



# Morpho-anatomical variations of *Parmotrema pilosum* (Parmeliaceae, Ascomycota) in fragmented forests of central Argentina: relationship between forest cover and distance to crops

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## Abstract

Forest vegetation is key for buffering microclimatic factors and regulating atmospheric deposition. Epiphytic lichens are sensitive to these factors and can indicate the overall health status of the ecosystem. Specifically, the analysis of morpho-anatomical variations allows us to understand the degree of tolerance or sensitivity of these organisms exposed to agricultural crops and how vegetation might buffer this response. We analyzed variations in vegetative and reproductive characters and injuries in thalli of *Parmotrema pilosum* as a response to distance to crops and forest cover. The study was conducted in forest patches of the Espinal in central Argentina, an ecosystem threatened by agricultural activity. We selected 10 sites with different forest cover areas and two collection points differing in distance to crops: sites adjacent to (0 m) and far from (150 m) crops. We collected five thalli from each collection point and analyzed variations in morpho-anatomical characters at macro- and microscopic levels. We found a lower number of algae and a higher proportion of simple cilia in individuals at points adjacent to crops. At points with low forest cover, a thinner upper cortex was observed, whereas at points with greater forest cover, an increase of necrosis and greater presence of apothecia were detected. Bleaching was the most frequent injury at sites adjacent to crops, decreasing with increasing forest cover. Conservation and reforestation of Espinal forest patches would promote the propagation of lichens affected by agricultural practices.

**Keywords** Espinal · Bleaching · Necrosis · Upper cortex · Forest vegetation, Agricultural

## Introduction

The expansion of the agricultural frontier and urban areas is one of the main drivers of deforestation at the global level. In Argentina, approximately 6 million ha of native forest has been lost in the last three decades due to the advance of agriculture, largely driven by the “soybean boom” and global changes in the precipitation regimes (Carranza et al.

8). The current industrial agriculture system, which relies on chemical pest control, poses a serious threat to the soil, since it reduces its capacity to transform, filter, buffer, and degrade the substances that reach the soil surface, as well as its capacity to regulate the biogeochemical cycles (Aparicio et al. 3). Moreover, soil erosion causes re-suspension of dust towards the atmosphere, making particulate matter one of the most damaging contaminants for health, as recognized by the World Health Organization (<http://www.who.int/>). The most affected ecoregions are seasonally dry forests from the north and center of the country, corresponding to the Chaco and the Espinal ecoregions (Agost 1). The conservation state of the Espinal forests is alarming; indeed, deforestation for agricultural use has reduced forest cover in the national territory to less than 0.03%, because these forests develop in plains with highly productive soils (Cabido et al. 5).

Vegetation plays an important role in the mitigation of atmospheric pollution. Some particles can be absorbed by trees, although most particles that are intercepted are

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retained on the plant surface. The intercepted particle is often resuspended to the atmosphere, washed off by rain, or dropped to the ground with leaf and twig fall (Nowak et al. 31). Fan et al. (16) demonstrated that the capacity to remove particulate matter increases with increasing patch size and with patches exhibiting a high conservation degree. Along with vegetation, epiphytic lichens also intercept atmospheric dust. These symbiotic organisms are formed by a mycobiont (fungi) and a photobiont (green algae and/or cyanobacteria), and unlike vegetation, lichens cannot regulate dust uptake, since they lack of cuticle or stomata and therefore are very sensitive to environmental changes (Will-Wolf et al. 47).

It is well known that lichen communities are structured as a function of land uses due to the sensitivity of some species to habitat fragmentation, and emissions from agriculture, livestock, or urban areas (Will-Wolf et al. 47; Rocha et al. 37; Palharini et al. 34). Previous works in forests from the Espinal in Argentina showed that the frequency of nitrophilous species increases in areas with high proportion of crops and grazing, whereas it decreases in areas with intensive livestock production (Filippini et al. 20). However, this tolerance depends on the distance to emission sources, adjacent vegetation cover, and phenotypic plasticity of each species (Jairus et al. 22; Cardós et al. 7).

The analysis of morpho-anatomical variations of lichen species allows us to explore the changes that they undergo in disturbed areas (Daimari et al. 10; Rangel-Orsorio et al. 36). In lichens with thalli arranged in layers (heteromorous lichens), variations in thickness or relative proportion of each layer with respect to the thallus allows us to know the relationship between autotrophic/heterotrophic processes and their ecological significance. The proportion of mycobiont and photobiont layers in a single species can vary, depending on physiological requirements. For example, the upper layer (mycobiont) can become thicker and produce pigments that protect algae from photoinhibition caused by solar radiation (McEvoy et al. 28; Palharini et al. 33). Likewise, in nitrophilous species, algal density can increase, and in less tolerant species, the photobiont layer may be decreased, involving a reduction of the photosynthetic activity.

