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RESEARCH ARTICLE

The combined effects of habitat fragmentation and life history traits on specialisation in lichen symbioses

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Abstract

1. Interactions between organisms are determined by life-history traits. Ecological strategies regarding species specialisation range from generalist to highly specialised relationships. Although it is expected that habitat fragmentation's effect on species abundance and survival depends on their degree of specialisation and life-history traits, few studies have delved into the interplay between interaction specialisation, life-history traits and habitat fragmentation.
2. Here, we investigate the combined effect of habitat fragmentation, forest structure and life-history traits (growth form and reproductive mode) on the specialisation of lichen-forming fungi (mycobionts) toward their photosynthetic partners (photobionts) in lichen symbioses.
3. We studied mycobiont specialisation in epiphytic lichen communities present in 10 fragments of *Quercus rotundifolia* forest embedded in an agricultural matrix. Both mycobionts and photobionts were identified DNA barcoding and mycobiont specialisation was measured through interaction parameters calculating the relative number of interactions (normalised degree; ND) and the specialisation of each species based on its discrimination from a random selection of partners (*d'*). Phylogenetic generalised linear mixed models were used to analyse the effect of patch size as well as the life history traits growth form (crustose, foliose, fruticose) and reproduction mode (sexual vs. asexual) on mycobiont specialisation.
4. Both mycobiont and photobiont richness along the patch size gradient followed a hump-back pattern, which was more pronounced in photobionts. Mycobionts forming crustose thalli established the largest number of interactions. Mycobiont specialisation (*d'*) was larger for fruticose and foliose forms and species with vegetative reproduction. Along the gradient of fragment size, the relative number of interactions decreased and the specialisation of mycobionts with vegetative reproduction increased.
5. *Synthesis*. The study of mycobiont specialisation towards their photobionts in epiphytic lichen communities in a fragmented Mediterranean forest revealed a complex interaction between species' life history traits and habitat fragmentation. In

particular, this interplay had a significant impact on the specialisation of mycobionts. The results show the ability of some species to modulate their specialisation according to habitat conditions, suggesting that some species may be more resilient to abiotic changes than expected.

KEYWORDS
epiphytic, lichen-forming fungi, Mediterranean forest, photobionts, selectivity

1 | INTRODUCTION

Habitat fragmentation is a landscape process in which natural homogeneous habitat is transformed into smaller fragments embedded in a matrix different from the original one (Wilcove et al., 1986). It represents one of the main drivers of biodiversity loss under the current scenario of global change (Haddad et al., 2015). Habitat fragmentation leads to a reduction in the amount of habitat available to species and connectivity between available fragments, as well as to changes in the abiotic and biotic conditions of the remaining habitat (Fahrig, 2003). This process usually entails a decrease in population sizes, increasing the risk of species extinctions, and subsequent reduction of ecosystem structure, functioning, and services (Isbell et al., 2011; Millennium Ecosystem Assessment, 2005). Furthermore, as species interact with other species in natural habitats, fragmentation jeopardises species interactions (Hagen et al., 2012; Tylianakis et al., 2008), especially those established between specialists (Devictor et al., 2008; Nordén et al., 2013).

Whereas the loss of taxonomic diversity has received large attention during the last decades, knowledge on the effect of habitat fragmentation on interactions is still scarce (Gonzalez et al., 2011; Xiao et al., 2016). Since the loss of interactions often precedes the loss of species diversity in ecosystems, it is seen as an early signal of ecosystem decay (Aizen et al., 2012; Valiente-Banuet et al., 2015). Not all kinds of interactions are equally affected by fragmentation, which has been shown to produce more negative effects on mutualisms than on antagonisms (Magrach et al., 2014). Most of the knowledge we have on the effect of fragmentation on mutualisms comes from non-intimate interactions (e.g. seed dispersal, plant pollination) whereas intimate mutualisms have been largely ignored. Independently of the interaction type, species show variability regarding the degree of specialisation towards their partners, ranging from generalists, interacting with many partners, to specialists, which interact with a few or even a single partner (Solé & Montoya, 2001; Thompson, 1988). These strategies involve different evolutionary and ecological advantages and constraints (Dennis et al., 2011; Ollerton, 2006), and they have been highly relevant for predicting the loss of interactions and species in gradients of habitat availability (Aizen et al., 2012). In addition, it is known that specialisation in terms of the range of suitable partners is influenced by morphological and reproductive traits (Maglianesi et al., 2014; Otálora et al., 2013; Reif et al., 2016; Santamaría & Rodríguez-Gironés, 2007) and that it is also constrained by species' evolutionary history (Webb et al., 2010). Fragmentation may modify the dynamics of interactions by changing partner densities and behaviours (Xiao et al., 2016), and in general, specialisation tends to decrease as habitat fragmentation increases (Hadley et al., 2018; Jauker et al., 2019). This decline may be due to the loss of specialists (Aizen et al., 2012) or due to the variation of specialisation within species, which may show plasticity to cope with new habitat conditions. However, it is largely unknown whether and to what extent species can modulate their specialisation under changing environmental conditions to survive.

Lichens, the symbiotic phenotype of lichen-forming fungi (hereafter, the mycobiont) associated with, at least, one photosynthetic partner (the photobiont) cyanobacteria and/or a green alga (Grube & Hawksworth, 2007) are paradigmatic examples of intimate mutualisms. Due to their high sensibility to subtle changes in abiotic conditions in the environment, they are renowned bioindicators of habitat conditions (Marmor et al., 2011; McCune, 2000; Nascimbene & Marini, 2015; Rivas Plata et al., 2008), including changes in land management and habitat fragmentation (Aragón et al., 2010; Brunialti et al., 2013; Matos et al., 2017; Svoboda et al., 2010; Trobajo et al., 2022). Habitat fragmentation leads to major changes in the richness, abundance, and distribution of vital features in epiphytic lichen communities, mainly due to changes in abiotic conditions (interior vs. edge), often leading to the emergence of ubiquitous tolerant species as edge conditions prevail and impoverishment of the lichen flora (Belinchón et al., 2007; Brunialti et al., 2013; Trobajo et al., 2022).

Lichen-forming fungi depend on their photobionts as a source of carbohydrates which play a role in fungal growth as well as their capacity to survive desiccation (Spribille et al., 2022). The last two decades have seen major advances in the knowledge of photobiont diversity and the range of fungal-algal interactions in lichen symbioses at different systematic levels, especially in understanding the breadth of compatible photobionts (Dal Grande et al., 2012; Fernández-Mendoza et al., 2011; Leavitt et al., 2015; Sanders & Masumoto, 2021; Thüs et al., 2011). During this time, it has become clear that mycobiont specialisation toward the photobiont varies among species (Magain et al., 2016; Pérez-Ortega et al., 2012; Singh et al., 2017) and that partnering different photobionts allows certain species to expand their climatic niche (Fernández-Mendoza et al., 2011; Rolshausen et al., 2018). Research has shown that certain life history of lichens traits may influence their specialisation towards the photobiont (Otálora et al., 2013; Wornik & Grube, 2010). However, our understanding of the extent

of this influence remains limited. For instance, lichen thalli show several growth forms named biotypes (Honegger, 2001), which have numerous implications for associated photobionts since different architectures and topologies imply the existence of different microniches (Hawksworth & Grube, 2020). The role of thallus morphologies on photobiont specialisation has not been formally tested, although it is known that, for instance, crustose species usually display a high phylogenetic breadth of photobiont partners (Blaha et al., 2006; Guzow-Krzeminska, 2006; Muggia et al., 2014). Lichens also show a wide range of reproductive strategies (Tripp & Lendemer, 2018), but they can be simply divided between asexual versus sexual reproduction. Most common asexual reproductive strategies involve vegetative clonal propagules in which both bionts are dispersed together ensuring the availability of compatible photobionts for the fungi at the expense of suppressing genetic variability (Buschbom & Mueller, 2005; Yahr et al., 2006). On the contrary, the dispersal of the mycobiont via ascospores implies the propagule to find a compatible photobiont after its establishment on the new substrate (Fernández-Mendoza et al., 2011; Yahr et al., 2006). The scarce studies in which specialisation towards the photobiont has been explored within a reproductive framework offer contradictory results (Otálora et al., 2013; Wornik & Grube, 2010), likely due to the possibility of photobiont switches after the vegetative propagule is settled (Nelsen & Gargas, 2008).

