Cover of *Ramalina* species as an indicator of habitat quality in threatened coastal woodlands

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Abstract. Coastal forests in the Mediterranean area are threatened habitats due to intense human influence. In the context of global change, a rapid tool is needed for the evaluation of habitat quality in woodlands by calculating lichen cover on twigs rather than weight biomass. We selected epiphytic *Ramalina* species as indicators of habitat quality and evaluated them in threatened woodlands dominated by *Olea europea* var. *sylvestris* on the island of Menorca, a UNESCO Biosphere Reserve in the Mediterranean area. We measured species richness, percentage cover and dry weight in nine sites grouped into urban, rural and naturalized areas to evaluate the effect of land uses, and regressed dry weight against percentage cover. Percentage cover, dry weight, species richness of *Ramalina* and of its species varied depending on land use, reaching higher rates in naturalized and rural areas rather than in urban areas. In addition, we showed for the first time that field estimates of *Ramalina* cover at the field were related to dry weight by applying a logarithmic transformation to dry weight. Percentage cover of *Ramalina* was a good indicator of land uses and will be a rapid, easy and non-destructive tool to assess the quality of woodland habitats in the coastal zone.

Keywords. Habitat quality, lichen ecology, Mediterranean region, lichen biomass, land use, bioindicator, Menorca.

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The woodlands of the hot lowlands and shores of the Mediterranean region are threatened habitats in Europe due to intense human influence (Janssen et al. 2016). These woodlands are dominated by *Olea europaea* and *Ceratonia siliqua* (Janssen et al. 2016), and their main threat is habitat fragmentation due to the extension of anthropogenic habitat, mainly agricultural and cattle lands, and urbanization (Rey et al. 2009). Despite the threat, these harbor epiphytic lichens tied to humid areas (Incerti & Nimis 2006). Epiphytic lichens are an important component of forest ecosystems (Brunialti et al. 2012), and can reach a high biomass on tree trunks and especially on branches (Arseneau et al. 1998). Some of these epiphytic lichen species growing on twigs of coastal shrubs and trees are considered aerohygrophylous, as for example those belonging to the genus *Ramalina* (Roux 2020), which are considered threatened in Italy (Nascimbene et al. 2013) and France (Roux 2020). However, most studies considered only presence or absence, and few data exist on their cover or biomass. We propose to use these to assess the health of these threatened forest ecosystems.

Quantitative studies of epiphytic lichens provide information on population dynamics (Gauslaa et al. 2005), lichen communities (Phinney et al. 2021), the role of lichens in ecosystem functioning (Miranda-González & McCune 2020), and have been used as

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indicators for sustainable forestry (Boudreault et al. 2015; Longan & Gómez-Bolea 2002), since epiphyte lichens are especially sensitive to forest habitat quality and, consequently, to management (Aragón et al. 2010). These studies have mainly focused on specific species and sometimes to a group of species as indicators of habitat quality and monitoring of forest ecosystems (Martínez et al. 2014; Otálora et al. 2015). Quantitative studies include several approaches to evaluate abundance, such as visual estimation of cover, measurement of maximum length and width of the largest individual of a species, or biomass (McCune 1990). Biomass is the accumulated growth of lichens in a given time, and is the variable that best explains how factors influence lichen growth, and how factors act directly to species distribution or indirectly by altering competitive balance among species (Armstrong 2015). Despite its biological value, measuring biomass is a tedious and can be destructive; however, estimates of lichen cover have been shown to be a good indicators of lichen biomass (Pike 1981). However, the relationship between cover and biomass is often species- or group-specific (McCune 1990), and data are lacking for groups of major ecological and conservation interest, such as the epiphytic fruticose lichens of the Mediterranean region.

Species of *Ramalina* have been used in several fields of research, as evaluation of climatic changes (Bannister et al. 2004), understanding lichen responses to industrial activities (Garty et al. 2002), and biomonitoring the impacts of these human activities to the environment (Garty 2001; Giralt 1996). Species of *Ramalina* can grow on rocks, trunks and branches (Spjut et al. 2020). Species growing on branches of Mediterranean woodlands were considered threatened due to the restriction of their habitat (Nascimbene et al. 2013; Roux 2020). Despite this concern, the island of Menorca, a UNESCO Biosphere Reserve in the Mediterranean region, shows a high richness of *Ramalina* species on trunks and branches (Stolley & Kappen 2002), although we lack high quality data on *Ramalina* populations.

