by fragmentation are associated with a reduction in the abundance, distribution and viability of species closely linked to these habitats (Bender et al., 2005; Fahrig and Merriam, 1994; Kleyer et al., 1996; Kupfer et al., 2006). However, not all plant species show the same response to habitat fragmentation. For instance, a number of studies have shown that the nature of the species-area relationship describing species loss from habitat fragments is confounded by differences in species traits (Cagnolo et al., 2006; Ewers and Didham, 2006; Godefroid and Koedam, 2003; Kolb and Diekmann, 2005). Some studies have shown that habitat fragmentation affected plants with specific dispersal modes (Kolb and Diekmann, 2005; Tabarelli et al., 1999), low frequency of occurrence and high habitat specificity (Hill and Curran, 2001; Iida and Nakashizuka, 1995). Plant species with different growth-forms (woody *vs.* herbaceous; short-lived *vs.* long-lived) can present different responses to fragmentation. Woody plants grow more slowly and devote the greater part of their photosynthesis to the production of structural materials for long-term survival (Chapin, 1991). Meanwhile, herbaceous plants

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doi:10.1016/j.jenvman.2011.06.024

grow and die more rapidly and devote the greater part of their photosynthesis to reproduction and rapid turn over. These characteristics can make species respond differently to fragmentation and, if they are affected, have different response times (Ewers and Didham, 2006). In fact, it has been postulated that short-lived species like herbs should be more sensitive to edge effects which would favor colonization by ruderal species (Cagnolo et al., 2006). Influence from surrounding vegetation may actually increase the total species richness of fragmented woodlands, but reduce the fraction of habitat specialists (Harrison, 1999). Thus, an assessment of the effect of fragmentation on plant communities should be based not only on species richness but also on species type, which can be defined in terms of conservation value or ecological traits (Honnay et al., 1999a; Hill and Curran, 2001).

In the north of the Iberian Peninsula the 'the native vegetation type mixed-oak forests, dominated by *Quercus robur* L. with *Fraxinus excelsior* L. and *Castanea sativa* Miller (Onaindia et al., 2004). However, since the beginning of the 20th century much of the area has been planted with fast growing exotic species, namely *Pinus radiata* and *Eucalyptus globulus*. This change has mainly affected forest specialist species (Amezaga and Onaindia, 1997). The aim of this research was to test whether the spatial configuration of those forests, namely size, form and degree of isolation of the patches, affects all of the plant species in the same way or whether the effect varies for different growth-forms (herbaceous, ferns, climbers, shrubs and trees) and forest specialist species (Aseginolaza et al., 1988).

2. Methods

2.1. Study area

This study was carried out in the Urdaibai Biosphere Reserve (UBR) (area 220 km^2) located in the north of the Iberian Peninsula (43°19′N, 02°40′W) (Fig. 1). The UBR is one of the most important natural areas of the Basque Country (Northern Spain) due to, among other features, its unique and diverse landscape which includes a craggy countryside occupied by meadow land, oak groves, deciduous woods and, especially, pine plantations.

The native vegetation for about 80% of the UBR is mixed-oak forests, dominated by *Q. robur* L. with *F. excelsior* L. and *C. sativa* Miller (Onaindia et al., 2004). Throughout the 20th century, these

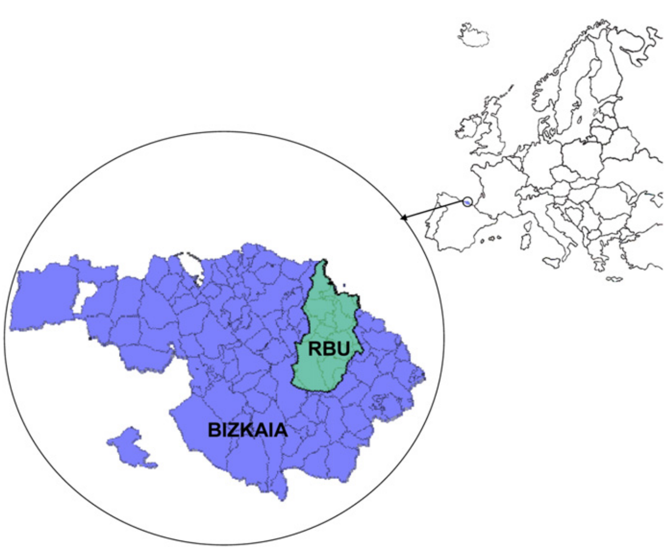

Fig. 1. Location of the study area.

native mixed-oak forests were heavily fragmented and, as a result, today they cover only about 6% of the total area of the Urdaibai Reserve (Rodríguez-Loinaz et al., in press) as has happened with other natural forests in other parts of the word (Schessl et al., 2008). Afterward, the traditional practices of timber and coal production were abandoned and the remaining forest patches started a process of regeneration (Michel, 2006).