As the growth form and the type of sexual reproduction tend to vary with contrasting environmental conditions (Ellis et al., 2021; Giordani et al., 2012; Hurtado et al., 2020; Trobajo et al., 2022), we hypothesise that trait-mediated specialisation towards photobionts is influenced by habitat fragmentation. To test so, we studied epiphytic lichen communities in a Mediterranean fragmented Holm oak (*Quercus rotundifolia*) forest in Central Spain along a fragment size gradient. More specifically, we first tested whether fragmentation equally affects mycobiont and photobiont diversity. Second, we analysed whether the specialisation of the mycobionts towards their photobionts is driven by variables related to fragmentation (fragment size, perimeter, shape and distance to the nearest fragment), as well as factors related to forest structure (tree density, mean diameter, canopy cover). Finally, we tested whether there could be a combined effect of life history traits (growth form and reproductive mode) and fragmentation, on specialisation towards photobionts.

2 | MATERIALS AND METHODS

2.1 | Study area

We studied a Holm oak (*Quercus rotundifolia*) archipelago placed in the plateau of central Spain (42°5–8′ N; 3°36–44′ W, 923–1093 m a.s.l.; Figure 1). This region currently has very few woodlands, since the use of the land for cereal production has reduced the forest mass to approximately 7%–8% of its original area (Santos & Tellería, 1998). In addition, the remaining forest stands have been used as a source of firewood. The fragmentation process occurred quickly in the study area being forest area reduced by 55.6% in the period 1946–1996 (Santos & Tellería, 1998). Fieldwork was carried out in the surroundings of the towns of Torrecilla del Monte, Mecerreyes and Villamayor de los Montes, within an area of c. 7000 ha. The dominant tree in most of the fragments of the area is Holm oak *Quercus rotundifolia*, with isolated Lusitanian oak *Quercus faginea*, prickly juniper *Juniperus oxycedrus*, and Spanish juniper *Juniperus thurifera*. Some forest patches in the northern part of the area were dominated by the Lusitanian oak and the Pyrenean oak *Quercus rotundifolia*. Most abundant understory shrubs are typical from the wetter and cooler supramediterranean belt, including *Cistus laurifolius*, *Genista scorpius*, *Thymus zygis* and *Lavandula stoechas*. Annual precipitation is 501.3 mm and annual mean temperature is 12.1°C (from AEMET data, mean 1961–2013 for precipitation, mean 1981–2003 for temperature: Villamayor de los Montes meteorological station at 1.59 km away from the closest studied forest fragment). Dominant soils are Cambisols (calcics) (WRB-FAO, 2015).

2.2 | Experimental design and lichen sampling

The selection of the forest fragments was made as follows: Orthorectified photographs were downloaded from the National Centre for Geographic Information (http://centrodedescargas.cnig.es/CentroDescargas/-index.jsp) corresponding to the year 2017, and using the Google Earth Pro software (available at https://www.google.com/earth/download/gep/agree.html) all forest fragments present in the area were identified. Fragment size and perimeter were calculated for each forest patch in Google Earth Pro. Fragments with highly irregular geometries (low area/perimeter ratio) were discarded to avoid disproportionate edge effects. The remaining fragments were inspected during autumn 2017 to establish the identity of the tree composition. Considering the large number of fragments of different sizes in the area, we focused on the fragments where *Quercus rotundifolia* was the dominant species (>85%) in order to avoid differences caused by substrate preference since slightly different epiphytic communities occurred on Pyrenean and Lusitanian oaks in the area (Pérez-Ortega, data not published). Finally, a total of 10 fragments were selected in a size gradient ranging from 0.002 ha (a single isolated tree) to 250 ha (Table S1).

In each fragment, epiphytic lichen communities were surveyed at the geographic center of the fragments. Lichen Inventory was carried out according to Asta et al. (2002). In summary, we identified the lichen community on a total of 10 trunks. Starting from the geographic centre of each forest fragment (except the single tree), we selected 10 trees that fulfilled the following conditions: diameter ≥12 cm, trunk inclination ≤10° and presence of a single stem. By eliminating trees with high inclinations, we excluded those with large bryophyte cover. Each trunk was sampled using a 10 × 50 cm grid divided into five 10 × 10 cm squares, which were placed in both north and south orientation, and we recorded all species present and their abundance, measured as the number of squares in which the

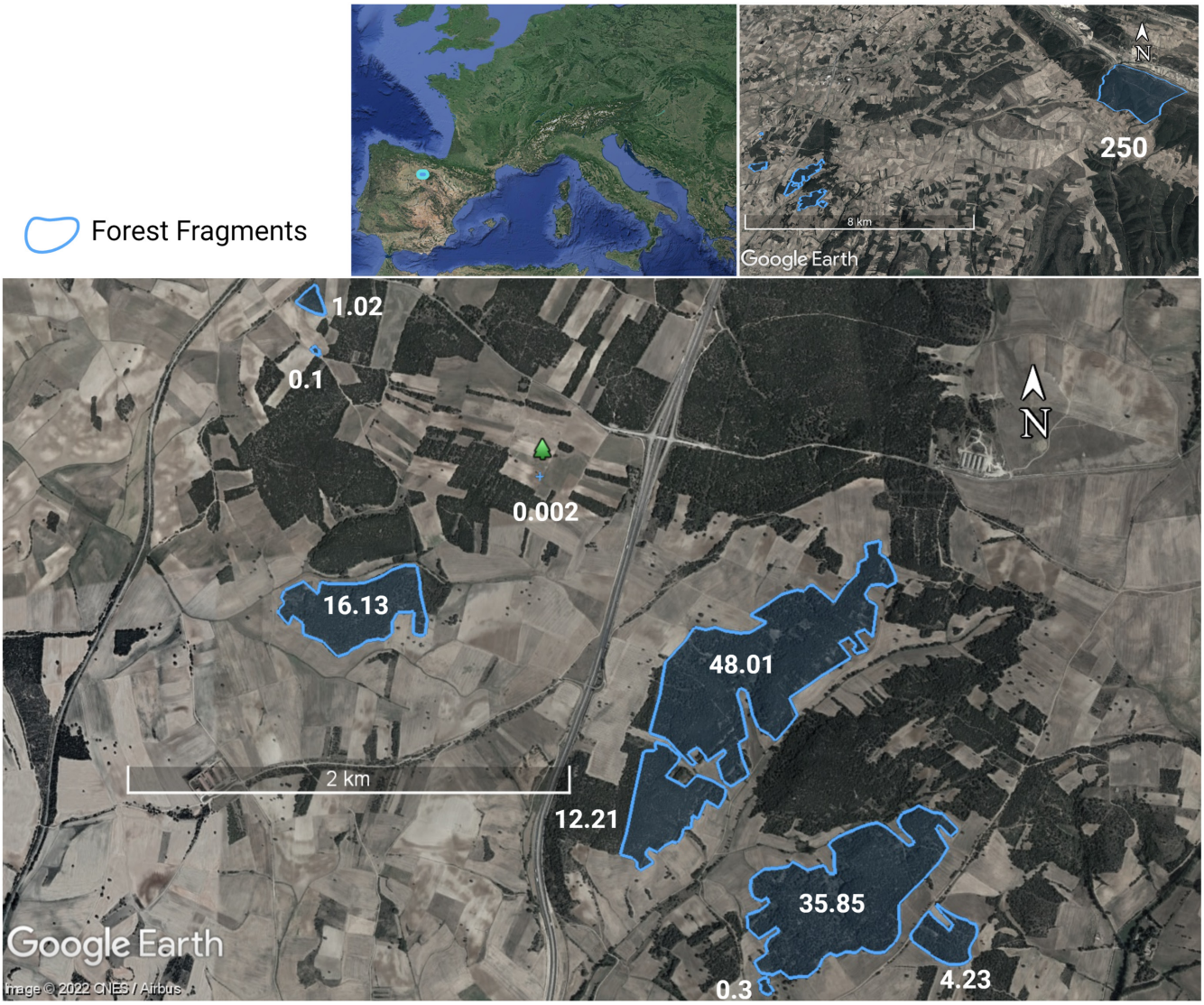

FIGURE 1 Map detailing the area where the study was carried out. Images obtained from Google Earth (14/12/2015) Europe (Landsat/Copernicus Data SIO, NOAA, U.S. Navy, NIGA, GEBCO, GeoBasis-DE/BKG (©2009) Map GISrael Instituto Geográfico Nacional; 45°09′57″ N 11°06′33″ E; https://earth.google.com/web/@45.16589093,11.10934345,1020.81870028a,3304630.75835109d,35y,0h,0t,0r). The second map (top right) is also obtained from Google Earth (20/08/2018; Madrigal del Monte. Instituto Geográfico Nacional. 42°07′17″ N 3°38′41″ W. https://earth.google.com/web/search/Madrigal+del+Monte/@42.1099131,-3.64460219,982.59816573a,17350.74198053d,35y,9.39648819h,0t,0r/data=CigiJgokCd4U-oSjR0ZAESMzrLf-pEJAGbzIL2UKER5AIaiywk9ffynA). The numbers inside or aside the fragments represent the hectares (ha) of each one.

