








Article

# Vitality and Growth of the Threatened Lichen *Lobaria pulmonaria* (L.) Hoffm. in Response to Logging and Implications for Its Conservation in Mediterranean Oak Forests

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**Abstract:** Forest logging can be detrimental for non-vascular epiphytes, determining the loss of key components for ecosystem functioning. Legal logging in a Mediterranean mixed oak forest (Tuscany, Central Italy) in 2016 heavily impacted sensitive non-vascular epiphytes, including a large population of the threatened forest lichen *Lobaria pulmonaria* (L.) Hoffm. This event offered the background for this experiment, where the potential effects of logging in oak forests are simulated by means of *L. pulmonaria* micro-transplants (thallus fragments <1 cm). Our working hypothesis is that forest logging could negatively influence the growth of the thalli exposed in logged stands compared to those exposed in unlogged stands. One hundred meristematic lobes and 100 non-meristematic fragments are exposed for one year on 20 Turkey oak trees (*Quercus cerris*), half in a logged and half in an unlogged stand. Chlorophyll (Chl) *a* fluorescence emission and total chlorophyll content are used as a proxy for the overall vitality of the transplants, while their growth is considered an indicator of long-term effects. Generally, vitality and growth of the transplants in the logged stand are lower than in the unlogged stand. Both vitality and growth varies between the meristematic and non-meristematic fragments, the former performing much better. Hence, irrespective of forest management, meristematic fragments show higher growth rates (0.16–0.18 cm<sup>2</sup> year<sup>-1</sup>) than non-meristematic ones (0.02–0.06 cm<sup>2</sup> year<sup>-1</sup>). Considering that a conservation-oriented management for this species should be tailored at the habitat-level and, especially, at the tree-level, our results suggest that for appropriate conservation strategies, it is necessary to consider the life cycle of the lichen, since the probability of survival of the species may vary, with meristematic fragments having more chance to survive after logging.

**Keywords:** biodiversity conservation; chlorophyll fluorescence; epiphytic macrolichens; forest management; growth rates; indicator species

## 1. Introduction

Biodiversity is increasingly threatened by several anthropogenic factors [1–5]. Among them, five main pressures have been recently pinpointed [6], including the overexploitation of species; the introduction of invasive alien species; pollution from industrial, mining and agricultural activities; changes in land use; climate change. Due to these pressures, some models predict that up to 50% of the species are expected to become extinct in the next 50 years [7–9]. Pollution, land use (including forest management) and climate change cause habitat loss and fragmentation, changes in species range, population size and abundance, vitality and reproductive capacity [10–12]. Epiphytes may be particularly at risk due to their dependence on trees for their entire life cycle [13]. Specifically, growing on other plants they depend on their host for physical support and on tree host-specific throughfall chemistry to satisfy their nutrient requirements [14,15]. Non-vascular epiphytes fulfill various ecological functions in forests. They contribute to water and nutrient cycling by intercepting and retaining nutrients from atmospheric humidity, with some also adding nitrogen by nitrogen fixation [16–18]. Moreover, they provide resources and microhabitats for bark-dwelling invertebrates, birds, and mammals [19,20].

Intensive logging and forest fragmentation can be particularly detrimental for epiphytic lichens, causing a break in the availability of their primary habitat [21,22], especially for species with low dispersal capacity (e.g., [23]). The foliose lichen *Lobaria pulmonaria* (L.) Hoffm. is considered an “umbrella” species requiring spatial and temporal continuity of the forest habitat to maintain viable populations [24–27]. It is a tripartite species in which the fungus is associated with both a green alga (phycobiont) and a nitrogen-fixing cyanobacterium (cyanobiont). It is often accompanied by other rare or endangered lichens [28–31] and sensitive to abrupt changes in light conditions (especially in the dry state [32]), such as those occurring after forest logging. Its vulnerability to logging may be exacerbated in dry environments, such as in the Mediterranean region, where oak-dominated forests represent its main habitat (e.g., [33]). Actually, the results of a recent work carried out in Italy suggest that oak (*Quercus* sp.pl.)-dominated forests provide more suitable habitat conditions for *L. pulmonaria* than montane mixed forests, with chestnut forests in an intermediate position [34]. Regarding Mediterranean Italy, the climatic niche of *L. pulmonaria* widely overlaps (>70%) with that of oak dominated forests [35], therefore the conservation of forest habitats with suitable ecological conditions (e.g., [36]) is important.