2.2. Patch selection and vegetation sampling

A total of 33 patches of mixed-oak forest situated in the UBR were selected by means of the land use map at a 1:10 000 scale (Fig. 2). The selection was made as a function of size, since a principal objective was to establish if the diversity of vascular plant species was affected by the size of the patch. Therefore, 18 patches of a size between two and 3 ha and 15 patches of a size between ten and 30 ha were selected. There was no difference in altitude, slope, soil type or geographical location between small and large patches (small patches: mean altitude: 133 ± 18.89 m, slope: 25 ± 2.10%, pH: 4.65 ± 0.10, UTM_X: 525 762 ± 687, UTM_Y: 4.7984 10^6 ± 1534 and large: mean altitude: 174 ± 17.36 m, slope: 30 ± 2.61%, pH: 4.76 ± 0.11, UTM_X: 526 363 ± 962, UTM_Y: 4.7965 10^6 ± 1693). This selection was determined after analysis of the distribution of patch sizes given that these were the only sizes that occurred in significant numbers. The following indices were determined for each patch: area, distance to the nearest patch of mixed-oak forests (edge to edge) (NND, measure of the degree of isolation) and the fractal dimension (FD, measure of the form) (Mc Garigal et al., 2002), for which the v-LATE software was used (Lang and Tiede, 2003).

Since sampling effort and number of species recorded are usually related (Magurran, 1988; Hill et al., 1994; Lomolino, 2001), the area sampled was kept constant in all sites in order to avoid sampling artefacts on the effects of habitat fragmentation (Hill et al., 1994). In each of the patches (large and small) one plot of 25 m × 25 m was located approximately in the center of each patch in order to minimize possible edge effects. Within each plot, five sub-plots of 2 × 1 m were delineated. One was in the center and the other four separated by 12 m, making a cross with an arm running with the slope and the other perpendicular to it. The number of sub-plots was determined according to the method of the species/area curve (Kent and Coker, 1992). In these sub-plots the pattern of vegetation during June and July 2005 was studied. In each sub-plot, plant species were identified and the percentage cover for each plant species, calculated through visual estimation, was determined. In order to determine percentage cover, five different strata (levels) were considered, i.e. 0–0.20, 0.20–1, 1–3, 3–7, >7 m, following Brower and Zar (1977) and Onaindia et al. (2004). The total percentage cover for each plant species was obtained by adding up its percentage cover in each of the five different strata. In addition, the cover of trees as an indirect measure of quantity of light was measured, as light condition is one of the main factors in forest habitats (Sarlöv-Herlin and Fry, 2000) and it is known to affect vegetation (Amezaga et al., 2006; Borchsenius et al., 2004).

Summing the cover in the five sub-plots, the total cover of each species in the sampled area was obtained. Using these data the indices of richness (S) and Shannon (H′) and Simpson (1-D) diversity were calculated. These indices were obtained for the overall vegetation and for the different growth-forms present. Five grownforms were considered: herbaceous, ferns, climbers, shrubs and trees. Not woody species were separated in herbaceous and ferns, due to their botanical difference, and woody species were separated in climbers, shrubs and trees. To classify not climber woody species as shrub or tree the following criteria was used: not climber woody species that usually do not reach heights higher than 3 or