In the Mediterranean Sea, Menorca Island was declared a Biosphere Reserve in 1993 (Lavola 2019). The Menorca Biosphere Reserve has an action plan to achieve sustainable development in a context of global change, which requires indicators to evaluate the health of terrestrial ecosystems (Lavola 2019). In Menorca, various land uses linked to human activity affect habitats of conservation interest (Fernández et al. 2021), such as the thermo-mediterranean coastal woodlands of *Olea europea* and *Ceratonia siliqua* (Janssen et al. 2016). The potential extension of these woodlands have been reduced due to agricultural and grazing practices and urbanization, especially in the Balearic Islands (Rey et al. 2009), including Menorca, when many of them are included in the Natura 2000 Network (IDE Menorca 2022). Lichens are good indicators of atmospheric quality (Garty 2001) and also of habitat quality (Asta et al. 2002; Bergamini et al. 2005). Therefore, the aim of this work was to provide a rapid and easy tool for the evaluation of habitat quality in these threatened woodlands by estimating percentage cover of *Ramalina* species on twigs instead of measuring biomass directly.

Materials and Methods

Study sites and gradients of land uses. This study was conducted in Menorca Island, at the eastern end of the Balearic Islands, located in the north-west of Mediterranean Sea. Menorca has a Mediterranean climate with mean annual temperature of 17.1°C, mean total annual rainfall of 593.6 $mm \cdot yr^{-1}$ and mean annual relative humidity of 72% (OBSAM 2021). The main landscape consists mostly of forests between farmlands and grasslands, which are composed by *Olea europaea* var. *sylvestris* or *Quercus ilex*, or mixed (Fernández et al. 2021).

To address the response of *Ramalina* species to the habitat quality, nine sites were selected with *Olea europaea* var. *sylvestris* as the dominant tree. Study sites were grouped into three types of land uses: sites with woodlands of *Olea europaea* close to an urban area, sites with current agricultural and extensive grazing practices (hereafter, rural), and sites with no agricultural or silvicultural practices for several years (hereafter, naturalized). We checked the categorization of each site by field visits and consulting vegetation cover maps and orthophotos of the last decades (IDE Menorca 2022). Their distribution throughout the island has been also considered (**Fig. 1**, **Supplementary Table S1**), selecting each of the three types of land use in the west, in the center and in the east of the island around the main cities of Menorca (Ciutadella, Es Mercadal, and Maó).