In several species-rich lichen communities in uncontaminated areas, plenty lichenicolous fungi are parasymbiotic and are not visibly detrimental to host populations. In contrast, in impoverished communities affected by air pollution, the surviving species are highly vulnerable to attack by these fungi (Lawrey and Diederich 24). In this way, simplification of the natural landscape and the increasing air pollution associated with urbanization can favor colonization by lichenicolous fungi (Lawrey and Diederich 24).

Lichenized fungi exhibit some of the greatest reproductive diversity across Eukaryotes, through sexual spores, asexual conidia, and specialized asexual propagules (Tripp and Lendemer 45). The ontogenic sequence of sexual and vegetative

reproduction structures is only known for a few species (Sanders 40; Martínez et al. 27); how environmental factors may affect those structures is even less known, due to the complexity inherent to the symbiosis and the slow growth of lichens. Some modifications in the reproduction patterns were reported indicating an increase of vegetative reproductive structures with respect to sexual ones along increasing environmental stress gradients (Koch et al. 23). Opposite to this, Stofer et al. (43) showed that species which usually produce fungal propagules were more often found in open and more intensively managed land use units, while forests or landscapes with a high proportion of forest cover harbored more species with vegetative symbiotic propagules.

On the other hand, gradual accumulation of pollutants in lichens due to atmospheric deposition can lead to ultrastructural injuries, which become visible to the naked eye over time, such as the appearance of necrotic zones due to fungus death or thallus “bleaching” due to algal death (Sillett et al. 41). Necrosis is the evidence of physiological stress processes undergone by the photobiont; indeed, chlorophyll degradation causes an increase in the concentrations of pheophytin, which turns thalli brownish (Nimis et al. 30). The appearance of discolored areas (or “bleachings”) shows a different type of injury, which is characterized by the loss of structure of the chloroplasts, leading to the disintegration of the membranes and subsequent death of the photobiont. This injury is macroscopically visible as completely discolored areas that appear almost simultaneously with ultrastructural damage (Holopainen, 21; Tarhanen et al. 44).

Previous morphological work of urban areas in central Argentina done by Estrabou et al. (14) reported no changes in upper cortex thickness nor cover of soredia in *Parmotrema pilosum*, but they find higher density of algae and a reduction in the length of cilia in polluted urban zones. In agricultural zones, woody vegetation regulates micro-site conditions and plays a key role in atmospheric deposition. In the Espinal forest, this function is threatened by the impact of agricultural activity and the scarce conservation policies. In this work, we aimed at investigating how different percentage of forest cover and distance to crops affect vegetative and reproductive characters and the occurrence of injuries in *Parmotrema pilosum*, expecting to find similar tendencies than was reported previously.

## Methods

### Lichen species selection

*Parmotrema pilosum* (Stizenb.) Krog & Swinscow is a corticolous foliose lichen, rarely saxicolous, loosely adhered to the substrate (Fig. 2A). Its margins have sub-irregular lobes, rounded at the apex and densely ciliated. The cilia can be simple or branched, and between 0.2 and 1.5 mm in length (Elix

and Hale, 12; Estrabou, 13). Its upper cortex is smooth, the medulla is white and has no secondary metabolites, and its lower cortex is black and densely rhizinate (Spielmann and Marcelli 42). Its reproductive structures include orbicular soralia at the beginning of development that become dispersed or coalescing at maturity, and rare, short-stipitate apothecia with spores 11.5–14.5  $\mu\text{m}$  long and 6.8  $\mu\text{m}$  wide (Estrabou, 13). No pycnidia have been reported. This species is common in the seasonally dry forests of the Chaco (Canton et al. 6; Quiroga et al. 35), even in forests degraded by fire (Rodríguez et al. 38), and fragmentation (Estrabou et al. 15; Filippini et al. 19). In addition, this lichen species shows a degree of tolerance to pollution in urban ecosystems and can accumulate atmospheric pollutants for a long time. This characteristic allows its use as a biomonitor for environmental mapping (Chaparro et al. 2013; Estrabou et al. 18).