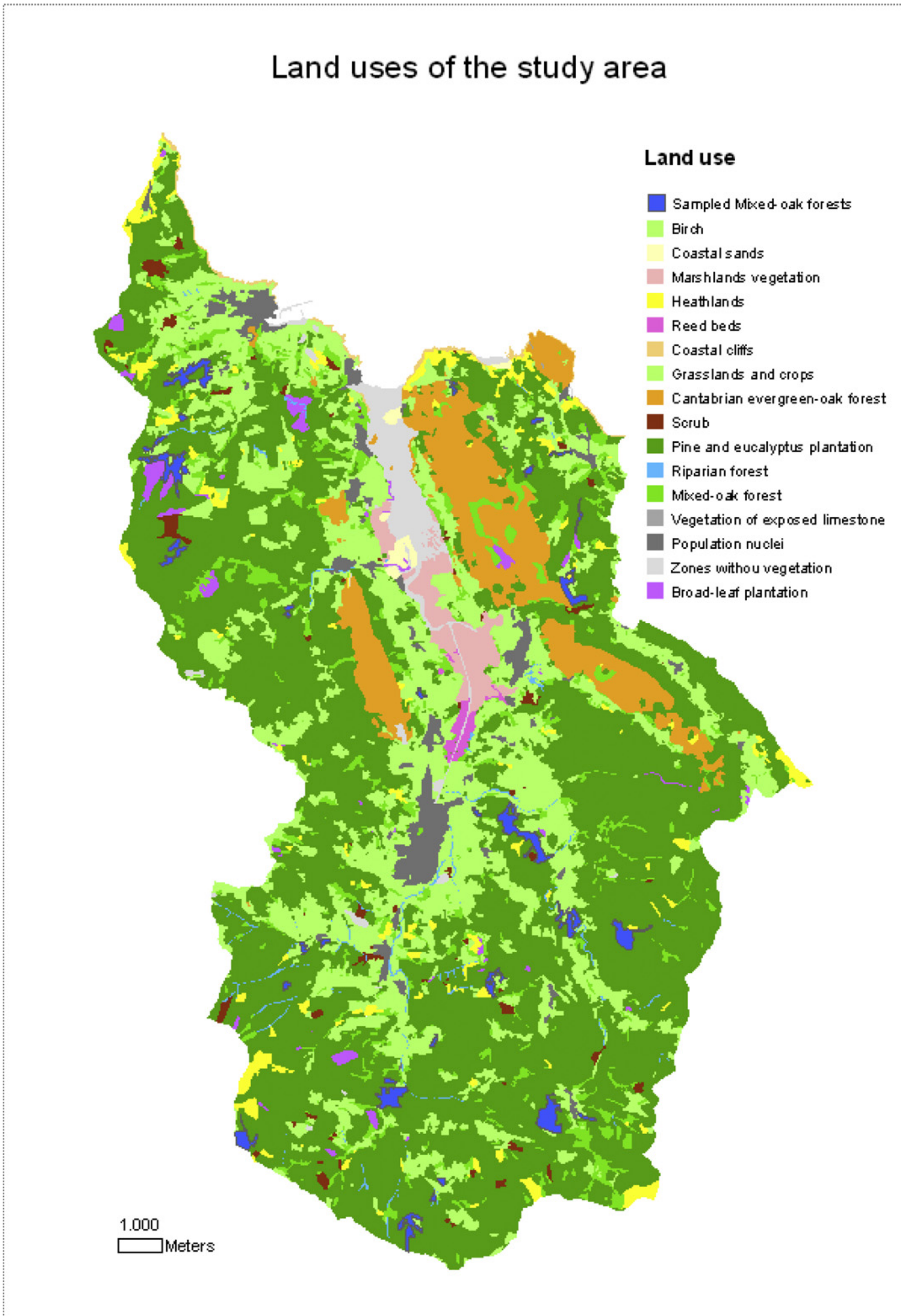

Fig. 2. Map of land uses and localization of studied mixed-oak forests in the study area.

4 m and are usually branched out from the base were classified as shrub; and not climber woody species that reach heights higher than 3 or 4 m and have a differentiated stem were classified as tree. In addition, the same indices were calculated for the overall forest specialist species and finally for the different growth-forms within the forest specialist species. To classify a species as forest specialist the "Illustrated keys of the flora of the Basque Country and bordering territories" (Aseginolaza et al., 1988) was used. In this book the natural habitat for every species is described. All those species whose natural habitat was described as nemoral forest, beech forest, oak forest or humid and shaded sites in forest, were classified as forest specialist species.

In addition, the overall vegetation similarity in relation to patch size and distance to the nearest missed-oak forest patch was calculated using the Sorensen's community similarity index. As the distance to the nearest patch was a continuous variable, the comparison was performed among the five patches with the smallest (<50 m) NND and the five patches with the largest (>200 m) NND.

2.3. Statistical analysis

As patch indices (patch size, patch isolation, fractal dimension) were not correlated (Spearman rank correlation, $P > 0.05$), a General Linear Model (GLM) was performed to analyze the effects of fragmentation on the richness and diversity of the vegetation. In this model the size (large or small) was introduced as a factor and the fractal dimension (FD), degree of isolation (NND) and cover of trees were introduced as co-variants.

Having analyzed the effects of fragmentation on richness and diversity, the effect of size and isolation (distance to the nearest patch of mixed-oak forest) on overall species composition was tested by means of the semi-parametric permutational multivariate analyses of variance (hereafter PERMANOVA) developed by Anderson (2001). Indicator Species Analysis (ISA; Dufrene and Legendre, 1997) was used to determine the characteristic species within patch size. Only species with $P < 0.05$ were considered (assessed using Monte Carlo randomizations with 999 permutations and INDVAL> 25).