species occur. Species accumulation curves reached a plateau in all fragments indicating that sampling 10 trees was sufficient to estimate species richness (Figure S1). Once the inventory was complete, we attempted to collect a total of 10 individuals of all species identified in each fragment to examine the photobiont diversity associated with each mycobiont species. This search, which lasted up to 4 h per plot (two people), started in the central part of each fragment, walking randomly through the forest fragment. In the smaller fragments, the search extended practically throughout the entire fragment, but trees at the edge of the fragment were avoided in all fragments. In this way, the sampling prevented the collection of closely located individuals, thereby reducing spatial correlation. Samples were dried and stored at room temperature until further processing.

2.3 | Environmental predictors

We used fragment size (ha), which was log-transformed—log(area + 1)—to avoid extremely skewed data and negative logs, as well as the distance to the nearest fragment as measures of habitat availability. We also used the perimeter (m), altitude (m a.s.l.), and coordinates (longitude, latitude). In addition, we calculated the shape of every fragment using the shape index (SI) (Laurance & Yensen, 1991), which represents how each fragment's shape differs from being perfectly circular. SI is calculated as $P/200[(\pi TA)^{0.5}]$, where P is the perimeter length in meters, π is the mathematical constant that approximately equals 3.14159, and TA is the total size of the fragment in hectares. Lower values of SI reflect more regular

fragments and vice versa. Two 10 × 10 m plots were established near the geographic center of each fragment to estimate forest structure variables: tree density per m^2, mean diameter at breast height (DBH) of all trees (cm), and canopy openness. The latter was inferred using three pictures taken in each plot using a Canon 70D camera with a Samyang 8 mm fish eye lens placed at ground level at the centre and two corners of each plot. Pictures were converted into binary images and the percentage of pixels not occupied by vegetation was calculated in the Fiji distribution of ImageJ (https://imagej.net/software/fiji/downloads). Average measures of the two plots in each forest fragment were used in subsequent analyses. All information about environmental variables for each forest fragment is available at Table S1.

2.4 | Mycobiont barcoding

Field identifications of mycobionts were subsequently checked using currently used methods in lichen taxonomy and available literature. Further, we sequenced the nrITS region, the universal fungal barcode for fungi (Schoch et al., 2012) to corroborate identifications in each of the identified morphospecies. Briefly, DNA extractions were performed using cationic exchange resin Chelex 100 (BioRad, Madrid) following Ferencova et al. (2017). PCRs were performed using the primers ITS1F and ITS4-Kyo2 (Gardes & Bruns, 1993; Toju et al., 2012). Reactions included 6.5 μL of MyTaq Red Mix (BioLine, UK), 2 μL of diluted (1:10) DNA template, 0.5 μL (10uM) of each primer, and 5.5 μL of sterile distilled H_2O. PCR conditions were set as follows: a denaturation step of 94°C for 5 min followed by 35 cycles of 95°C for 30 s, 57°C for 30 s and 72°C for 1 min ending with an elongation of 72°C for 7 min. Amplicons were sequenced in Macrogen (Madrid, Spain) using the same primers as in the amplification reaction.

2.5 | Mycobiont phylogeny

We constructed the phylogenetic tree depicting the evolutionary relationships of mycobionts to account for statistical non-independence derived from common ancestry in the models. To determine phylogenetic affinities, we used DNA of three nuclear genomic regions, i.e. the ribosomal internal transcribed spacer (nrITS) obtained in the previous section, the nuclear large subunit region (nrLSU), and the largest subunit of RNA polymerase II (RPB1), as well as the mitochondrial small subunit locus (mtSSU). When available, sequences were retrieved from GenBank. For those species lacking information for some of the regions, we obtained sequences from our own specimens either using extractions obtained in the previous section or extracting DNA using E.Z.N.A.® Forensic Kit (Omega Bio-Tek, Norcross, Georgia, USA) according to the protocol defined by the company. The primers used for the amplification of these regions are provided in Table S2. PCR reactions were performed in a total volume of 15 μL, containing 2 μL of template DNA for amplification of the nuLSU, mtSSU (or 4 μL for the amplification of the RPB1 region), 0.5 μL of each primer (10 mM), and 6.5 μL of MyTaq Mix which contains MyTaq DNA Polymerase (Bioline, UK) and dNTPs; distilled water was added to reach the final volume. The PCR conditions used for amplification for RPB1, nuLSU, and mtSSU were the same as described in Pérez-Ortega et al. (2016). PCR products were sequenced by Macrogen Inc. (Madrid, Spain) using the same primers as in the amplification reaction.

The four regions were aligned through the MAFT tool v 1.4.0 (Katoh & Standley, 2013) using the algorithm 'auto' implemented in Geneious Prime v 2019.0.3 (https://www.geneious.com/prime/). To eliminate the ambiguously aligned regions of those alignments, Gblocks v 0.91b (http://molevol.cmima.csic.es/castresana/Gblocks_server.html) has been used (Castresana, 2000) allowing the least stringent parameters smaller final blocks, gap position within the blocks and less strict flanking positions. In order to select the best-fit partitioning schemes and nucleotide evolution models, we used PartitionFinder v 2.1.1 (Lanfear et al., 2017). Phylogenetic relationships among taxa were calculated using Bayesian inference in BEAST2 v 2.6 (Bouckaert et al., 2014) as implemented in CIPRES Science Gateway v 3 (https://www.phylo.org/) (Miller et al., 2010). We used an uncorrelated relaxed model with a log-normal prior for modelling clock and a Yule process to model tree prior (Drummond et al., 2006). The MCMC chain length was run during 5×10^9 generations and results were logged every 5000 generations. Trace plots and effective sample sizes (ESS) were examined by TRACER v 1.7 (Rambaut et al., 2018). Finally, after discarding the first 25% sampled trees (burning), the results were summarised and annotated in a maximum clade credibility tree (MCC) through TreeAnnotator v 1.8.4 (https://beast.community/treeannotator) (Drummond & Rambaut, 2007). For the visualisation of the resulting consensus tree and comparison of retrieved phylogenetic relationships with previous literature, we used Figtree v 1.4.4 (Rambaut, 2012). Accession numbers for the sequences generated and used during this study are available in Table S3.