Due to its heterothallic nature, self-incompatibility, poor dispersal capacity, long generation cycles (up to 25 years) and susceptibility to environmental parameters (such as air pollution) [37–39], this sensitive species is decreasing across Europe [40]. Considering all the above reasons, it is deemed (and often used as) a model species to assess the response of epiphytic lichens to multiple environmental factors [41]. Current forest management practices can hardly sustain future viable populations of this species [42,43]. Additionally, air pollution still may limit recolonization of potentially suitable forest habitats [44]. However, despite being declining and threatened in Southern Europe [43,45–47], the species is not often recognized in conservation policies in Mediterranean regions [48].

This research began in 2016 with a legal logging in a Mediterranean mixed oak forest in Tuscany, Central Italy, that heavily impacted a large population of *L. pulmonaria*. It was estimated that 40% of *L. pulmonaria* biomass (8.5–12.3 kg ha<sup>-1</sup>) was lost (in the mostly colonized area, up to 1.8 kg 100 m<sup>-2</sup>), including large and fertile thalli [48]. More than one year later, the analysis of chlorophyll *a* fluorescence emission revealed a significant reduction of the vitality of the thalli left on retained-isolated trees [49]. Here, the potential effects of logging on *L. pulmonaria* in this oak forest have been simulated by means of micro-transplants (thallus fragments <1 cm). Our working hypothesis is that forest logging could negatively influence the growth of the thalli exposed in logged stands compared to those exposed in unlogged stands, with potential consequences for the conservation of the species. Since the viability of *L. pulmonaria* populations in relation to forest management often depends on the regenerative capacity of the thalli, we focus our attention on the behavior of thalli (fragments) with meristematic (young) and non-meristematic (adult) properties. Hence, healthy young and adult fragments of *L. pulmonaria* are

transplanted for one year to a logged and an adjacent unlogged mixed oak stand, two and half years after the conclusion of logging. Afterwards, ecophysiological responses (vitality and growth) of the species are recorded. To optimize survival and growth, the response to logging is tested under the most suitable conditions for lichen growth (north) side of the trunk, breast height [34,50]. The following questions were addressed: (i) does forest management influence the growth capacity of the model species in logged and unlogged stands? (ii) do vitality and growth vary between meristematic and non-meristematic thalli?

## 2. Materials and Methods

### 2.1. Study Sites

The study was carried out in two forest sites, a logged and an adjacent unlogged stand dominated by *Quercus cerris*, *Q. pubescens* and *Q. ilex*, (Tuscany, Central Italy, WGS84: N 43.1851°; E 11.3602°) (Figure 1).