## Study area

The study was conducted in the Espinal ecoregion, province of Córdoba, central Argentina (32°21' S—63°25' W), during spring–summer 2019. Mean temperature in the study area is 23 °C in summer and 8 °C in winter; annual precipitation was 558 mm in 2019 according to the CLIMA weather database (<https://magya.omixom.com>). The Espinal ecoregion is a matrix of small forest patches surrounded by fields used for transgenic crops (forage, oilseed, and cereal) and domestic livestock production (goats, sheep, horses, and cattle),

covering about 70% of Córdoba province (Agost 1; Zeballos et al. 48). The dominant vegetation is a xerophytic mosaic of deciduous to semideciduous forests of *Prosopis* spp., *Celtis ehrenbergiana*, and shrublands intermingled with grasslands (Zeballos et al. 48). The remaining small forest patches are in a poor state of conservation (Fig. 1), with a high density of adult individuals and scarce regeneration, as has been reported for other Espinal fragments (Lewis et al. 25); patches are currently used for domestic livestock grazing and firewood extraction (Peirone-Cappri et al. 26).

## Sampling design and field work

Ten sampling sites were selected from Google Earth (2019) satellite images; the sites had a homogeneous floristic composition and physiognomy, and were far from the river (more than 400 m) to avoid the influence of humidity. Sites were located in patches with plant cover typical of the Espinal and that were also close to crops in an area of approximately 60 km<sup>2</sup> (Fig. 1). In each site, we established a 150-m distance from the edge of the patch adjacent to the transgenic crop towards the interior of the patch.

We determined the 150-m distance based on forest patch area and shape; thus, the greatest distance to crops that could be assessed in all the selected patches was 150 m. In each sampling site, two lichen collection points were determined: one at the edge of the crop (0 m) and the other 150 m away



**Fig. 1** Study area in southeast of Córdoba, central Argentina (right). Spatial distribution of the 10 sampling sites with their corresponding forest covers and the two lichen collection points (left)

from the crop, hereafter referred to as adjacent to and far from the crop, respectively. We established a circular area around the collection points adjacent to the crop (300-m radius buffer) of 0.28 km<sup>2</sup>, where we estimated and digitized forest cover using Google Earth (2019) and Qgis 3.16; we then visually corroborated the forest cover in the field. The data were expressed as percentage of forest cover.

We collected samples from the dominant tree species of this forest, *Prosopis* spp. and *Celtis ehrenbergiana*; lichen communities were not found to show preference for the bark of either species (Filippini et al. 19). Lichens were collected only from branches; we did not collect lichens from the bark of trunks because bark is more influenced by wet deposition through stemflow from the canopy to the trunk than branches. Five (5) thalli per point were collected from branches of five trees located both adjacent to and far from crops. Branches were more than 2 cm in diameter, at between 0.5 and 2 m from the ground; this distance to the ground was selected considering the airborne particle dynamics. In addition, to reduce the influence of thallus age—which can be estimated from thallus size—on morpho-anatomical characters, we removed young thalli from analysis and selected only thalli of 3 to 8 cm in diameter. Four thalli from two collection points were excluded because their size slightly exceeded the studied range. Of each collected thallus, we randomly selected three independent lobes that were 1.5 cm away from the thallus end for permanent histological slides and further microscopic analysis of the mycobiont and photobiont layers. In turn, other three lobes of each thallus were randomly selected to analyze variations in cilia, whereas the remaining thalli were frozen for stereoscopic observations.

### Sample preparation for microscopic analyses

The selected lobes were fixed in FAA (formaldehyde, ethanol, acetic acid), dehydrated with an increasing series of alcohol-xylene, and then embedded in Paraplast®. The resulting blocks were transversally sectioned (10–15 µm thick) with rotary microtome, and stained using triple staining (active hematoxylin, safranin and fast green). Permanent slides were observed under stereoscopic microscope Olympus BX61 equipped with a camera and light microscope Zeiss Primo Star with Nikon Coolpix S630 camera; 30 fields (10 per lobe) were photographed per collection point. Measurements on images were taken using the software ImageJ 1.50 version.

### Observation of specimens and data collection

From each image, total thallus thickness was measured, and two measurements of the upper cortex (one from the widest part and the other from the narrowest part). The latter were averaged and divided by thallus thickness to obtain relative

thickness of the upper cortex. The number of algae was obtained by counting the cells of the photobiont within four fields of 62 × 62 µm (3844 µm<sup>2</sup>) per image. Four measurements per image were made, and then calculated the average. All the algal that were completely included in the field of view were considered. To determine the proportion of short/long cilia and simple/branched cilia, images of the youngest lobes of thalli were taken using stereoscopic microscope, and the length and branching of all cilia that did not exhibit curvatures were recorded for an easier measurement. Based on measurements, we grouped length data into “long cilia,” those exceeding 0.4 mm, and “short cilia,” those below that value. Both length and branching of cilia were calculated as a proportion based on the total number of cilia observed. Proportion of soredia was visually estimated as the percentage of each thallus that is sorediate. Finally, the presence of apothecia and pycnidia and the number of necrotic spots, bleached spots, and infections by lichenicolous fungi in the five thalli per collection point were recorded. The morpho-anatomical variations analyzed are characterized in Fig. 2 and the variables obtained are detailed in Table 1.