3. Results

3.1. Vegetation structure

A total of 110 plant species of which 53 (27 forest specialist) were herbaceous, 5 (4 forest specialist) climbers, 18 (6 forest specialist) trees, 23 (7 forest specialist) shrubs and 11 (7 forest specialist) ferns were found in this study (Table 1). Of these 110 species, 84 were found in the large patches and 90 in the small ones.

The vegetation similarity results showed that 78% of the species were the same for the large and small patches and 50% for the patches with the smallest and largest NND. Those species only present in the large patches were usually (80%) forest specialist species such as *Asplenium adiantum-nigrum, Asplenium trichomanes, Helleborus viridis* or *Lamiastrum galeobdolon* while those only present in the small patches were more ubiquitous and generalist such as *Cardamine pratensis*, *Cirsium* sp., *Pulmonaria longifolia* or *Rumex acetosella*, and the same happened when the NND was applied. Species such as *L. galeobdolon*, *Frangula alnus*, *Hypericum androsaemum*, *A. adiantum-nigrum* or *Athyrium filix-femina*, appeared only in short distance (<50 m) patches while more generalist species, namely *Cirsium* sp., *Calluna vulgaris*, *Erica arborea* or *Ulex* sp., only appeared in the long distance (>200 m) patches.

3.2. Environmental effect

The General Linear Model (GLM) was applied to the 36 calculated indices (Table 2) but significant results were obtained only in 14 cases: richness and diversity of forest specialist species considered as a whole ($S_{f.e.}$, $H'_{f.e.}$ and $1\text{-}D_{f.e.}$); richness and diversity of overall and of forest specialist ferns (S ferns, H′ ferns, 1-D ferns, S ferns $_{f.e.}$, H′ferns $_{f.e.}$ and 1-D ferns $_{f.e.}$); diversity of overall herbaceous species (H′herbaceous and 1-D herbaceous); and richness and diversity of forest specialist herbaceous species (S herbaceous $_{f.e.}$ H′herbaceous $_{f.e.}$ and 1-D herbaceous $_{f.e.}$).

The model explaining the highest percentage of variance was related to the richness of forest specialist species considered as

Table 1
Plant species composition and percentage cover (mean ± SE) of the plant species for the large and small patches of mixed-oak forest (small = 2–3 ha, and large ≥ 10 ha).