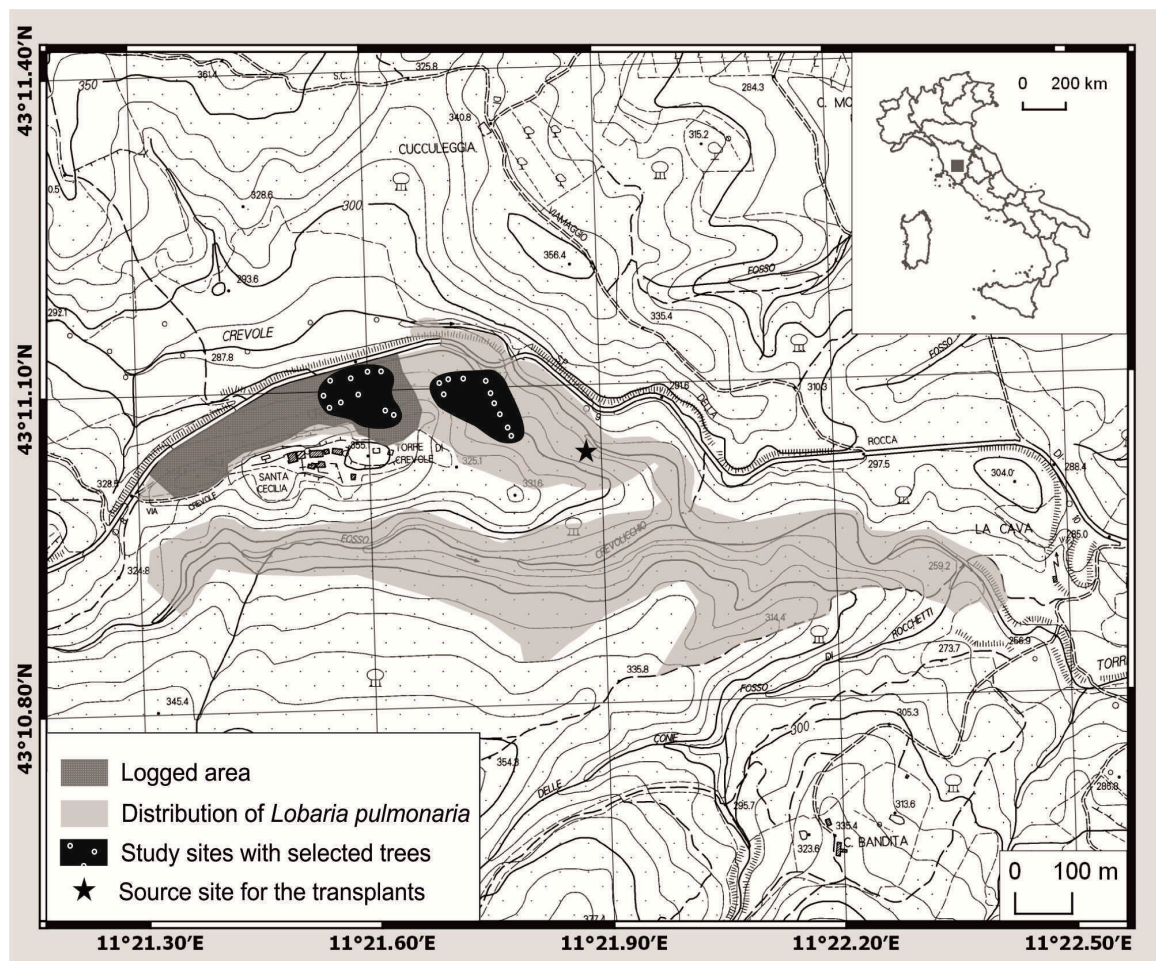


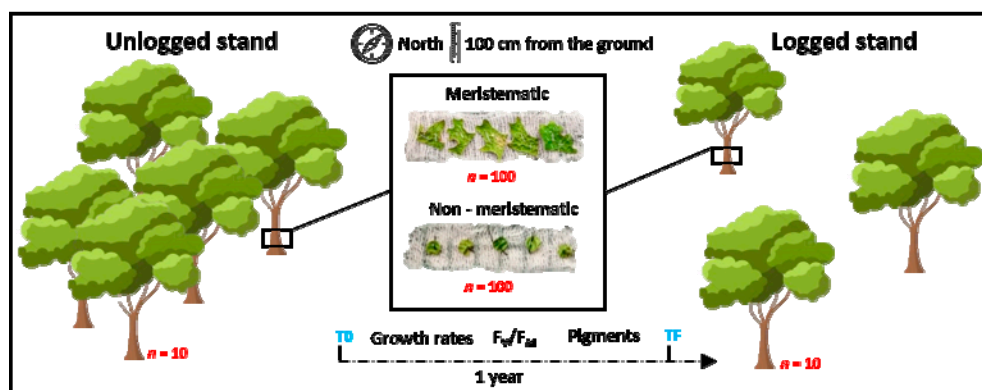
Figure 1. Map of the study sites. Taken from Paoli et al., [48] (modified).

Both study sites were located along a narrow valley with comparable orientation (North), soil type, tree age (average around 40 years, with scattered older trees) and composition, moisture, and distance from the closest stream. The density of the stems in the unlogged area was about 1100 ha<sup>-1</sup>, decreasing to 165 ha<sup>-1</sup> in the logged stand, with a consequent increase of sun irradiance all around retained-isolated trees (from 130–1100 to 900–1550 μmol m<sup>-2</sup> s<sup>-1</sup> PAR at noon) [49]. The logged stand (about 4.4 ha) was part of a local hotspot of *L. pulmonaria*, which had a patchy distribution and

colonized more than 1000 trees. Prior to logging, the overall biomass of *L. pulmonaria* in the study area was 15.8–19.6 kg ha<sup>-1</sup> [48]. Oak forests in Tuscany are mainly managed by a coppice system with standards and rotation cycles of 18–20 years [51]. Some stands with low management intensity have a longer logging cycle, which dates back to more than 40 years ago, as in our case. Since *L. pulmonaria* is not protected by law in Italy, logging operations did not take into account the presence of the species. The exposure lasted from March 2019 to March 2020. During the experimental (1-year) period, the study area was characterized by an average temperature of 14.1 °C, with the hottest period between June and August (average of daily maximum temperature 32 °C), the coldest in January and February (average of daily minimum temperature 0 °C); precipitation was about 920 mm, distributed over 72 rainy days (precipitation ≥1 mm), 18 of which occurred in November.