### Statistical analysis

To understand the effects of forest cover and proximity to crops, we used linear mixed models (LMM) and generalized linear mixed models (GLMM) after check ANOVA assumptions and found non-normal distributions of the data using Shapiro-Wilks tests and QQ-plot. We ran LMM for the upper cortex thickness and number of algae, since the residuals are normally distributed, and GLMM for the rest of morpho-anatomical variables. We used Poisson (P) and binomial (B) error family, or negative binomial (NB) when overdispersion was detected. Using diagnostics plots, we checked that all models met the assumptions.

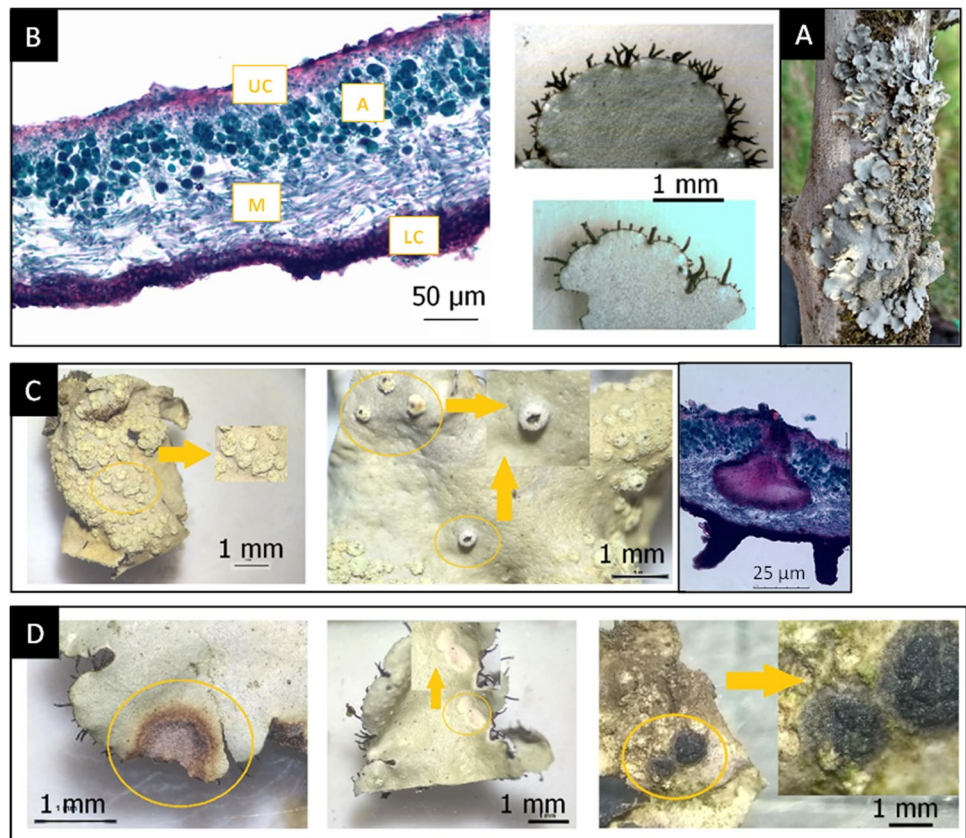
Forest cover proportion in each site and distance to crop (2 levels: far and adjacent) were the explanatory variables, and we included the interaction between both terms. Sampling sites were included as a random factor to model the dependence of the data.

All the models, from the null model without independent variables to the full model that included the interaction, were compared and selected using the Akaike information criterion (AIC). Analyses were performed using the software Infostat, and a connection to an R interpreter.