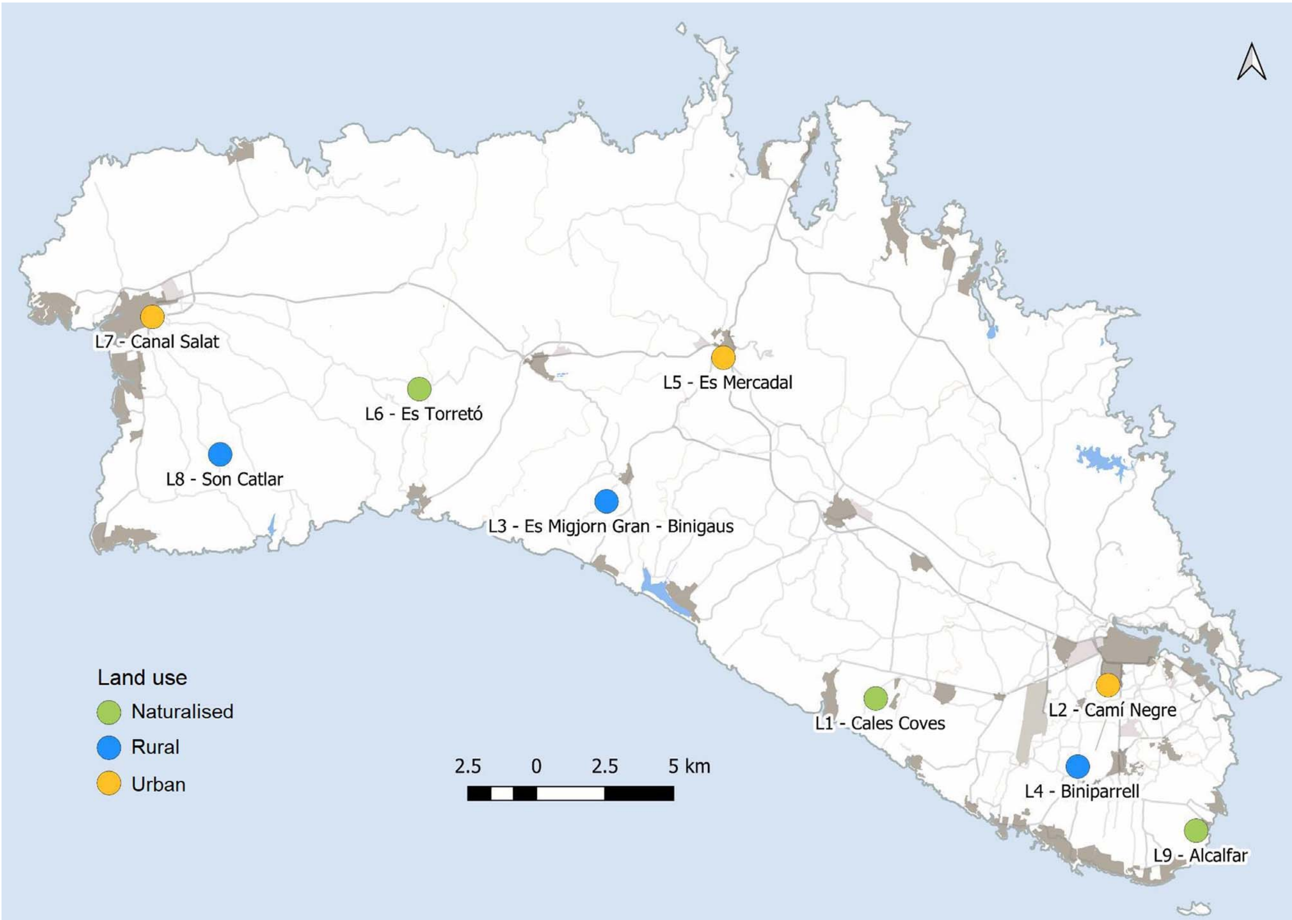

Figure 1. Localities in Menorca colored according to type of area. Green are naturalized areas, blue are rural areas, and orange are urban areas. Online pdf in color.

Lichen surveys–Ramalina identification and lichen cover and biomass. We conducted lichen surveys in 2019. In each site, we selected five *Olea europaea* var. *sylvestris* trees. In each tree, we studied ten fragments of different twigs at the breast height. Each fragment measured, approximately, a length of 10 cm and a diameter of 1 cm, a minimum area between 100–200 cm^2. We selected fragments between the 4^{th} and 6^{th} twig node, starting from the terminal bud. Healthy twigs with their foliage exposed to full sunlight were selected.

Ramalina species were identified at the field. However, chemical compounds were identified by thin-layer chromatography (Elix & Ernst-Russell 1993) in two specimens per species to check the correct identification at the field. Percent cover was visually estimated in the field per each branch segment and dry weight of *Ramalina* species per each segment was measured in the laboratory. Each specimen of *Ramalina* was separated using tweezers under a stereo microscope, and subsequently dried to a constant weight at 50°C for 5 days and weighted in a precision scale (0.00001 g, MS105DU, Mettler Toledo, Columbus, OH, U.S.A.). Dry weight was expressed as $mg{\cdot}cm^{-2}$.

Statistical analyses. All statistical analyses and graphics were performed using R version 4.1.2. The effect of land use on *Ramalina* species was evaluated using Generalised Linear Mixed Models. To evaluate the effect, we modelled five response variables: species richness, percentage cover and dry weight of genus *Ramalina*, and percentage cover and dry weight for each *Ramalina* species. Response variables were sampled at twig level ($n=450$), although summarized to tree level using the mean ($n=45$). Trees are the sub-replicates of the type of land use, which is the fixed effect in our study ($a=3$). We selected three replicates for each type of land use, named sites ($n=9$). To include the heterogeneity due

Table 1. Species of genus *Ramalina* found, their lichen substances and reproductive strategy in studied localities.