2.6 | Photobiont

A small fragment (1–2 mm^2) of the thallus was taken from each specimen. Special care was taken to avoid thallus areas with clear signs of epiphytic fungi or algae as well as necrotic areas. Up to 10 fragments per species in each forest fragment were pooled in a single microcentrifuge tube. DNA extractions were performed using E.Z.N.A. Forensic DNA Kit (Omega Bio-Tek, Norcross, Georgia, USA) following the manufacturer's instructions. The second part of the internal transcribed spacers (ITS2) of the photobiont was amplified using the primers FDGITS2-f y FDGITS2-r (Dal Grande et al., 2018) to which the Illumina adapters CS1 and CS2 respectively had been attached (available in: https://rtsf.natsci.msu.edu/-genomics/sequencing-services/sample-requirements-for-illumina-sequencing/). PCR reactions were performed using

a mix of 25 μL which contained 0.625 U PrimerSTAR GXL DNA Polymerase (Takara BioInc, Japan), 5 μL of Buffer, 2 μL of dNTP Mixture (2.5 mM), 3 μL of DNA template, 0.5 μL of each primer at 10 mM and 13.5 μL of sterile distilled H_2O. PCR conditions were set as follows: a 94°C denaturation step for 1 min followed by 30 cycles of 95°C for 15 s, 52°C for 15 s, and 72°C for 30 s ending with an elongation of 72°C for 1 min. PCR products were visualised in agarose gels and quantified by using a Qubit® Fluorometer (Life Technologies, Darmstadt, Germany) and normalised. Samples were indexed and pooled in a single Illumina MiSeq run (2 × 250 bp paired-end sequencing, v2 Standard 500 cycle) at the Research Technology Support Facility Genomics Core at Michigan State University (USA).

2.7 | Bioinformatic analysis

A total of 8,478,480 paired-end reads were obtained from the photobiont ITS2 amplicons. MiSeq reads were merged, demultiplexed and filtered using the package DADA2 v 1.8.0 (https://benjjneb.github.io/dada2/index.html) for R studio (v 3.4.3 https://cran.r-project.org/bin/windows/base/old/3.4.3/). The first-left 22 bp corresponding to primer sequences were trimmed using the *filterAndTrim* function. Based on quality plots forward and reverse reads were trimmed to 200 and 170 bp respectively. Sequence filtering parameters were set to maxN = 0, maxEE = c (1,4), trunQ = 2. The remaining parameters were operated as default. After denoising, merging, and chimera removal, we kept a total of 4,956,878 reads, with an average of 19,248 reads per sample. Finally, amplicon sequence variants (ASVs) were generated and a data matrix was built in which the rows represented mycobiont samples analysed, columns the ASVs of the photobiont and each cell contained the number of reads of each ASV inside the lichen, being the last a proxy for the interaction strength. The table containing the data was rarefied using the function *rrarefy* from the package R package *vegan*.

We could not rule out the presence of contaminations despite the special care taken during sample tissue collection, as the thallus surface may dwell single algae cells not visible under the stereoscope. Further, some algal species may occur inside the thallus but not take part in the symbiosis (Moya et al., 2017). In order to overcome this problem, we performed a double filtering of the obtained results. First of all, we collected information available in the literature about the photobionts associated with the species and/or genera found in our study (Blaha et al., 2006; Muggia et al., 2014; Sanders & Masumoto, 2021; Tschermak-Woess, 1988; Wornik & Grube, 2010). Secondly, we established a limit of reads (100) below which we considered that the signal corresponded to contaminations of transients or algae not primarily associated with the mycobiont. Thus, sequences of taxa that based on the available literature do not represent the actual photobiont of the species, and/or those with less than 100 reads were eliminated from subsequent analyses. All sequences obtained in this study are available in the SRA (NCBI) under BioProject PRJNA939089 (https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA939089).

2.8 | Species specialisation

An interaction matrix between mycobionts and photobionts was built to account for interactions in each forest patch. Mycobiont specialisation towards their photobionts was explored using two metrics, normalised degree and the parameter *d'*. Normalised degree (Nd) is the number of different photobiont ASVs with which a mycobiont species interacts (degree, d) at the forest fragment level divided by the total number of total ASVs present in that forest patch. The lower the value of ND the higher the degree of specialisation of the species. The parameter *d'* measures the specialisation of each mycobiont species based on its discrimination from a random selection of partners. The index *d'* is based on the Kulback-Leibler distance and reflects how a species deviates from a random sampling of the available partners (Blüthgen et al., 2006). It ranges from 0, no specialisation, to 1, a perfect specialist, a species that interacts only with another partner, which interacts only with that same species. Thus, if one species interacts with only one ASV but that ASV is widespread as a partner of other mycobiont species in the same forest fragment, it is considered that there is limited evidence of specialisation for the former species. (Blüthgen et al., 2006). Both metrics were calculated for every species at all studied forest fragments using the function *specieslevel* from the *bipartite* v. 2.16 R package (Dormann et al., 2008).

2.9 | Life-history traits

All species recorded during the inventories fitted well within the main three growth forms recognised in lichens (Nash, 1996): crustose, whose thalli are strongly attached to the substrate and cannot be separated from it; foliose, whose thalli have a more or less flat appearance and are attached to the substratum by specialised structures in the lower cortex; and fruticose, whose thalli have a three-dimensional architecture, similar to tiny bushes and are attached to the substratum from a single point. Secondly, we categorised all species based on their reproduction modes. Although several reproductive strategies may be found in lichens (Tripp & Lendemer, 2018), species may be easily divided between those producing meiotic propagules (ascospores) which once settled in a new substrate must find a compatible photobiont to re-establish the symbiosis, and those producing asexual propagules in which both partners, fungus and alga, are dispersed together, and the new thalli correspond to clones of the source thallus. It should be noted that we have applied rather broad categories for both growth forms and reproductive modes. In recent years, ecological studies tend to apply more detailed categories (e.g. Giordani et al., 2012; Trobajo et al., 2022). However, in our study, considering the low

number of fragments analysed, the use of narrower categories meant a highly uneven comparison between groups. The use of broader categories, still biologically meaningful, made the analysis more balanced. To test for significant differences in specialisation (ND and *d'*) among life-history traits (reproduction type and growth form) a post hoc Tukey test was performed.

2.10 | Statistical analyses

We first explored the changes in mycobiont and photobiont richness along the fragment size gradient. To reduce the number of environmental variables and collinearity we carried out a Spearman correlation test, discarding variables for subsequent analyses with $\rho > 0.7$ and $p < 0.05$. Shape Index was highly correlated with fragment size ($\rho = 0.917$, Table S4) so it was excluded from the analyses. Also, tree density and DBH showed a significant inverse correlation ($\rho = -0.84$) in which log density is lower the larger the diameter of the trunks, so only DBH was considered. Latitude and longitude were also discarded since the study area is small and no differences are expected due to geographic position. The smallest fragment of the gradient, which included a single tree, was removed from all analyses using stand structure variables. Thus, altitude, distance to the closest fragment, DBH, canopy openness and fragment size (both log and quadratic transformed) were used for fitting generalised linear models (GLMs) (McCullagh & Nelder, 2019) to predict biont richness using a Poisson distribution for errors with a 'log' link function. All GLM analyses were carried out using the function *glm* of the *stats* v. 3.6.2 R package.

Second, we explored changes in ND and *d'* along the fragment size gradient at the mycobiont species level. To do so, we selected those species present in at least five fragments and used Pearson's correlation tests to see if there was any trend between specialisation levels and fragment size in each of the selected species. Then, we explored the effect of the two life-history traits, growth form and reproduction mode, in the mycobiont specialisation towards their photosynthetic partners independently and in the context of a gradient of habitat availability. We used generalised linear mixed models in a Bayesian framework (Hadfield & Nakagawa, 2010) implemented in the package MCMCglmm v. 2.29 (Hadfield, 2010) in order to control the phylogenetic effects on these relationships. Life history traits and fragment size (log(area + 1)) were used as predictors and species identity and phylogenetic relationships were considered as random effects. The uncertainty in the phylogenetic reconstructions was accounted for by running three MCMCglmm's, each one using a phylogenetic tree randomly chosen from the distribution of tree topologies obtained in BEAST after discarding 25% of samples as burnin, and integrated over the posterior samples by drawing 1000 random samples across models and using *HPDinterval* function from the *coda* R package (Plummer et al., 2006). Models priors follow de Villemereuil and Nakagawa (2014) using an inverse-Gamma distribution with shape and scale parameters equal to 0.01 as priors for the random effects and the residual variance.

All graphs for the analyses performed were created with the R packages *ggplot2* (Wickham, 2016), *gridExtra* (https://CRAN.R-project.org/package=gridExtra) and *wesanderson* (https://CRAN.R-project.org/package=wesanderson).