## 2.2. Experimental Design

A graphical representation of the experimental design is presented in Figure 2.



**Figure 2.** Graphical representation of the experimental design.  $n = 10$  number of trees;  $n = 100$  number of thallus fragments for each type; T0—time zero, beginning of the experiment; TF—final time, harvesting of the samples;  $F_V/F_M$  = the maximum of the quantum yield of primary photochemistry.

A total of 200 thallus fragments (half meristematic and half non-meristematic) were randomly cut from a batch of about 100 healthy thalli of *L. pulmonaria* randomly selected from a nearby oak forest (Figure 1). Particularly, the former are upward-growing young lobes with intact apical meristems, and the latter are fragments of the inner sorediate or non-sorediate parts of the thallus, lacking apical growth [52,53]. The source habitat for collected fragments has the same characteristics as the unlogged stand, being adjacent to the study sites and extending on a hillside with a N slope, where *Quercus cerris* L., *Q. ilex* L. and *Q. pubescens* Willd. are the most common trees colonized by *L. pulmonaria*. To minimize the harvesting of material from the native population, meristematic fragments were cut respecting the natural shape of the meristematic lobes (dimensions 0.55–0.95 cm for most of the samples). Non-meristematic fragments were obtained with a hole-puncher by selecting discs of approximate diameter (0.50–0.65 cm) from internal adult parts of healthy adult thalli. *Lobaria pulmonaria* was exposed using a specific transplant device (“barella”) composed of a sterilized bandage supported by a plastic net (10 × 2 cm). Concerning practical reasons, each device brought five meristematic or five non-meristematic fragments of the thalli tied on the bandage using cotton threads, in a way that each lichen fragment did not overlap with the others. The following experimental conditions were considered (explanatory variables) to infer the implications of logging on the conservation and distribution of the model species in Mediterranean oak forests: forest management (logged versus unlogged stand) and type of thallus fragment (meristematic versus non-meristematic). Two hundred thallus fragments (each representing a sample) were transplanted on the north side of twenty randomly selected Turkey oak trunks (*Q. cerris*) (reciprocal distance >10 m), at about 100 cm from the ground, half in the logged and half in the unlogged stand: 50 meristematic and 50 non-meristematic fragments

in each forest type. The selection of the Turkey oak was justified by its presence and distribution after logging. Growth rates, chlorophyll (Chl) *a* fluorescence emission and total chlorophyll content were assessed. The vitality of the samples was measured monthly in terms of chlorophyll *a* fluorescence emission and seasonally (every three months) for chlorophyll content. Pre-exposure and final values were considered for the purpose of this article. Final values for chlorophyll *a* fluorescence emission were represented by the average of the last three months (winter season), to account for monthly fluctuations. Measurements were taken in the morning to minimize the variability of external conditions during the day. One measurement was taken for each sample (one measurement per visit, with one visit per month).

### 2.3. Vitality of the Lichen Photobiont

Photosynthetic parameters were previously used to assess the vitality of *L. pulmonaria* [54–59]. Here, chlorophyll (Chl) *a* fluorescence emission and total chlorophyll content were used as a proxy for the overall vitality of the transplants. The measurements of chlorophyll *a* fluorescence were carried out by a Plant Efficiency Analyzer Handy PEA (Hansatech Ltd., Norfolk, UK). Thalli were kept hydrated (sprayed with mineral water) and dark-adapted for at least ten minutes (covered with a black velvet cloth) before the measurements. Each sample was illuminated using the clip for 1 s with a saturating excitation pulse ( $3000 \mu\text{mol}(\text{photon}) \text{s}^{-1} \text{m}^{-2}$ ) of red light (650 nm) from a LED into the fluorometer sensor. All fluorescence induction curves were recorded up to 1 s. The condition of the samples was expressed by the maximum of the quantum yield of primary photochemistry as inferred from fluorescence data:  $F_v/F_M = (F_M - F_0)/F_M$ , where  $F_v = (F_M - F_0)$  is the variable fluorescence,  $F_0$  is the calculated basal fluorescence and  $F_M$  is the maximum Chl *a* fluorescence.