### Results

Of the 10 characters analyzed in *P. pilosum*, six showed a significant response to the explanatory variables. Upper cortex thickness, number of necrotic spots, and the presence of apothecia varied according to forest cover percentage,

**Fig. 2** Morpho-anatomical characters analyzed in *Parmotrema pilosum*. **A** Thallus of *P. pilosum* on branch. **B** Transverse section of thallus showing the upper cortex (UC), algal layer (A), medulla (M), and lower cortex (LC), and branched cilia (above) and simple cilia (below). **C** Soralia, apothecia, and pycnidia (left to right). **D** Injuries in the thalli: necrosis (left), bleaching (center), and infections by lichenicolous fungi (right)



**Table 1** Description of the morpho-anatomical variables analyzed in *Parmotrema pilosum*

Morpho-anatomical variations	Anatomical character	Unit	N	Description
Vegetative	Upper cortex thickness	Proportion (0–1)	590 images (1180 measurements)	Upper cortex thickness (μm) divided by total thallus thickness (μm)
	Number of algae	Number of algae/3844 (μm <sup>2</sup> )	590 images	Number of algal cells within a 62 × 62 μm field (four fields per image)
	Proportion of long cilia	0–1	288 images	Number of long cilia divided by total number of cilia
	Proportion of branched cilia	0–1	288 images	Number of branched cilia divided by total number of cilia
Reproductive	Proportion of soralia	0–1	96 observations	Thallus proportion with soredia (visual estimation)
	Presence of apothecia	0–1	96 observations	Record of the presence of apothecia in each thallus
	Presence of pycnidia	0–1	96 observations	Record of the presence of pycnidia in each thallus
Injuries	Number of necrotic spots	Number of necrotic spots	96 observations	Number of necrotic spots in each thallus
	Number of bleached spots	Number of bleached spots	96 observations	Number of bleached spots in each thallus
	Number of lichenicolous fungi	Number of lichenicolous fungi	96 observations	Number of lichenicolous fungi in each thallus

whereas the number of algae and the proportion of branched cilia varied with respect to distance to crops (Tables 2 and 3).

The number of bleached spots responded significantly to both predictors, whereas the proportion of long cilia, the presence

**Table 2** Results of statistical tests for each morpho-anatomical variable. The superscript letters indicate *LMM*, linear mixed models and *GLMM*, generalized linear mixed models, and the error family used for estimation *NB*, negative binomial; *B*, binomial; *P*, Poisson. Significance level: \* =  $p < 0.05$ 

Thallus part	Anatomical character	Proportion of forest cover		Adjacent/far to crops	Interaction	Intercept ± SD	Std. error (fixed effects)	
		<i>p</i> value	Slope ± SD				Proportion of forest cover	Adjacent/far to crops
Vegetative	Relative thickness of upper cortex <sup>LMM</sup>	0.0442*	0.02 ± 0.01	0.0552	0.0595	0.13 ± 4.6E-03	8.590e-03	1.870e-05
	Number of algae <sup>LMM</sup>	0.1667	1.81 ± 1.19	0.0001*	0.8314	1895.22 ± 134.82	0.5478	0.0011
	Proportion of long cilia <sup>B</sup>	0.3102	3.90 ± 3.83	0.1367	0.7569	− 5.00 ± 1.83	3.83	1.10
	Proportion of branched cilia <sup>B</sup>	0.4808	0.63 ± 0.89	0.0449*	0.6775	− 1.75 ± 0.42	0.89	0.29
Reproductive	Proportion of soralia <sup>NB</sup>	0.3581	− 0.31 ± 0.34	0.1540	0.3057	4.09 ± 0.16	0.34	0.11
	Presence of apothecia <sup>B</sup>	0.0474*	6.07 ± 3.02	0.7069	0.1327	− 4.48 ± 1.50	3.02	0.58
	Presence of pycnidia <sup>B</sup>	0.2159	10.75 ± 8.63	0.9771	0.9951	− 8.22 ± 4.44	17.23	1.18
Injuries	Number of necrotic spots <sup>NB</sup>	0.0081*	1.24 ± 0.46	0.2266	0.7120	1.36 ± 0.21	0.46	0.14
	Number of bleached spots <sup>P</sup>	0.0123*	− 2.55 ± 0.64	0.0019*	0.0008*	2.13 ± 0.26	0.64	0.24
	Number of lichenicolous fungi <sup>NB</sup>	0.0737	1.94 ± 1.07	0.9396	0.4388	− 0.53 ± 0.50	1.07	0.33

of pycnidia, and the number of lichemicolous fungi were not related to these variables (Table 2).

Among vegetative characters, upper cortex was thinner at sites with scarce vegetation than at sites with higher cover, where cortices were slightly thicker. The highest cortex thickness values were observed under intermediate forest covers only at points far from crops. However, we found that this vegetative trait does not show variation to proximity to crop, according to our significance level of analysis (Fig. 3). Algae were less abundant at points close to crops (Table 3), regardless of tree cover; the same trend was observed in proportion of simple cilia.

Regarding sexual reproduction of *P. pilosum*, we found that apothecia (scarce seen in this species) were present at points with great forest cover, regardless of distance to crops (Fig. 3). Vegetative reproduction by soredia did not show differences with forest cover nor distance to crops.