Species	Lichen substances	Reproductive strategy
Ramalina canariensis J.Steiner	Usnic and divaricatic acids	Mainly asexual (soredia)
Ramalina implectens Nyl.	Usnic, salazinic and norstictic acids	Mainly sexual
Ramalina lacera (With.) J.R.Laundon	Bourgeanic acid	Mainly asexual (soredia)
Ramalina lusitanica H.Magn.	Usnic and divaricatic acids	Mainly sexual
Ramalina pusilla Duby	Usnic, sekikaic, homosekikaic and salazinic acids	Mainly sexual

to experimental design, we included site and tree nested to site as random factors (i.e., (1|site) + (1|site: tree). To evaluate the models, over- and under-dispersion of model residuals were detected using *simulateResiduals* function in DHARMa package version 0.3.1 (Hartig 2017). *lmer* function was applied when normality of residuals was fulfilled, otherwise the Gamma distribution or Negative Binomial were applied using the *glmer* function in the lme4 package version 1.1.5 (Bates et al. 2007) or, in case residuals were over- or under-dispersed, we used the *glmmTMB* function in the glmmTMB package version 1.1 (Magnusson et al. 2019). We evaluated the differences between types of land use with an analysis of variance. When differences were statistically significant, we assessed multiple comparisons among levels with the *glht* function in multcomp package version 1.4-13 in R (Hothorn et al. 2009).

We also evaluated the effect of topographical factors, such as distance to the sea, and the effect of twig or tree size on species richness, dry weight and percentage cover of genus *Ramalina*, to better understand the heterogeneity of the experimental design. Twig size was characterized as the exposed surface, and the tree size as the volume of tree canopy. Response variables were also sampled at twig level ($n=450$), although summarised to tree level using the mean ($n=45$). We evaluated this effect using Generalised Linear Mixed Models with site and tree as random factors as described with the effect of land use. In this case, we evaluated the correlation between response variables and covariates, and did not run an analysis of variance.

To evaluate the relationship between cover and dry weight, we performed a linear regression. Variables were also sampled at twig level, but we used the mean at tree level as individual sample units in the regression. The *lm* function was applied when normality of residuals and homoscedasticity of variances were fulfilled, otherwise we first applied a log transformation to dry weight's variable, because variance increases with mean in our data (McCullagh & Nelder 1989).

Results

Five species of *Ramalina* were found on twigs of *Olea europea* at nine localities (**Table 1**, **Fig. 1**). *Ramalina canariensis*, *R. lacera* and *R. pusilla* were found in all localities. *R. implectens* was found in six localities and *R. lusitanica* in eight. Differences in species richness, cover and dry weight of genus *Ramalina* (**Table 2**, **Figs. 2, 3**) were explained by land use, and those variables were not correlated with topographical factors, such as distance to the sea, nor with twig volume or tree canopy volume (**Table 3**). Also, tree canopy volume did not differ among land uses (*Chisq*=3.16, *P value*=0.206). Species richness of *Ramalina* per twig varied by type of land use ($P<0.05$), being richest in naturalized areas, then rural areas, and least in urban areas (**Table 2**). Mean cover of *Ramalina* was 33.55 %, but this varied by type of land use ($P<0.05$). Cover was highest in naturalized and rural areas, and lowest in urban areas (**Table 2**).

Table 2. Generalized linear models and averages with standard errors of cover, biomass and species richness for each type of area. Letters indicate significant differences between areas.