The chlorophyll content of the samples, expressed as total chlorophyll per  $\text{m}^2$  of biological material ( $\text{mg m}^{-2}$ ), was measured by a Chlorophyll Content Meter-300 (Opti-Sciences CCM-300, Hudson, NH, USA), which gauged the chlorophyll content based on reflectance and/or absorbance of radiation by chlorophyll molecules. The method provided accurate readings also in lichens, comparable to those obtained using the classical dimethyl sulphoxide (DMSO) extraction method [60].

### 2.4. Lichen Growth

Lichen growth was used as an indicator of potential long-term effects. We are aware that growth rates may vary on a seasonal basis, depending on several environmental factors; however, for the purpose of this research, the attention was focused on the annual growth rate of the thallus. Therefore, both at the beginning and at the end of the transplant experiment, each thallus fragment was fully hydrated with mineral water and carefully flattened to avoid the folding of the lobes before scanning by Canon i-SENSYS MF4320d (Canon Inc., Tokyo, Japan). The area (*A*) of the meristematic and non-meristematic fragments was assessed using Photoshop CS6 extended (Adobe Systems, San Jose, CA, USA). The lichen growth comparing the same samples before and after the exposure was quantified as percentage increases  $A (\%A) = [(area T_F - area T_0) / area T_0] \times 100$ . After one year, the growth of the surface of each individual thallus was assessed by subtracting the initial area from the respective area at harvest. All transplant devices remained attached to the bark during the transplant period. Only a few samples (both meristematic and non-meristematic) detached, likely due to the presence of wildlife. The loss was similar in the two stands (unlogged and logged). Thus, the final sample size for statistical analyses comprised 90 meristematic fragments (40 in the logged stand and 50 in the unlogged one) and 97 non-meristematic ones (49 in the logged stand and 48 in the unlogged one).

### 2.5. Data Analysis

The datasets of meristematic and non-meristematic transplanted fragments were processed separately, accounting for the irregular shape of meristematic lobes and possible dimensional differences with non-meristematic ones. The non-parametric rank-sum Wilcoxon–Mann–Whitney test was used to test the significance of the differences in the pairwise comparisons between samples transplanted

respectively in the logged and in the unlogged stand. A Principal Component Analysis (PCA) was used as an explorative unsupervised multivariate analysis to study the relationships among variables. Multiple linear regression models were applied to fit the relationship between the above-mentioned predictors (delta Chl, delta FV/FM and forest management) and the response variable 'growth rate'. Two models were performed accounting separately for meristematic and non-meristematic thallus fragments. Regarding each model, R<sup>2</sup>, adjR<sup>2</sup> and F statistics were considered.

The following variables were considered for data processing:

- Growth rate =  $(\text{area } T_F - \text{area } T_0) \text{ area } T_0^{-1}$ , where:  
 area  $T_F$ : area of the transplanted thallus fragment at the end of the exposure.  
 area  $T_0$ : area of the transplanted thallus fragment at the beginning of the experiment.
- Delta fluo =  $[(F_V/F_M)_{TF} - (F_V/F_M)_{T0}] (F_V/F_M)_{T0}^{-1}$ , where:  
 $(F_V/F_M)_{TF}$ :  $F_V/F_M$  of the transplanted thallus fragment at the end the exposure.  
 $(F_V/F_M)_{T0}$ :  $F_V/F_M$  of the transplanted thallus fragment at the beginning of the experiment.
- Delta Chl =  $(\text{Chl}_{TF} - \text{Chl}_{T0}) \text{ Chl } T_0^{-1}$ , where:  
 Chl  $T_F$ : chlorophyll content of the transplanted thallus fragment at the final time of the exposure.  
 Chl  $T_0$ : chlorophyll content of the transplanted thallus fragment at the beginning of the experiment.