3 | RESULTS

3.1 | Characterisation of the forest fragments

Table S1 provides all information on the characterisation of the studied forest fragments. Fragments ranged from 0.002 ha (single tree) to 250 ha. The altitude ranged between 923 and 1093 m a.s.l. Distance to the closest fragment was between 9.09 and 28 m. Tree density was between 1.05 and 2.85 trees per m^2, DBH between 10.1 and 18.98 cm, and canopy openness between 19.04% and 50.38%. None of the forest structure variables were correlated with fragment size (Table S4). Only the shape index was correlated with fragment size, due to the regular shape of the chosen fragments. Tree density and mean trunk diameter were negatively correlated. We also observed that, in general, fragment size increased towards the east, with a slight increase in altitude along this gradient (Table S4; Figure 1).

3.2 | Changes in mycobiont and photobiont diversity

We detected a total of 44 species of lichen-forming fungi in the study area (Table S3). The number of mycobiont species per forest fragment ranged from 9 in the 0.002 ha fragment to 28 in the 12.21 ha fragment, with an average of 22 species per fragment (Table 1). The number of exclusive taxa was low in all fragments, ranging from 0 in the 16.13 ha

TABLE 1 Mycobiont and photobiont richness and mean mycobiont specialisation in each studied forest fragment.

A	M_d	M_e	P_d	P_e	d	ND	*d'*
0.002	9	1	25	3	9	0.33	0.50
0.1	17	1	34	8	7.64	0.22	0.55
0.3	22	1	42	6	9.13	0.217	0.52
1.02	23	1	63	12	10.83	0.17	0.57
4.23	27	1	51	13	8.37	0.17	0.49
12.21	28	1	75	20	9.54	0.13	0.59
16.13	23	0	57	8	11.87	0.21	0.59
35.85	23	1	52	9	8.39	0.16	0.56
48.01	27	0	61	12	8.89	0.15	0.57
250	20	2	46	7	2.9	0.06	0.66

Abbreviations: A, size of each forest fragment, in hectares; d, mean degree; *d'*, mean specialisation parameter *d'* of the mycobiont species in the forest fragment; M_d, number of mycobiont species; M_e, number of exclusive species of mycobionts in a given forest fragment; ND, mean normalised degree of the mycobiont species in the forest fragment; P_d, number of photobiont ASVs; P_e, number of exclusive ASV species in a given forest fragment.

and 48.01 ha fragments to 2 in the 250 ha fragment (mean = 0.9) (Table 1). Fragment size was the only variable significantly explaining mycobiont richness along the size gradient, showing a negative quadratic relationship with a higher number of species in intermediate fragment sizes (Figure 2; Table S5).

A total of 2272 thalli were collected, corresponding to 229 unique pools sequenced in Illumina MiSeq. The average number of thalli per species and fragment was 9.92. A total of 985 amplicon sequence variants (ASVs) were recovered in DADA2. Afterward, we applied a manual filter removing those ASVs with less than 100 reads, obtaining a final number of 178 ASVs. Furthermore, the total number of reads remaining after this manual filter was 4,903,976 with an average of 21,415 reads per sample. The number of reads for each ASV-species-fragment combination is provided in Table S6. BLAST searches of those remaining ASVs resulted in 158 being in the genus *Trebouxia*, 13 to *Dictyochloropsis*, and 4 to *Asterochloris*. *Asterochloris* ASVs were restricted to *Cladonia*, while the genus *Dictyochloropsis* was mostly found in the species *Phlyctis argena*.

The number of ASVs per forest fragment ranged from 25 at 0.002 ha to 75 at 12.21 ha (Table 1). GLM analyses showed that fragment size and canopy openness were the only significant variables explaining photobiont richness along the size gradient, showing a negative quadratic relationship regarding fragment size (Figure 2; Table S5). The number of exclusive ASVs was relatively high, ranging from 3 in the fragment consisting of a single tree to 20 in the 12.21 ha fragment (mean = 9.8) (Table 1). Fragment size was the only significant variable explaining the number of exclusive ASVs showing a negative quadratic relationship.

3.3 | Effect of fragmentation on life history traits

Of the total number of species found in the study, 17 species formed crustose thalli, 19 foliose, and 8 fruticose (Table S7). Twenty species reproduced mainly sexually, while 24 showed asexual reproduction (Table S7). GLM analyses showed that fragment size was the only significant predictor of crustose species richness, increasing in number as fragment size increased (Table S5; Figure S2A). Although the number of foliose and fruticose species also appeared to increase along the fragment size gradient, no significant predictors related to fragmentation or forest structure were found to explain their richness. Regarding reproduction, while no significant predictors were found to explain the richness of asexually reproducing species, fragment size significantly predicted the richness of sexual species, which decreased as fragment size increased (Table S5; Figure S2B).

3.4 | Effect of fragmentation on specialisation

The mean number of ASVs per mycobiont species ranged from 2.9 at the 250 ha fragment to 11.87 at the 16.13 ha fragment (Table 1). The normalised degree, which accounts for the range of partners, was the lowest (0.01) in *Evernia prunastri* at the 12.21 ha forest fragment and the highest (0.54) in *Blastenia xerothermica* at the 0.3 ha forest fragment (Table S7). The specialisation parameter *d'*, varied across different lichen species and forest fragment sizes. For instance, *Cladonia fimbriata* in the 563.9 ha forest fragment exhibited a *d'* value of 1, indicating that it exclusively interacted with an ASV unique to that species. On the other hand, *Lecanora subcarpinea* in the 1.02 ha forest fragment had a lower *d'* value of 0.16, signifying that it interacted with a large number of ASVs present in that specific fragment (Table S7).

Nineteen species occurred in at least five forest fragments (Table S8). The correlation analyses between normalized number of partners (ND) and fragment size in these species showed negative correlations for all species, showing a decrease in the number of

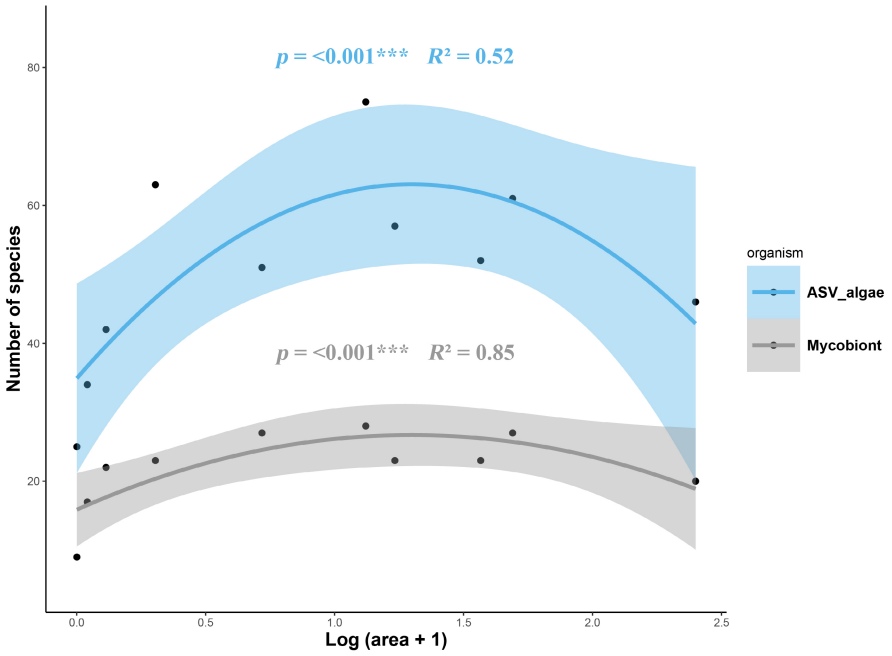

FIGURE 2 Distribution of the number (N) of mycobiont species and ASVs along the patch size gradient (log(area + 1), in which area is given in hectares). Quadratic adjusted models (bold line) and confidence intervals (paler coloured area) are represented for the number of mycobiont species in red and green for ASVs.

partners as fragment size increased (Table S8). This correlation was only statistically significant in five of them, i.e. *Candelariella xanthostigma*, *Melanelixia subaurifera*, *Parmelina tiliacea*, *Physconia perisidiosa* and *Ramalina fastigiata*, all but the last of which are asexually reproducing species. Regarding correlations between *d'* and fragment size, we observed both positive and negative correlations although they were only significant in *Physconia enteroxantha* and *Physconia perisidiosa*, for which *d'* increased along the forest fragment size gradient (Table S8). *Buellia griseovirens* showed a quadratic relationship with fragment size in terms of ND, and similarly, *Candelariella xanthostigma*, *Parmelia sulcata* and *Physconia enteroxantha* exhibited a quadratic relationship with fragment size for *d'* (Table S8).