Bleaching, a frequent type of injury in all the sites, was abundant at points with scarce forest cover, even at points adjacent to crops, gradually decreasing at points with increasing forest cover. However, we detected a significant interaction between both predictor variables; therefore, this effect of forest cover on both collection points (higher number of bleached spots at the point adjacent to crops and lower number far from crops) was clearly observed up to points with 35% of cover (approximately 0.1 km<sup>2</sup>) (Fig. 3). Necrosis increased with increasing forest cover, regardless of distance to crops.

## Discussion

Our results showed that the analysis of morpho-anatomical variations of the lichen *P. pilosum*, both in vegetative and reproductive characters and in some injuries at the thallus level, allows us to have an insight into how tolerant this species can be to exposure to crops and how vegetation might buffer this response by regulating atmospheric deposition, high temperatures, lack of moisture, solar radiation, and winds, among other factors. These aspects are discussed in detail below.

## Variations of vegetative characters

In edge areas or areas of canopy openness, upper cortex thickening has been reported as a response of the mycobiont to protect the phycobiont from high solar radiation, increasing the water retention capacity of the thallus; on the other hand, lichens growing in shady areas have a thinner cortex, including the medulla (mycobiont) (Jairus et al. 22; Palharini et al. 33). In contrast to this and previous findings in *P. pilosum*, our results reveal that the cortex was slightly thinner in open sites, where climatic conditions can be more

**Table 3** Descriptive statistics of morpho-anatomical variables analyzed in *Parmotrema pilosum*

Morpho-anatomical character	Units	N	Distance to crops	Mean	S.D	Min	Max
Relative thickness of upper cortex	0–1	300	Adjacent	0.14	0.04	0.05	0.29
		290	Far	0.15	0.03	0.08	0.28
Number of algae	Cells/3844 ( $\mu\text{m}^2$ )	300	Adjacent	8.04	2.12	1.75	16.25
		290	Far	9.07	2.26	4.50	16.75
Proportion of long cilia	0–1	143	Adjacent	0.18	0.15	0.00	0.83
		141	Far	0.19	0.14	0.00	0.57
Proportion of branched cilia	0–1	143	Adjacent	0.30	0.23	0.00	0.90
		141	Far	0.38	0.22	0.00	0.83
Proportion of soralia	0–1	49	Adjacent	0.53	0.23	0.05	0.90
		46	Far	0.46	0.21	0.10	0.85
Presence of apothecia	0–1	49	Adjacent	0.14	0.35	0	1
		47	Far	0.17	0.38	0	1
Presence of pycnidia	0–1	49	Adjacent	0.04	0.20	0	1
		47	Far	0.04	0.20	0	1
Number of necrotic spots	Number	49	Adjacent	6.49	5.73	0	27
		47	Far	5.47	3.44	1	13
Number of bleached spots	Number	49	Adjacent	3.96	5.12	0	11
		47	Far	3.36	2.58	0	10
Number of lichenicolous fungi	Number	49	Adjacent	1.31	1.86	0	9
		47	Far	1.32	2.13	0	9

stressful for these lichens and the scarce vegetation cannot retain the atmospheric dust. These conditions do not affect the establishment of this species but might affect thallus growth (Fernández-Salegui et al. 17); hence, future studies should analyze a wider range of thallus size.

The algal layer did not seem to be modified by the conditions generated by the different forest covers; however, in collection points adjacent to crops, we found a lower number of cells. This finding might be due to the direct application of herbicides, since they affect especially algal growth in lichens (Anupam et al. 2). Carrera and Carreras (9) showed that field applications of different glyphosate formulations on *Usnea amblyoclada* causes degradation of chlorophylls to pheophytins, even at doses lower than those usually applied. By contrast, other authors found an increase in algal density in *P. pilosum* in large urban centers compared to less polluted areas (Estrabou et al. 14). These authors interpreted the increase of algae as a tolerance response in *P. pilosum*, which would use the nitrogen inputs typical of these environments for a better photosynthetic and growth activity. However, the oxidative capacity of glyphosate is much higher than that of the atmospheric pollutants commonly occurring in urban areas (Carrera and Carreras 9); therefore, the pollution tolerance of a species may vary among ecosystems within their distribution range or in the presence of different pollutants.