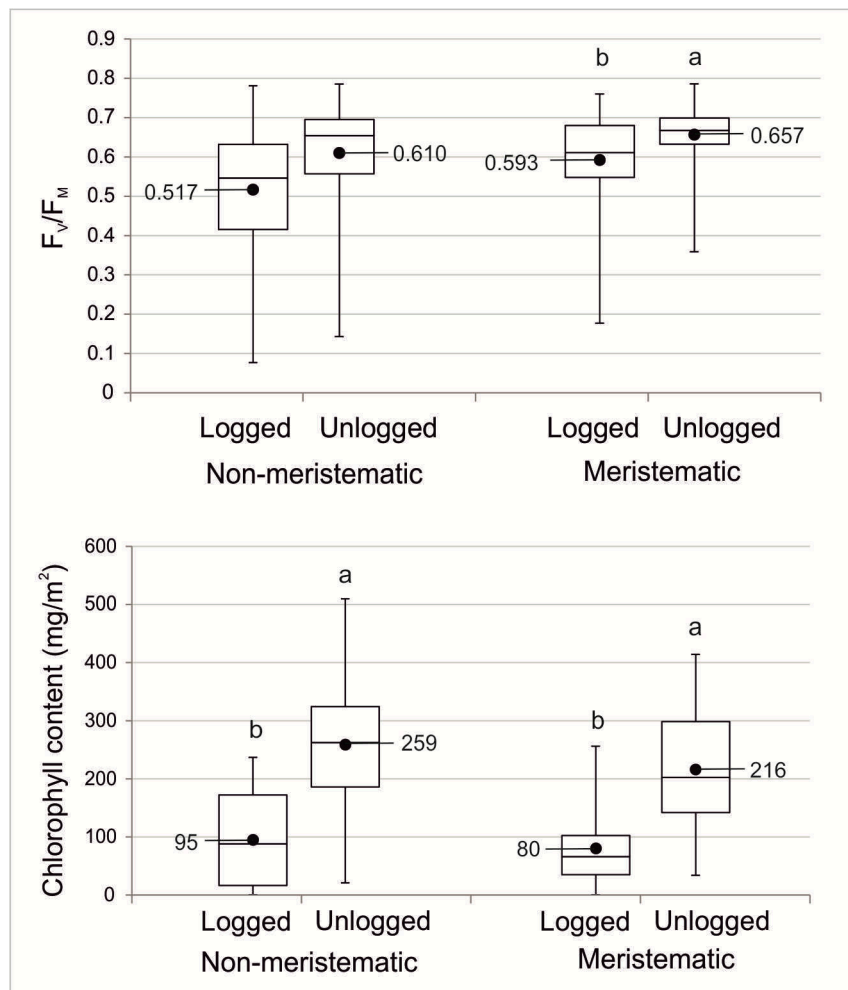
The R package *Stats* was used for all the analyses [61].

### 3. Results

#### 3.1. Lichen Vitality

A summary of the results concerning lichen vitality is represented in Figure 3. Inferred from fluorescence data, the lichens performed better in the unlogged area. The transplant experiment induced a decrease of  $F_V/F_M$  in both stands ( $p < 0.05$ ) as compared with pre-exposure conditions ( $F_V/F_M = 0.749 \pm 0.040$  for both meristematic and non-meristematic fragments). After one year of exposure, a decrease in chlorophyll concentrations was observed both in the logged and unlogged stand, compared with pre-exposure concentrations (Chl =  $403 \pm 96 \text{ mg m}^{-2}$  for meristematic and  $437 \pm 106 \text{ mg m}^{-2}$  for non-meristematic fragments) ( $p < 0.001$ ).

Upon ending the experiment, according to the parameter  $F_V/F_M$ , meristematic fragments performed better in the unlogged area ( $p < 0.05$ ). Concerning non-meristematic fragments, the difference between  $F_V/F_M$  values in the logged and unlogged stands was not significant ( $p > 0.05$ ). Both types of fragments in the unlogged stand were characterized by significantly higher ( $p < 0.05$ ) chlorophyll contents compared with those in the logged stand. Notwithstanding the variability in shape and dimensions existing between meristematic and non-meristematic fragments (which can limit a full comparison between the two groups in the same forest stand), it was observed that meristematic lobes had higher photosynthetic performances in the logged area than did the non-meristematic ones.