Phylogenetic generalised linear models showed significant differences in the normalised number of partners (ND) between crustose and fruticose species, with the latter having a significantly lower number of partners (Figure 3a; Table S9). On the contrary, no significant differences in ND were found between reproduction modes (Figure 3b; Table S9). Regarding the specialisation parameter *d'* we found significant differences between crustose species and foliose and fruticose species, with the former being significantly less specialised in terms of the number and exclusivity of their partners (Figure 3c; Table S9). Likewise, we found differences between reproduction modes, with asexually reproducing species showing higher average *d'* (Figure 3d; Table S9).

Phylogenetic generalised linear models also showed that fragment size had a significant and negative effect on the normalised degree (Figure 4a,b; Table S10). There were significant differences between crustose taxa and foliose and fruticose species and these differences were independent of the fragment size (Table S10). No significant differences were found between reproduction modes regarding normalised degree along the fragment size gradient (Table S10). Concerning the specialisation parameter *d'*, the fragment size had a significant effect, with the larger fragment patch showing large *d'* when considering species biotypes (Figure 4c,d; Table S10). In addition, fruticose species had larger *d'* than crustose species. There was a significant interaction effect between fragment size and fruticose growth mode, in which the increase of the parameter *d'* along the fragment size gradient was lower than in crustose species (Figure 4c; Table S10). When considering the combined effects of fragment size and reproductive modes on *d'* we found differences between sexual and asexual species, with significant effects of interactions (Figure 4d; Table S10). Asexually reproduced species

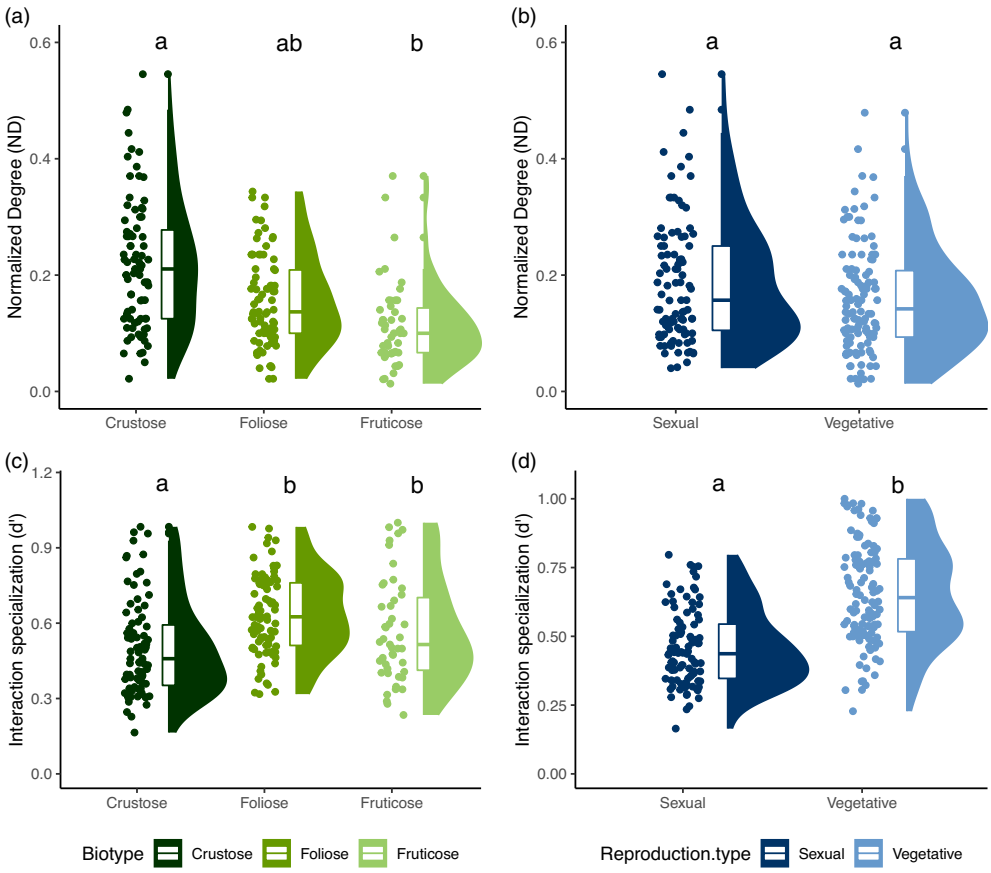

FIGURE 3 Violin plots (representing the distribution of points) show differences in specialisation among species with different life-history traits. (a) Differences among biotypes in the normalised degree (ND); (b) differences between reproduction modes in the normalised degree (ND)'; (c) differences among growth modes in specialisation parameter *d'*; (d) differences between reproduction modes in specialisation parameter *d'*. The letters above the boxplots depict significant differences (Tukey test) among life-history traits. Significance is based on phylogenetic-controlled MCMCglmm models (see Table S8).

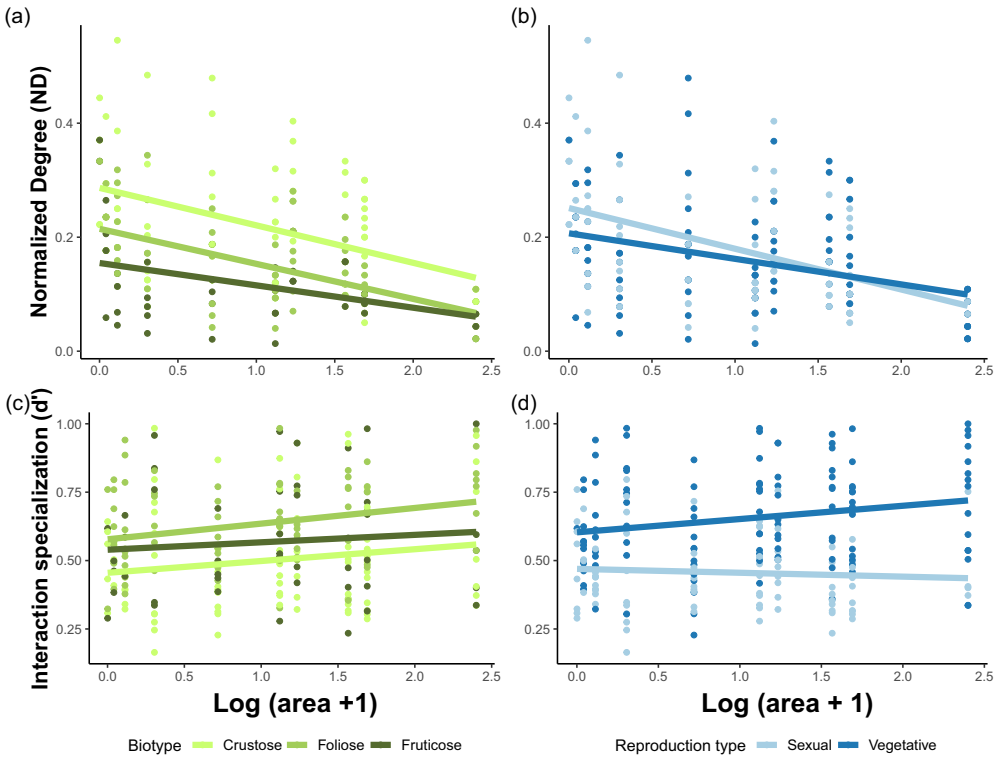

FIGURE 4 Representation of the interaction effect between fragment size and reproduction type on ND (a) and *d'* (b), as well as the interaction effect between fragment size and biotype on ND (c) and *d'* (d). Area is represented in hectares.

increased their *d'* along the fragment size gradient, but the pattern was the contrary in sexual species.

4 | DISCUSSION

Our study represents the first attempt to investigate the effect of fragment size on interaction specialisation in symbiotic organisms. The obtained results shed light on how fragmentation interplays with life history traits to affect fungal-algal interactions in epiphytic lichen symbioses.