Plant species	%Cover	
	Small patches	Large patches
Herbaceous plants		
Ajuga reptans L. [b]	1.72 ± 0.63	1.13 ± 0.65
Euphorbia amygdaloides L. [b]	0.83 ± 0.46	3.01 ± 1.75
Euphorbia sp.	2.39 ± 1.15	1.41 ± 1.07
Geranium robertianum L.	0.63 ± 0.31	0.47 ± 0.40
Gramineae	52.44 ± 9.62	30.27 ± 5.73
Helleborus viridis L. [b]	0 ± 0	0.27 ± 0.11
Hypericum pulchrum L. [b]	0.28 ± 0.16	0.41 ± 0.21
Lamiastrum galeobdolon (L.) Ehrend. & Polatschek [b]	0 ± 0	7.16 ± 3.33[a]
Lathyrus linifolius (Reichard) Bässler [b]	0.57 ± 0.24	0.40 ± 0.21
Potentilla erecta (L.) Raeuschel	1.74 ± 1.07	0.13 ± 0.13
Potentilla sterilis (L.) Garcke	1.00 ± 0.58	0.30 ± 0.21
Ranunculus tuberosus Lapeyr.	1.29 ± 0.84	1.08 ± 0.47
Rubia peregrina L. [b]	2.29 ± 1.24	3.95 ± 1.63
Saxifraga hirsuta [b]	0 ± 0	1.28 ± 0.90
Solidago virgaurea L.	1.39 ± 0.65	0.13 ± 0.13
Stachys officinalis (L.) Trevisan	6.56 ± 2.28	2.83 ± 1.50
Symphytum tuberosum L. [b]	1.72 ± 1.11	0.93 ± 0.46
Teucrium scorodonia L.	6.36 ± 2.34	7.27 ± 2.99
Vicia sepium L. [b]	0.39 ± 0.39	1.67 ± 0.87
Viola riviniana Reichenb.	3.90 ± 1.60	2.35 ± 1.08
Ferns		
Asplenium adiantum-nigrum L. [b]	0 ± 0	1.27 ± 0.61
Asplenium trichomanes L. [b]	0 ± 0	0.72 ± 0.28
Athyrium filix-femina (L.) Roth [b]	5.29 ± 2.86	12.33 ± 5.76
Blechnum spicant (L.) Roth [b]	8.78 ± 2.51	15.75 ± 5.96
Dryopteris affinis (Lowe) raser- Jenkins [b]	2.44 ± 1.39	18.05 ± 5.24[a]
Polystichum setiferum (Forsskål) Woynar [b]	3.06 ± 1.29	12.80 ± 4.93[a]
Pteridium aquilinum (L.) Kuhn	31.11 ± 7.69	11.60 ± 4.37
Climbing plants		
Hedera helix L. [b]	59.10 ± 7.26	44.94 ± 7.93
Lonicera periclymenum L. [b]	16.18 ± 3.31	23.05 ± 5.36
Smilax aspera L. [b]	16.78 ± 6.14	25.23 ± 7.88
Tamus communis L. [b]	5.94 ± 2.12	3.52 ± 1.32
Trees		
Acer campestre L. [b]	1.29 ± 1.08	4.56 ± 3.30
Arbutus unedo L.	1.50 ± 1.03	1.27 ± 1.27
Betula alba L.	3.00 ± 2.57	3.93 ± 3.17
Castanea sativa Millar [b]	19.67 ± 6.78	31.93 ± 9.69
Fraxinus excelsior L. [b]	11.01 ± 4.37	9.86 ± 4.41
Laurus nobilis L. [b]	18.68 ± 12.6	10.60 ± 6.31
Prunus avium L. [b]	1.33 ± 0.66	2.33 ± 1.88
Quercus ilex L.	4.00 ± 2.64	0 ± 0
Quercus robur L. [b]	98.53 ± 8.48	93.90 ± 7.70
Salix atrocinerea Brot.	9.06 ± 5.72	8.13 ± 4.38
Shrubs		
Cornus sanguinea L.	9.56 ± 5.64	8.81 ± 3.43
Corylus avellana L. [b]	33.39 ± 9.10	52.67 ± 15.7
Crataegus monogyna Jacq.	0.56 ± 0.28	6.24 ± 3.71
Daboecia cantabrica (Hudson) C. Koch	1.18 ± 0.48	1.08 ± 1.00
Erica vagans L.	0.46 ± 0.26	0 ± 0
Euonymus europaeus L.	1.56 ± 1.18	0.47 ± 0.32
Frangula alnus Miller [b]	2.52 ± 1.27	2.81 ± 1.54
Hypericum androsaemum L. [b]	1.44 ± 0.72	2.56 ± 1.02
Ilex aquifolium L. [b]	0.74 ± 0.43	3.64 ± 3.18
Rosa sp.	8.73 ± 3.48	8.01 ± 1.99
Rubus sp.	43.17 ± 6.83	67.81 ± 9.07
Ruscus aculeatus L. [b]	2.39 ± 1.39	5.27 ± 2.79
Ulex sp.	1.61 ± 1.02	1.13 ± 0.77

Only those species that were found in more than 20% of the patches of at least one of the sizes have been included.
[a] Large patch indicator species.
[b] Forest specialist species.

Table 2
Diversity indices of vegetation composition.

	Indices	Small patches (Mean ± SE)	Large patches (Mean ± SE)
Overall Species	S	22.78 ± 1.14	24.67 ± 1.77
	H′	3.46 ± 0.08	3.63 ± 0.09
	1-D	0.87 ± 0.01	0.89 ± 0.01
	S herbaceous	7.78 ± 1.00	7.40 ± 0.97
	H′herbaceous	1.82 ± 0.17	1.81 ± 0.23
	1-D herbaceous	0.59 ± 0.04	0.57 ± 0.07
	S trees	3.94 ± 0.35	4.27 ± 0.36
	H′trees	1.15 ± 0.13	1.37 ± 0.13
	1-D trees	0.43 ± 0.05	0.52 ± 0.04
	S shrubs	5.22 ± 0.31	5.60 ± 0.34
	H′shrubs	1.61 ± 0.09	1.65 ± 0.11
	1-D shrubs	0.59 ± 0.03	0.59 ± 0.03
	S ferns	2.67 ± 0.29	4.20 ± 0.54
	H′ferns	0.94 ± 0.15	1.59 ± 0.17
	1-D ferns	0.38 ± 0.06	0.58 ± 0.05
	S climbing plants	3.17 ± 0.17	3.20 ± 0.20
	H′climbing plants	1.14 ± 0.09	1.13 ± 0.16
	1-D climbing plants	0.48 ± 0.03	0.45 ± 0.06
Only Tipical Forest Species	$S_{f.e.}$	12.83 ± 0.84	15.80 ± 1.35
	$H'_{f.e.}$	2.65 ± 0.10	2.90 ± 0.13
	1-D $_{f.e.}$	0.57 ± 0.06	0.66 ± 0.07
	S herbaceous $_{f.e.}$	3.94 ± 0.64	4.67 ± 0.77
	H′herbaceous $_{f.e.}$	1.22 ± 0.23	1.51 ± 0.22
	1-D herbaceous $_{f.e.}$	0.43 ± 0.07	0.53 ± 0.07
	S trees $_{f.e.}$	2.72 ± 0.23	3.27 ± 0.30
	H′trees $_{f.e.}$	0.86 ± 0.10	1.10 ± 0.13
	1-D trees $_{f.e.}$	0.36 ± 0.04	0.43 ± 0.05
	S shrubs $_{f.e.}$	0.83 ± 0.22	2.27 ± 0.34
	H′shrubs $_{f.e.}$	0.42 ± 0.10	0.55 ± 0.10
	1-D shrubs $_{f.e.}$	0.17 ± 0.04	0.23 ± 0.05
	S ferns $_{f.e.}$	1.78 ± 0.30	3.07 ± 0.42
	H′ferns $_{f.e.}$	0.54 ± 0.16	1.16 ± 0.20
	1-D ferns $_{f.e.}$	0.22 ± 0.06	0.45 ± 0.07
	S climbing plants $_{f.e.}$	2.56 ± 0.12	2.53 ± 0.19
	H′climbing plants $_{f.e.}$	0.98 ± 0.06	1.00 ± 0.14
	1-D climbing plants $_{f.e.}$	0.43 ± 0.03	0.42 ± 0.06