**Figure 3.** Boxplots of the potential quantum yield of primary photochemistry ( $F_v/F_M$ ) and the total chlorophyll content ( $\text{mg m}^{-2}$ ) at the end of the transplant experiment as indicators of the vitality of meristematic and non-meristematic fragments, in logged and unlogged stands. The average value is shown at the side of each box. Regarding each type of fragment, different small letters indicate significant differences according to forest management ( $p < 0.05$ ).

### 3.2. *Thallus Growth*

Following one year of exposure, the transplants in the unlogged stand (both non-meristematic and meristematic) were characterized by larger surfaces and a comparable increase in the thallus area (by 25% and 21%, respectively). Transplants exposed in the logged stand showed a significantly lower ( $p < 0.05$ ) growth in the case of non-meristematic fragments (their increase was about 6%). The growth of meristematic fragments (14%) did not significantly differ between the logged and unlogged stand. Irrespective of forest management, meristematic fragments showed higher growth rates (surface increase  $0.16\text{--}0.18 \text{ cm}^2 \text{ year}^{-1}$ ) as compared with non-meristematic fragments ( $0.02\text{--}0.06 \text{ cm}^2 \text{ year}^{-1}$ ). The presence of regeneration lobules along the cut edges of meristematic fragments was not observed (except for one case in the unlogged stand).

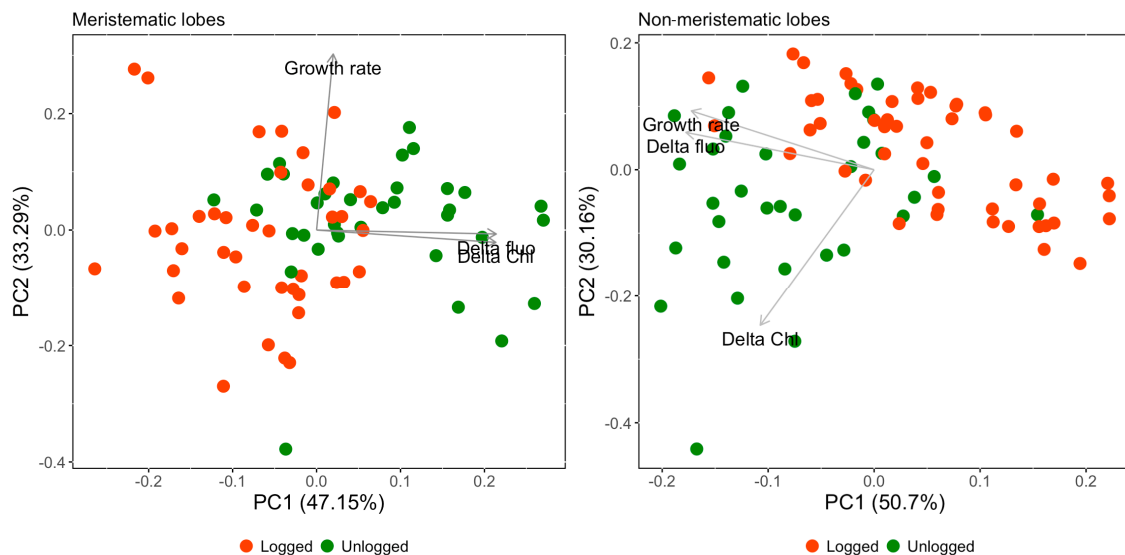
### 3.3. *Principal Component Analysis (PCA)*

A 3-dimensional solution was found both for meristematic and non-meristematic datasets, explaining 100% of the total variance (Table 1). Figure 4 shows the score plots of the first two axes, that explained  $>80\%$  of the variance. Regarding meristematic fragments, the first two principal components (PC) cumulatively explained 80.44% of the total variance. PC1 (explained variance:

47.15%) showed an increasing gradient of vitality (delta fluo,  $R^2 = 0.706$ ) and chlorophyll content (delta Chl,  $R^2 = 0.705$ ) in relation to the meristematic thalli that were transplanted in the unlogged stand (26 out of the 35 samples, 74%). The growth was positively correlated with PC2 (explained variance: 33.29%), irrespective of forest management. Concerning non-meristematic fragments, the first two PC cumulatively explained 80.86% of the total variance. The fragments transplanted in the unlogged stand were distributed (26 out of the 33 samples, 79%) for negative values of PC1 (explained variance: 50.70%), showing an increasing gradient of growth rate ( $R^2 = -0.639$ ) and vitality (delta fluo,  $R^2 = -0.658$ ), while the values of chlorophyll content were highly related to the negative values of PC2 (explained variance: 30.16%).