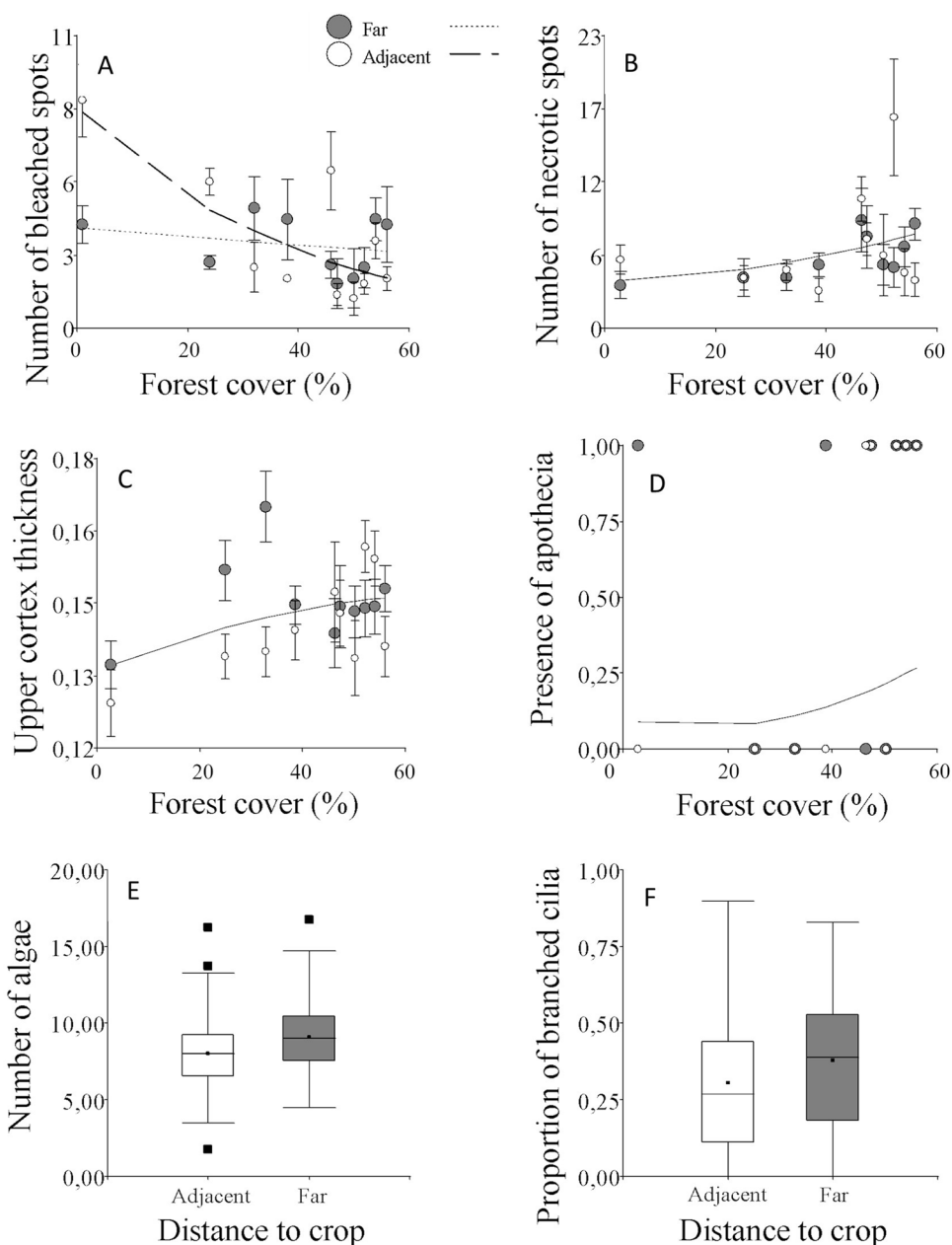
While the specific role of cilia is still unknown, they have been shown to increase the area of contact with air, allowing thalli to rehydrate and rapidly lose water, as occurs in

other lichen genera present in regions with scarce moisture (*Heterodermia*, *Parmelia*, *Trichoramalina*, *Tornabenia*) (Rundel 39). Likewise occurs in other species with hairy thalli, where the presence of these structures protects the thallus from light irradiance, oxidative stresses and the lipid peroxidation, and could offer passive and selective water control (Bianchi et al. 4). Estrabou et al. (14) observed a drastic decrease in the length of these structures in urban and industrial areas as an adaptive strategy to contaminated environments. Similarly, we consider the decrease in the degree of branching as a reduction in structural complexity of cilia, which means a reduction in the contact area of thalli. This phenomenon might be interpreted as a response to the application of agrochemicals and/or atmospheric dust, since these structures tend to be simple in highly exposed sites.