4.1 | Biont diversity along the fragment size gradient

The size of the forest fragment, both in its linear and quadratic forms, emerged as the sole significant predictor for the richness of mycobiont species, exclusive photobiont ASVs, crustose taxa, and sexually reproducing species. Forest structure attributes did not prove to be statistically significant predictors for any variable, except for canopy openness, which was associated with photobiont richness. Biont richness showed a humped-shaped distribution along the patch size gradient, being this pattern more pronounced for algal ASVs. Previous studies dealing with lichen diversity along gradients of habitat loss in different forest ecosystems have reported a gradual loss of diversity as the available habitat diminished (Svoboda et al., 2010), with changes in abiotic conditions associated with an increasing fragment edge effect proposed as the main reason for such reduction (Asplund et al., 2014; Belinchón et al., 2007; Boudreault et al., 2008; Esseen & Renhorn, 1998). Hump-shaped diversity patterns have been reported for lichen-forming fungi along stand age (Asplund et al., 2014; Miller et al., 2020) and elevational gradients (Nanda et al., 2021). Brunialti et al. (2013) found a hump-shaped pattern in the diversity of lichen-forming fungi in a gradient from the forest edge to the interior, which they explained by the encounter of two distinct lichen communities, a more xerophilic one typical of the edge of the fragments and a more hydrophilic one from the interior of the fragments. In our study, intermediate-sized forest fragments likely act as a transitional zone, an ecotone, between two contrasting environmental conditions. On the one hand, the natural conditions found in larger fragments, characterised by minimal influence from the agricultural matrix and the absence of edge effects in the centre of the fragment, along with more stable microclimates (Chen et al., 1993), tend to diminish as fragment size decreases. On the other hand, elevated levels of eutrophication resulting from the surrounding agricultural land (Ortuzar-Iragorri et al., 2018) and the effects of forest edge are also expected to decrease as forest fragment size increases (Forman & Godron, 1986; Meeussen et al., 2021). It is widely recognised that ecotones typically exhibit higher levels of biodiversity due to the coexistence of species from adjacent regions or environmental conditions (Shmida & Wilson, 1985). Ecotones can also harbour a significant number of species unique to those transitional zones (Kark et al., 2007). In our study, in addition to the highest

diversity of mycobionts and photobionts, we observed the greatest number of exclusive photobionts in intermediate-sized forest fragments. Future studies should take into consideration the influence of abiotic factors like microclimatic conditions, as well as nitrogen and phosphorous deposition. This comprehensive approach will provide a clearer understanding of the hump-shaped patterns observed in both the total and exclusive diversity of mycobiont species and photobiont ASVs across the fragment size gradient. Such insights will contribute to a better understanding of how habitat fragmentation impacts the diversity of bionts within epiphytic lichen communities.

4.2 | Life history traits and fragment size gradient

Our results showed that fragment size influences the mean richness of some life history traits in the studied lichen communities. While the richness of foliose and fruticose remained unaltered, crustose richness mirrored the pattern of total mycobiont richness by exhibiting a quadratic correlation with fragment size. Changes in the distribution of life history traits in epiphytic lichen communities are directly related to abiotic conditions such as solar radiation, water availability, and pollution (Giordani et al., 2014; Koch et al., 2019; Matos et al., 2015; Paoli et al., 2017). Previous studies in fragmented landscapes showed that the richness of foliose and fruticose species, but not crustose species were affected by fragment size in addition to other environmental variables such as fragment slope, mean DBH and precipitation (Trobajo et al., 2022). The reason behind this difference between growth forms is not clear since no correlation was found with forest structure variables. Regarding reproduction modes, we did not find any significant correlation between the richness of asexually reproduced species and the measured environmental variables. In contrast, a correlation was found between the abundance of sexual species, which reproduce through ascospores, and both the size of the fragments and the openness of the canopy. Stofer et al. (2006) similarly noted that sexually reproducing species tend to be more abundant in open landscapes compared to denser forests. Consequently, it is plausible that the beneficial impact of canopy openness in our study area is linked to enhanced opportunities for the dispersal and colonisation of ascospores, facilitated by a more open forest fragment canopy that allows the ingress of new propagules.

4.3 | Life history traits and specialisation

Specialisation is affected by life-history traits in all known organisms (e.g. Armbruster, 2017; Dehling et al., 2016). Our results showed differences in specialisation among growth forms and between reproductive modes, both in terms of the normalised degree (ND) and in the specialisation parameter d'. Crustose species interacted on average with a higher number of ASVs than the other growth forms (higher ND) and, in addition, used photobionts tend to be more widely shared by other mycobiont species (lower d'). Until now, there has not been any research that has directly compared variations in specialisation among different biotypes. However, most studies investigating photobionts associated with crustose species have consistently revealed their interactions with a broad range of species spanning a wide phylogenetic spectrum (Blaha et al., 2006; Guzow-Krzeminska, 2006; Muggia et al., 2014), although instances of high specialisation have also been documented (Hauck et al., 2007; Pérez-Ortega et al., 2012; Singh et al., 2015). However, the reason why most of the crustose lichens studied so far show a higher number of associated photobionts remains unclear. A possible factor to consider is the continuous exposure to potentially suitable, free-living photobionts during lichen growth via the prothallus (Asmtrong & Bradwell, 2010; Sanders, 2005). This process could lead to the assimilation of new photobionts into the lichen thallus over the course of its development (Sanders & Lücking, 2002). On the contrary, chances to incorporate new algal strains in foliose and fruticose species are much more reduced due to the scarce contact (single fixation points in fruticose species, rhizines in foliose not adnate species) with substrate and global isolation due to cortical tissues. As we analysed pools of individuals at each site, it is not possible to determine whether the higher number of photobiont partners found on average in crustose species is due to the presence of multiple photobiont ASVs on each individual thallus, or whether this pattern originates at the population level. In terms of reproduction, sexually and asexually reproducing species had on average similar numbers of partners, but the latter showed considerably higher values of d'. This difference in d' indicates that asexual species interact with a set of photobionts, which are more restricted in terms of their interactions in the context of the community than the photobionts with which sexually reproducing species associate. Contrary to our findings, previous studies comparing closely related species pairs had shown that species of lichen-forming fungi with clonal reproduction via joint dispersal propagules tend to have lower associated algal diversity and are more selective than sexual species (Cao et al., 2015; Otálora et al., 2013). While so far, no study had analysed a high number of species to have a broad overview, Wornik and Grube (2010) showed that the ease to switch photobiont in the mostly asexually reproduced *Physconia grisea*, a Mediterranean species dispersed via soredia and also found in our study area, was the only suitable explanation for comparable photobiont diversity between a sexual and an asexual species. However, asexual species showed higher specialisation when compared to sexual reproductive species. Steinová et al. (2019) studied photobiont selectivity in several species of *Cladonia* in Europe and showed that asexual species were more selective towards their photobionts than sexually reproducing species, which behaved as generalists. Cao et al. (2015) suggested that the decrease of selectivity in sexual species may be due to the need to find a new photobiont before the ascospores perish. However, in this context, the similarity in partner numbers between both groups does not align with that hypothesis. Rather, it appears that the pattern arises from a mutual specialization of the bionts, possibly influenced by the clonal reproduction of both partners. The greater specialization found in asexually dispersing species may then reflect

the higher fitness of a particular myco-photobiont combination at the local scale which is favoured by clonal reproduction (Buschbom & Mueller, 2005).