	GLM family	Chisq (P-value)	Df	Naturalized	Rural	Urban
Cover (%)	Gaussian	16.37 (0.001)	2	48.8±4.13 A	34.95±2.52 A	16.91±2.07 B
Dry weight (mg/cm^2)	Gamma	26.93 (0.001)	2	18.28±3.26 A	10.25±1.45 A	3.80+0.74 B
Species richness	Gaussian	29.86 (0.001)	2	4.67±0.13 A	3.80±0.20 B	3.07±0.21 C

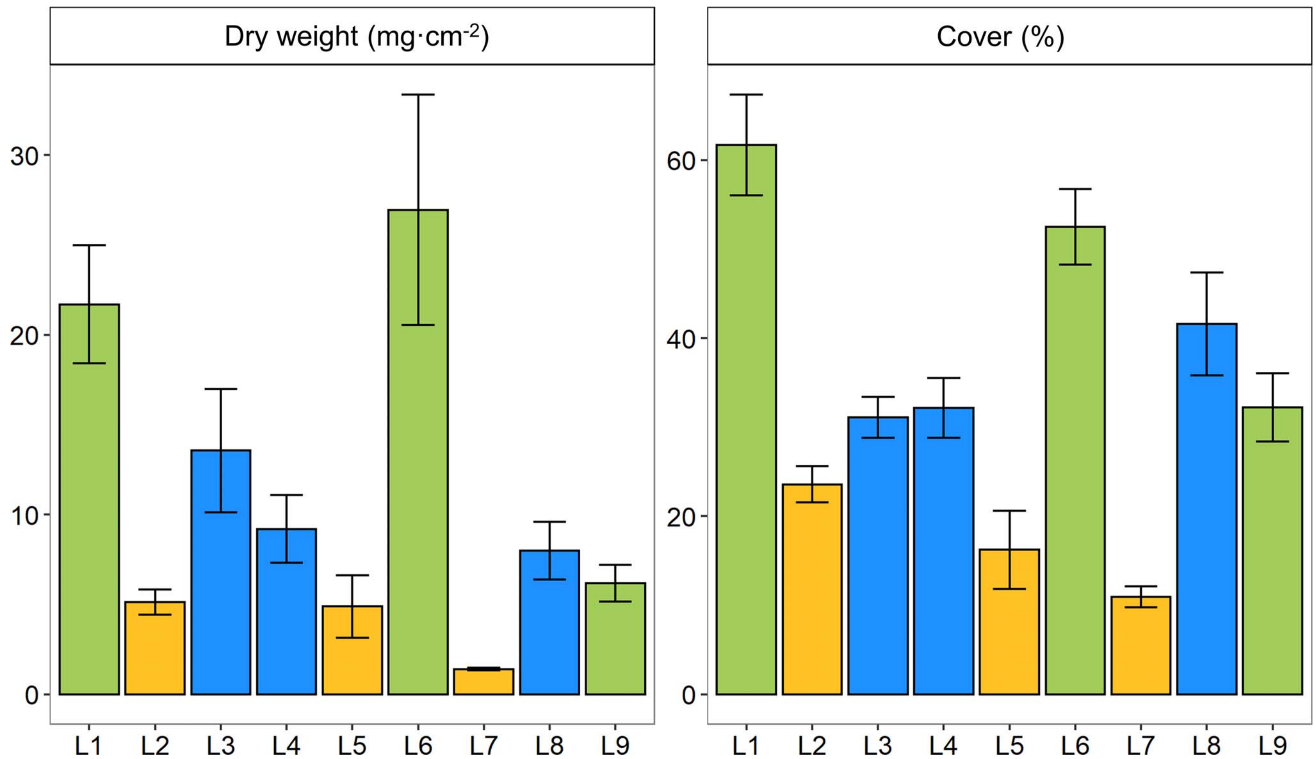

Figure 2. Averages and standard errors of cover (%) and dry weight (mg·cm-2) for genus *Ramalina* on twigs by locality. Green are naturalized areas, orange are rural areas and blue are urban areas. Online pdf in color.

Dry weight of genus *Ramalina* conformed to trends of species richness and cover ($P<0.05$). Naturalized and rural areas showed the highest biomass per unit area of genus *Ramalina*, with the lowest found in urban areas. Analyzing each *Ramalina* species separately (**Supplementary Table S2**) demonstrated different relationships with land use. Although all species decreased from naturalized to urban areas, the intensity of the response (sensitivity) varied among species. *Ramalina implectens* was the most sensitive species of all, with a