Both for the overall vegetation as well as for the forest specialist species (f.e.), totals and by growth-form (mean ± SE) for both sizes. S = richness, H′ = Shannon diversity, 1-D: Simpson diversity.

a whole (adjusted $r^2 = 0.43$, $P = 0.003$). Patch size had a positive effect on the number of forest specialist species ($S_{f.e.}$ (mean ± SE): 15.80 ± 1.35 for large and 12.83 ± 0.84 for small patches) while degree of isolation (NND) had a negative effect (Table 3). The same happened with the diversity of forest specialist species considered as a whole ($H'_{f.e.}$ and 1-D $_{f.e.}$) (Tables 2 and 3).

In the case of fern species richness, overall and forest specialist (f.e.), 39.8% and 36.5% respectively of the total variance was captured by the model (adjusted r^2, $P = 0.007$ and $P = 0.011$ respectively). Both were negatively affected by isolation (NND) and positively by patch size (Table 3). Thus, large patches had higher overall and forest specialist fern species richness than small ones (S ferns (mean ± SE): 4.20 ± 0.54 and 2.67 ± 0.29 respectively and S ferns $_{f.e.}$ (mean ± SE): 3.07 ± 0.42 and 1.78 ± 0.30 respectively for the forest specialist species). The same happened for the diversity of overall and those forest specialists (f.e.) fern (H′ ferns, 1-D ferns, H′ferns $_{f.e.}$ and 1-D ferns $_{f.e.}$) (Tables 2 and 3).

In relation to the herbaceous species the model for the overall and forest specialist herbaceous species diversity accounted for 37% of the total variance (adjusted r^2, $P = 0.012$) and 24% (adjusted r^2, $P = 0.043$), respectively. These diversities decreased only with patch isolation (NND) and were not significantly affected by patch size (Table 3). Moreover, forest specialist herbaceous species richness was also negatively affected by patch isolation (adjusted $r^2 = 0.29$, $P = 0.045$). The same happened with the forest specialist herbaceous species diversity (H′ herbaceous $_{f.e.}$ and 1-D herbaceous $_{f.e.}$) (Tables 2 and 3).

Finally, patch form and tree cover did not have any significant effect on the model for any of the 36 studied indices.

Table 3
Significant results of the general linear model for the vegetation indices analyzed.