4.4 | Specialisation along the gradient of fragment size

The variation of species specialisation in fragment size gradients has only been scarcely studied (Aizen et al., 2012). Our results showed that the average specialisation at the species level decreased with fragment size, as had been described for pollinator-plant interactions (Aizen et al., 2012). In addition, we found significant evidence for changes in specialisation along the fragment size gradient in certain species. Six mycobiont species showed significant changes in the number of partners along the gradient, reducing the number of ASVs with which they interact. Five out of six species were asexually reproducing species which could indicate, as stated above, a better performance of certain combinations as the habitat quality increases (Buschbom & Mueller, 2005). Although specialisation is often considered a species-level trait conserved across the phylogeny (Gómez et al., 2010) and with relatively little variation, it is known to vary at geographical and phylogenetic scales (Poisot et al., 2015; Trøjelsgaard et al., 2015). We observed a strong decrease in the average number of photobionts (ND) associated with each fungal species as the fragment size increased together with an overall higher specialisation (*d'*). The higher values of the normalised degree found in smaller patch sizes regarding larger ones could be an artefact derived from the lower number of photobionts available in small fragments (Figure 2), although we found a similar number of photobiont ASVs in the 1.02 and 250 ha forest fragments. The specialisation parameter *d'* did point towards higher mycobiont specialisation in larger fragments. Singh et al. (2019) analysed myco-photobiont interactions in three areas with different management regimes finding a large number of unique interactions in the managed and preserved areas which were absent from the more disturbed habitat. The authors also reported changes in the interactions in several mycobiont species which changed their photobionts regarding the management regime. Thus, in our study, although the number of available partners increased along most of the fragment size gradient, fungal species became more selective. It is likely that smaller fragments have greater homogeneity of abiotic conditions across the area (higher solar radiation, desiccating winds, etc.) and are also subject to seasonal nutrient pulses (nitrogen and phosphorus inputs from the agricultural matrix) due to the higher edge effect. Assuming local adaptation of the photobiont (Yahr et al., 2006), these smaller fragments would harbour only photobiont species that could be called 'weeds', adapted to the higher intensity of disturbance. New fungal propagules arriving in the fragments may only have a pool of similarly adapted photobionts, perhaps interchangeable in terms of holobiont fitness, so the degree of selectivity would be low. In contrast, within larger fragments, although abiotic conditions may show more localised spatial heterogeneity, they tend to remain stable over an extended period. Consequently, specialised interactions that contribute to increased holobiont fitness can persist for longer periods.

4.5 | The interplay between specialisation, traits and fragmentation

Variations in specialisation across the fragment size gradient were not consistent among different morphological groups and between the reproduction modes. This indicates that changes in specialisation along the gradient are additionally influenced by the life history traits of these species. For example, although the number of interacting photobionts decreased along the fragment size gradient in all biotypes, there was a significant difference between how this number decreased in foliose and fruticose species and how it did in crustose species. In addition, we found a significant interplay between the fruticose biotype and fragment size regarding the specialisation parameter *d'*, showing that changes in fragment size do not affect similarly all biotypes in terms of discriminating among their photosynthetic partners. It is recognised that different growth forms represent distinct primary strategies for utilizing water sources in lichens (Gauslaa, 2014), and that fruticose species are more dependent on atmospheric moisture than foliose and crustose species (Kidron & Kronenfeld, 2022; Rundel, 1978). Therefore, it is likely that possible changes in wind speed, humidity and temperatures along the patch size gradient do not similarly affect the interactions formed by species with different growth forms and their photobionts.

Regarding reproductive modes, it is certainly notable that although the number of associated partners (ND) of both functional groups decreased similarly along the fragment size gradient, they showed differences in the specialisation parameter *d'*, with sexual species becoming on average less specialised as the fragment size patch area increased and asexually reproducing species becoming more specialised. The mode of reproduction has relevant implications concerning constraints on the dispersal and colonisation of new habitats (Ellis et al., 2021; Walser, 2004). This is because large vegetative propagules can colonise substrates over shorter distances, in contrast to ascospores, which have the capability to colonise substrates over much greater distances (Ronnås et al., 2017). Ascospores, the sexual mycobiont propagules, are constrained by the necessity to locate a compatible photobiont once they have settled, whereas clonal propagules have the flexibility to develop a lichen thallus with the same photobiont or switch to a different one after settlement (Wornik & Grube, 2010). Koch et al. (2019) demonstrated that air pollution and urbanisation had differential effects on lichens that reproduce asexually via isidia and soredia. Regarding sorediate species, which constitute 21 out of 24 asexually reproducing species in our area, Koch et al. (2019) observed that the prevalence of sorediate species increased with urbanisation. This trend was attributed to the fragmented landscape in urban areas and the superior dispersal capabilities of soredia. Individual species models indicated the ability to modulate specialisation along the fragment size gradient, with a significant increase in specialisation (lower ND and higher *d'*)

for several species, most of which reproduce asexually. The explanation for this different trend of specialisation in sexual and asexual species along the fragment size gradient may be related to the more stable and natural environmental conditions of the larger fragments, together with the different modes of photobiont acquisition in species with different reproductive modes. Sexual species have to acquire their photobionts anew each time they disperse, whereas in asexual species the fungus and alga are dispersed together in clonal propagules, which favours the persistence of algae-fungus interactions (Cao et al., 2015; Steinová et al., 2019). Thus, although the average number of photobionts with which species interact decreases with increasing forest fragments for both reproductive modes, the need of ascospores to find a compatible photobiont in sexual species versus the production of clonal propagules of locally adapted myco-photobiont interactions increases the frequency of such interactions in the fragment, creating a pattern of higher specialisation in asexually reproducing species. The modulation of specialisation under contrasting abiotic conditions at the species level is certainly a phenomenon that should be considered in future studies, as it can provide relevant information on the extent of the ecological niche of species.

4.6 | Implications for conservation

Our results have clear implications in conservation biology. Interactions among species are certainly influenced by local abiotic conditions, so a certain biont combination would not have the same fitness in two patches with different sizes. Small changes in forest conditions can have drastic consequences, causing existing biont combinations to lose their ability to persist. In addition, if some mycobiont species can modulate their specialisation towards the photobionts to some extent, then these species would be more resilient to environmental changes such as those derived from habitat fragmentation than other species. Their ability to adapt to changes in local conditions through changes in specialisation towards their photobionts may buffer the effect of more stressful abiotic conditions due to the increase of edge effect in small fragments, at least until phylogenetic constraints prevent interactions in some species. Further studies will show whether the analyses of interaction dynamics (partner fidelity, link conservatism, and rewiring) in myco-photobiont symbioses in the context of interaction bipartite networks may be used as suitable tools to understand and predict long-term changes in epiphyte lichen communities.

AUTHOR CONTRIBUTIONS

Sergio Pérez-Ortega and Alejandro Berlinches de Gea carried out the field sampling. Alejandro Berlinches de Gea and Mar Villar de Pablo collected laboratory data. Miguel Verdú, Sergio Pérez-Ortega, Alejandro Berlinches de Gea and Mar Villar de Pablo analysed the data. Sergio Pérez-Ortega and Alejandro Berlinches de Gea led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/1365-2745.14229.

DATA AVAILABILITY STATEMENT

All NGS sequences obtained in this study are available in the SRA (NCBI) under BioProject PRJNA939089. Sequences obtained through Sanger sequencing are available in Gen Bank with the following accession numbers: OR101790–OR101806 (both included), OR105741–OR105783 (both included), OR12269, OR12270, OR12271, OR122711, OR126897–OR126906 (both included). Accession numbers are also provided in Table S2.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1: Environmental variables describing each forest fragment.

Table S2: Loci used in the phylogenetic reconstruction and the primers used in their amplification.

Table S3: Accession numbers for each species and loci used to infer phylogenetic relationships between species.

Table S4: Correlation matrix between the environmental variables of the studied forest fragments showing Spearman's *rho* in the upper half matrix and *p*-values in the lower half matrix.

Table S5: Summary of the generalized linear models at the fragment level.

Table S6: Readings recovered in the MiSeq Illumina sequencing for each photobiont ASV associated with mycobiont species in each forest fragment.

Table S7: Specialization values (ND and *d'*) for each species in each forest fragment. identified as fragment size (ha).

Table S8: Pearson correlation tests between specialization parameters normalized degree (ND) and *d'* and area (log(area + 1)) for those species occurring in ≥5 forest fragments.

Table S9: Results of Phylogenetic Generalized Linear Models explaining Normalized degree and specialization (*d'*) as a function of biotype and reproductive mode.

Table S10: Results of phylogenetic generalized linear models explaining normalized degree and specialization (*d'*) as a function of biotype, reproductive mode and area.

Figure S1: Species accumulation curves for every fragment, except for the solitary tree.

Figure S2: Distribution of growth forms and reproduction modes along the gradient of fragment sizes.

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