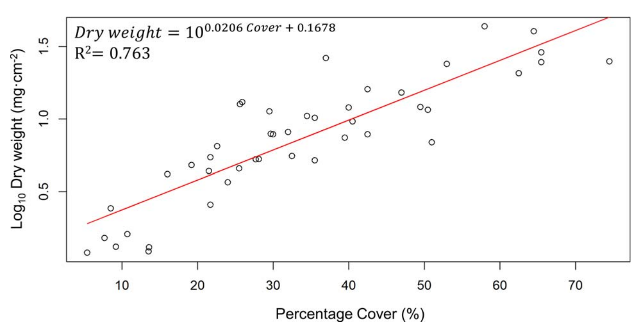

Figure 3. Predicted relationship from a linear regression between percentage cover and log-transformed dry weight based on genus *Ramalina* data. Points are sampled trees (n=44).

decrease of 98.2% in cover and 99.5% in dry weight in urban areas compared to naturalized areas. It was followed in sensitivity by *R. pusilla* with an 89% decrease in cover and 93% decrease in dry weight. The most resistant species was *R. lacera* with only 47.3% decrease in cover and 58.5% decrease in dry weight from naturalized areas to urban areas (**Supplementary Table S2**).

In general, percentage cover and log(dry weight) of *Ramalina* were strongly related ($R^2=0.763$, **Fig. 3**). Cover and dry weight were strongly related for *R. lacera* when analyzed separately ($R^2=0.796$, **Supplementary Fig. S3**), while the other four species had strong relationships of log(dry weight) against cover (see **Supplementary Figs. S1,S2,S4,S5**). The strongest relationship between cover and dry mass per area was for *R. lacera* ($R^2=0.796$), and the weakest for *R. canariensis* ($R^2=0.480$).

Discussion

We demonstrated that cover, dry weight and species richness of epiphyte lichens of the genus *Ramalina* and its species in Menorca vary by land use, reaching higher values on naturalized and rural

Table 3. Generalised linear models of cover, biomass and species richness related to twig volume, tree canopy volume and distance to the sea.

		Twig volume		Tree canopy volume		Distance to the sea	
	GLM family	Estimate	Z value (Pr > z)	Estimate	Z value (Pr > z)	Estimate	Z value (Pr > z)
Cover (%)	Gamma	-0.024	-1.09(0.28)	-0.002	-1.56(0.12)	0.000	-0.59(0.55)
Dry weight (mg)	Gamma	-0.019	-0.56(0.58)	-0.003	-1.16(0.25)	0.000	-0.15(0.88)
Species richness	Gamma	-0.003	-0.18(0.85)	-0.001	-0.56(0.58)	0.000	-1.17(0.24)

areas rather than urban areas. Results agree with the described ecology of *Ramalina* species growing on twigs of open woodlands in the Mediterranean area, especially in undisturbed localities (Nimis & Martellos 2017). On the other hand, we showed for the first time that estimated cover at the field and dry weight were correlated for *Ramalina* species as Pike (1981) and McCune (1990) had previously proved for other taxa.

Responses of Ramalina epiphytes in Menorca. Previous to this study, six epiphytic species of the genus *Ramalina* had been found in Menorca (Stolley & Kappen 2002). However, we found five species on twigs of *Olea europea* var. *sylvestris*, adding two new species for Menorca, *R. implectens* and *R. lusitanica*. Of these five species found, *R. pusilla*, *R. implectens* and *R. lusitanica* are considered threatened species in Italy (Nimis & Martellos 2017). *Ramalina implectens* and *R. lusitanica* are also considered endangered in France (Roux 2020), while their status in other Mediterranean countries is unknown due to lack of lichen red lists (e.g., Spain and Greece). Despite this *Ramalina* conservation concern, we found almost all species in the nine sites surveyed, except *R. implectens*, which was missing in two urban sites and a rural site and was almost undetectable in the third urban site. In addition, we have been able to verify that the species most sensitive to habitat alteration are *R. implectens* and *R. pusilla*, and the most resistant is *R. lacera*. These results indicate that Menorca is favorable habitat for epiphytic *Ramalina*. We hypothesize that this favorability derives from the extent of woodlands composed by *Olea europea* and *Ceratonia siliqua* (Fernández et al. 2021) and by the high relative humidity of the island, since these species appear on sites with frequent humid winds or fogs (Nimis & Martellos 2017).