Dependent Variables	β	r^2	F	p
H′ferns (adjusted r^2:0.364, $p = 0.011$*)				
FD	0.091	0.011	0.313	0.580
NND	−0.359	0.162	5.432	0.027*
Area	0.481	0.262	9.940	0.004**
Tree cover	−0.245	0.074	2.236	0.146
S ferns (adjusted r^2:0.398, $p = 0.007$**)				
FD	0.117	0.019	0.547	0.466
NND	−0.412	0.214	7.623	0.010**
Area	0.455	0.252	9.410	0.005**
Tree cover	−0.332	0.130	4.031	0.055
H′herbaceous (adjusted r^2:0.370, $p = 0.012$*)				
FD	0.358	0.135	4.171	0.052
NND	−0.491	0.275	10.617	0.003**
Area	−0.006	0.000	0.002	0.969
Tree cover	−0.330	0.133	3.797	0.062
$H'_{f.e.}$ (adjusted r^2:0.365, $p = 0.011$*)				
FD	−0.028	0.001	0.030	0.864
NND	−0.546	0.310	12.606	0.001**
Area	0.321	0.136	4.409	0.045*
Tree cover	−0.062	0.005	0.142	0.709
$S_{f.e.}$ (adjusted r^2:0.433, $p = 0.003$**)				
FD	0.176	0.044	1.301	0.264
NND	−0.527	0.319	13.122	0.001**
Area	0.352	0.175	5.951	0.021*
Tree cover	−0.238	0.072	2.094	0.159
H′ferns $_{f.e.}$ (adjusted r^2:0.309, $p = 0.03$*)				
FD	0.017	0.000	0.009	0.923
NND	−0.353	0.147	4.834	0.036*
Area	0.435	0.210	7.437	0.011*
Tree cover	−0.242	0.067	1.995	0.169
S ferns $_{f.e.}$ (adjusted r^2:0.365, $p = 0.011$*)				
FD	0.075	0.008	0.214	0.647
NND	−0.402	0.196	6.815	0.014*
Area	0.448	0.235	8.606	0.007**
Tree cover	−0.275	0.092	2.823	0.104
H′herbaceous $_{f.e.}$ (adjusted r^2:0.244, $p = 0.043$*)				
FD	0.243	0.066	1.980	0.170
NND	−0.384	0.165	5.551	0.026*
Area	0.171	0.039	1.123	0.298
Tree cover	−0.320	0.099	2.968	0.096
S herbaceous $_{f.e.}$ (adjusted r^2:0.294, $p = 0.045$*)				
FD	0.359	0.119	4.521	0.052
NND	−0.377	0.167	5.625	0.025*
Area	0.125	0.022	0.631	0.434
Tree cover	−0.365	0.133	4.149	0.052

The model was applied to the 36 calculated indices but only those for which significant results were obtained were included in this table. Results for Simpson diversity (1-D) have not been included since they are similar to those for Shannon diversity (H′). H′ = Shannon diversity, S = richness, f.e. = forest specialist, FD = fractal dimension, NND = distance to the nearest patch, Area = patch area, β = standardized beta coefficient, r^2 = r^2 coefficient of regression, p = level of significance.
*$p \leq 0.05$; **$p \leq 0.01$.

3.3. Fragmentation effect on individual species

PERMANOVA conducted on the overall species composition showed only a significant effect of patch size ($F_{(1,29)} = 1.80$, $P < 0.05$) and the Indicator Species Analysis identified three species, namely *L. galeobdolon*, *Polystichum setiferum* and *Dryopteris affinis* as indicators of large patches (Table 1).

4. Discussion

Worldwide, land use change and habitat fragmentation caused by human beings have been identified as the most important processes that affect forest species richness and composition (Guirado et al., 2007; Hobbs, 2000; Van der Veken et al., 2004; Wood et al., 2000). In the situation studied here, the positive effect of larger patch size on vegetation richness and diversity was evident when considering only the forest specialist species rather than the total vegetation, which we know not to be a result of habitat diversity due to the sampling method (Petit et al., 2004). This positive effect of patch area on forest specialist species richness has also been shown by other studies (Godefroid and Koedam, 2003; Honnay et al., 1999b). This type of vegetation has some habitat quality requirements which, when patch size is reduced, are lost (Amezaga and Onaindia, 1997; Levenson, 1981; Peterken and Game, 1984; Petit et al., 2004). Moreover, this effect of size was mainly seen for the diversity and richness of the group of ferns. These results were consistent with those obtained by Murakami et al. (2005). This relationship could be due to the fact that the ensemble of the forest specialist ferns was made up of only seven species, most of which show very similar ecological and life cycle characteristics. They need certain conditions, particularly humidity, which are found in mature forests but are modified upon reduction of the patch size because of the increase of the edge effect (reduction of habitat quality) (Petit et al., 2004). Thus, when the species were individually considered, patch size clearly showed an effect on the cover of some forest specialist fern and herbaceous species, namely *L. galeobdolon*, *P. setiferum* and *D. affinis*. These species are considered indicators of good forest conservation and mature forest and have been shown to increase with patch age (Bossuyt et al., 1999; Grime et al., 1988; Honnay et al., 1999a; Onaindia et al., 2004; Verheyen and Hermy, 2001). When the overall species, not forest specialist species only, were considered, richness and diversity might have not changed because species identity does not influence this factor and ubiquitous and generalist species such as *C. pratensis*, *Cirsium* sp., *P. longifolia* or *R. acetosella* have replaced species that are more intolerant to changes of forest conditions because of loss of habitat quality (Onaindia et al., 2004), namely *A. adiantum-nigrum*, *A. trichomanes*, *P. setiferum*, *D. affinis*, *H. viridis* and *L. galeobdolon*.