### Variations in reproductive characters

Our results evidence a major presence of apothecia at sites with increasing forest cover, while the proportion of soredia and presence of pycnidia did not show variation to our predictors, according to previous results in urban zones. Sexual reproduction is not the main reproductive trait in *P. pilosum*. However, we found that zones with higher forest cover offer a more favorable habitat for development of sexual structures, which is a key factor for the maintenance of the genetic variability of population. In addition, it is important to note that, under different habitat conditions, some species

**Fig. 3** Variation of morpho-anatomical characters in *Parmotrema pilosum* as a function of forest cover (proportion within a 300-m radius buffer) and distance to crops (adjacent: 0 m, far: 150 m). Boxplots and scatter plots show the significant response of characters to the explanatory variables: **A** number of bleached spots, **B** number of necrotic spots, **C** upper cortex thickness, **D** presence of apothecia, **E** number of algae, and **F** proportion of branched cilia (Table 2)



may grow more slowly and initiate reproduction at a smaller size (Merinero et al. 29). This idea leads us to think that our results on reproductive strategies in *P. pilosum* could be clearer if we consider all thallus sizes in future work.

### Injuries in thalli

Our results show that both bleaching and necrosis are common injuries in the thalli of *P. pilosum* in agricultural environments. These two injuries did not show any pattern in size or distribution along thalli; they appeared randomly all along the analyzed thalli and were variable in size.

Previous works reported an increase in necrosis related to different disturbances, such as forest fires (Rodríguez et al. 38), thermal power stations (Fernández-Salegui et al. 17), and high levels of particulate matter (Estrabou et al. 14). In turn, increased bleached spots were associated with atmospheric pollution in urban areas (Wakefield and Bhattacharjee 46), emissions from industrial facilities producing agricultural fertilizers, and field application of pesticides (Anupam et al. 2). Our results show that bleaching in *P. pilosum* might be caused by agrochemical applications, since this injury was most frequently observed at collection points adjacent to crops, where lichens are exposed to pesticide drift. This influence of agricultural practices on the extent of bleaching is evident at reduced forest

covers like 0.01% and could be attenuated by increased forest covers of up to 35% (0.1 km<sup>2</sup>). At these sites with greater cover, bleaching decreases, regardless of the distance to crops. Forest cover, mainly canopy cover, plays an important role in mitigating the impact of atmospheric dust on ecosystems (Nowak et al. 32); therefore, conservation and even expansion of these Espinal forest patches might reduce injuries in *P. pilosum*. Future works should explore the variations of the photobiont of *P. pilosum* exposed to crops, incorporating physiological and histological analyses, since the frequency of bleaching (easily observed in the field) might be an indicator of damage by agrochemicals, especially considering our results on the decrease of algal cells.

The increased necrosis associated with a greater forest cover might be due to colonization of thalli by lichenicolous fungi, which had a marginally significant *p* value ( $p < 0.07$ ). Many of the observed small areas or dark spots may have been at early stages of infection by lichen fungi. The lack of conservation present in Espinal and the effect of direct application of agrochemicals and air pollution may make this species highly vulnerable to attack by these fungi (Lawrey and Diederich 24). Therefore, future work that considers monitoring of the occurrence of small dark areas associated with the initial stages of infection process by lichenicolous fungi could shed light on the role of these fungi in different situations. Such study should be conducted using confocal laser scanning microscopy or transmission electron microscopy, since the hyphae of the host and the lichenicolous fungi can be easily confused using only transmission light microscopy (De los Ríos et al. 11).

## Conclusions

The Espinal forest remnants that are found isolated in central Argentina provide substrate for the permanence of *P. pilosum*. However, the conditions for growth and development of these lichens are increasingly stressful, as shown by the high frequency of injuries observed in their thalli. This phenomenon is especially evident in the increase of bleaching in lichens adjacent to crops, which decreased with increasing forest cover. Regarding vegetative and reproductive characters, our results show that at collection points adjacent to crops, thalli have a lower number of algal and a higher number of simple cilia. We found thinner upper cortex in response to decreasing forest cover, and an increase in necrosis and greater presence of apothecia with increasing forest cover.

The most important actions to promote the propagation of lichens threatened by agricultural practices will be conservation and reforestation of Espinal forest patches. Moreover, maintaining healthy epiphytic lichens in the managed forests will not only increase biodiversity but also benefit a wide variety of other organisms using these lichens.

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**Author contribution** All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by González Argüello and Filippini. The first draft of the manuscript was written by González Argüello, and Filippini and Machado commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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**Data availability** The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Declarations

**Ethics approval** Not applicable.

**Consent to participate** Not applicable.

**Consent to publish** Not applicable.

**Competing interests** The authors declare no competing interests.

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