The differences in *Ramalina* responses to land use may be related to the availability of light and humidity (McCune 1993), the development of a stand structure favorable for lichen establishment and growth (Boudreault et al. 2009), and other habitat conditions such as presence of atmospheric pollutants (Garty 2001). The *Ramalina* species that we studied require high humidity, sun-exposed sites, and slight eutrophication (Nimis & Martellos 2017). Pending a more exhaustive study by analysing the structure of the woodlands in Menorca and analysing habitat quality, which were not our main aim in this work, the differences between *Ramalina* populations of different land uses could be explained to a large extent by the regime of human disturbance, similar to other lichen indicators of habitat quality in coastal environments as *Seirophora villosa* in dunes with *Juniperus* spp. (Benesperi et al. 2013). Trees in urban areas have been more disturbed by humans than those in naturalized areas in recent decades; percentage cover, species richness and dry weight decreased when human disturbance increased, from naturalized to urban areas. Human disturbances that impact lichens include increasing pollutants in urban areas (Llop et al. 2017), increasing eutrophication in rural areas (Pey et al. 2020), and changing the density or structure of tree canopy with the removal of tree mass (Lehmkuhl 2004; McCune 1993). This latter impact would open up the tree canopy and woodland, leading to an increase of wind penetration (Boudreault et al. 2009), changing the humidity conditions (McCune 1993), especially as Menorca is subjected to strong northerly winds and sometimes severe thunderstorms (Estarellas et al. 2019). However, despite these limitations, in Mediterranean climate with summer drought such as Menorca Island, the low percentage cover and biomass in urban areas can be better explained by the dry microclimate of urban areas than by atmospheric pollution (Tretiach et al. 2012) or other disturbances, especially in the case of *Ramalina* species, which have a high humidity requirement (Spjut et al. 2020).

Correlation between cover and dry weight in Ramalina. Cover of *Ramalina* species has been shown to be a good indicator of dry weight, being a proxy to transform field data of lichen cover into lichen biomass (Miranda-González & McCune 2020). The relationship between percentage cover and dry weight from *Ramalina* data was fitted a linear regression after logarithmic transformation of dry weight, indicating that at higher cover values, the growth is less related to an expansion in the surface covering than to lichen volume. Growth of fruticose species is three-dimensional, being greater at the tips of lamina (Armstrong 2015), while the surface covering is two-dimensional. Despite this general trend, there is interspecific variability, as *Ramalina lacera* fits better without log transformation and residuals had a Gaussian distribution (McCullagh & Nelder 1989), and *R. canariensis* showed a low coefficient of determination (R^2) applying a logarithmic transformation to dry weight, requiring further studies to better understand their growth pattern.

Conclusions

Percentage cover of *Ramalina* species is a good indicator of land use in Mediterranean woodlands of the Natura 2000 Network, since it decreased when human disturbance increased. Percentage of cover is correlated with biomass, being a rapid, easy and non-destructive measure rather than directly measuring biomass to evaluate habitat quality. This new approximation will help the management of threatened coastal woodlands in the Mediterranean area using fewer species, offering new indicators for long-term monitoring of forest disturbance.

Acknowledgments

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Supplementary documents online:

Supplementary Table S1. Summary of study sites.

Supplementary Table S2. Generalized linear models and averages with standard errors of percentage cover, dry weight for each type of area. Family of GLMM is Negative Binomial.

Supplementary Fig. S1. Predicted relationship from a linear regression between percentage cover and log-transformed dry weight based on *Ramalina canariensis* data. Points are sampled trees (n=39).

Supplementary Fig. S2. Predicted relationship from a linear regression between percentage cover and log-transformed dry weight based on *Ramalina implectens* data. Points are sampled trees (n=13).

Supplementary Fig. S3. Predicted relationship from a linear regression between percentage cover and log-transformed dry weight based on *Ramalina lacera* data. Points are sampled trees (n=35).

Supplementary Fig. S4. Predicted relationship from a linear regression between percentage cover and log-transformed dry weight based on *Ramalina lusitanica* data. Points are sampled trees (n=32).

Supplementary Fig. S5. Predicted relationship from a linear regression between percentage cover and log-transformed dry weight based on *Ramalina pusilla* data. Points are sampled trees (n=33).