The external variables that affect the richness and diversity of forest plants are related to the context of the landscape in which those patches are found, for example, the degree of isolation and the characteristics of the surrounding matrix (Grashof-Bokdam, 1997; Laurence and Yensen, 1991; Petit et al., 2004; Schmidt et al., 2009; van Ruremonde and Kalkhoven, 1991). In this study, a negative effect of the degree of patch isolation on vegetation richness and diversity has been shown and, as in the case of size, it has been mainly detected when the forest specialist species were considered, especially for ferns and herbaceous species. Once these species have disappeared from a patch, they depend upon colonization from the surrounding patches and the probability of colonization decreases with increasing spatial isolation (Di Giulio et al., 2009; Jacquemyn et al., 2001). However, the generalist species or broad ranged species such as *Cirsium* sp., *C. vulgaris*, *E. arborea* or *Ulex* sp. are not influenced by the distance to the nearest patch since they are distributed throughout the territory. This is probably why the richness of the overall vegetation did not show the effect of the degree of isolation. Godefroid and Koedam (2003) also found a lack of isolation effect on richness of woodland flora (excluding ruderal species) but once species that usually exist in the matrix were removed the effect of isolation became significant. However, Cagnolo et al. (2006) did not found any effect of isolation on the richness of native plant species richness since the isolation range included in their study may have been too narrow (75–200 m) for effects to be detected. In our case the range was large enough, from 25 to 740 m, to detect the isolation effect.

As for trees, shrubs and climber species no effect of patch size or isolation was found, which could be due to the fact that woody plants have longer response times, possessing a greater "ecological inertia" (López et al., 2002). It is also now apparent that the effects of fragmentation can take many decades to be expressed (Ewers and Didham, 2006). Some authors (e.g. Renjifo, 1999) consider time-scales of 50–90 years as 'long-term' and sufficient to ensure that diversity patterns have reached a dynamic equilibrium. However, this time frame may not be long enough to allow all fragmentation effects to be exhibited (particularly for long-lived organisms). In our case the actual spatial pattern of the mixed-oak forest is the result of the expansion of fast growing exotic species plantations, namely *P. radiata* and *E. globulus*, that started at the beginning of the 20th century and was accentuated in the 1950s. This time period may be too short for the effect of fragmentation to be exhibited in these long-lived growth-forms. López et al. (2002) also found this lack of effect of fragmentation on woody vegetation in wetlands. However, other authors (e.g. Mikk and Mander, 1995; Cagnolo et al., 2006) found a negative effect of patch area of forest patches on tree and shrub diversity.

Patch form determined the extent of the internal habitat. Thus, it was expected to affect vegetation richness and diversity. However, it did not show any effect, perhaps because the studied patches were fairly homogenous (from 1.28 to 1.40). Previous works show contradictory results. In some cases the result is a positive effect (Honnay et al., 1999a; Mikk and Mander, 1995); in others the contrary was found (Bastin and Thomas, 1999; Dzwonko and Loster, 1992; Lovett-Doust et al., 2003); and in others, as in our case, no effect at all (Guirado et al., 2007; Petit et al., 2004).

5. Conclusions

Fragmentation of mixed-oak forest was found to mainly negatively affect the diversity of forest specialist species, particularly ferns and herbaceous growth-forms, due to reduction in patch size and increase in patch isolation. Based on these results, conservation policies should try to keep large, well-connected patches in the landscape in order to maintain mixed-oak forest biodiversity. However, the need for large woods highlighted in this study should not be taken as an argument against smaller patches. Nowadays, large patches are not easy to maintain due to economic competition with other land uses, and as such, smaller patches could perform as stepping stones for the former. Moreover, the fact that fragmentation did not affect the different species equally, shows the importance of weighing them rather than counting them, as the question is not which wood patch contains more species in total, but which contain more vulnerable species, i.e. forest specialist, which would be doomed to extinction if the particular forest conditions are changed.

Acknowledgments

We gratefully acknowledge financial support from the Spanish Ministry of Education and Science (CGL2005-08046-C03 01 and CGL2008-05579-C02-01), the Department of Universities, Research and Education of the Basque Government (Groups bursar), UNESCO Chair of the University of the Basque Country and Basque Government (Industry Department-Etortek Project). We would also like to thank Sir Marrack Goulding for his contribution to the editing of this document.

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