**Table 1.** Matrix of variable loadings reporting the eigenvalues of the three principal components of the PCA ordinations (explained variance in brackets), which were performed separately for meristematic and non-meristematic thallus fragments. Values  $>0.5$  are reported in bold.

PCA with Meristematic Dataset			
	PC1 (47.15%)	PC2 (33.29%)	PC3 (19.56%)
Growth rate	0.065	<b>0.997</b>	-0.034
Delta fluo	<b>0.706</b>	-0.022	<b>0.707</b>
Delta Chl	<b>0.705</b>	-0.070	<b>-0.706</b>
PCA with Non-Meristematic Dataset			
	PC1 (50.70%)	PC2 (30.16%)	PC3 (19.14%)
Growth rate	<b>-0.639</b>	0.344	<b>-0.688</b>
Delta fluo	<b>-0.658</b>	0.218	<b>0.721</b>
Delta Chl	-0.398	<b>-0.913</b>	-0.087



**Figure 4.** Score plots of the Principal Component Analysis obtained for meristematic (ordination on the left) and non-meristematic fragments (on the right). Distribution of meristematic lobes in logged and unlogged stand: 40 and 35 samples, respectively; non-meristematic fragments: 49 and 33 samples, respectively.

### 3.4. Multiple Linear Regression Models

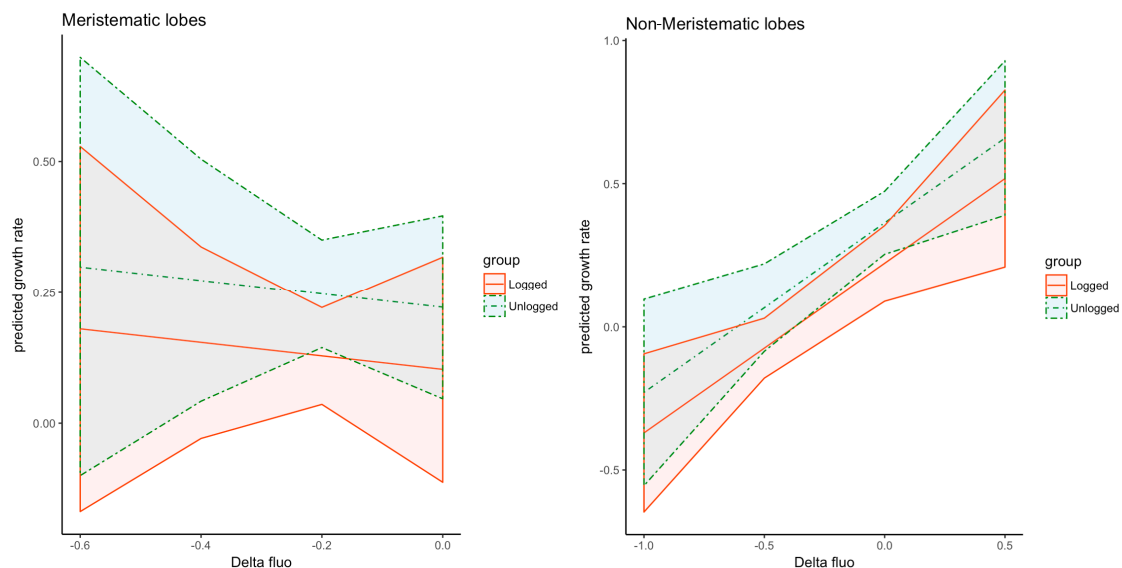
Considering the growth of meristematic fragments as the response variable, a non-significant model was obtained, thus showing that it was not affected by forest management and by photobiont vitality and chlorophyll content (Table 2 and Figure 5). The model for non-meristematic fragments was significant ( $AdjR^2: 0.197; p < 0.001$ ; Table 2), with an increasing gradient of growth with respect



to fragments transplanted in the unlogged stand ( $p < 0.05$ ), corresponding to an average of 0.142 cm estimated higher linear growth. The growth also was positively related to higher values of delta fluo ( $p < 0.01$ ; Figure 5).

**Table 2.** Multiple Linear Regression Models describing the effects of logging and vitality on growth rates both in meristematic and non-meristematic fragments. Estimates, Standard Errors,  $t$  values and  $p$  values ( $* p < 0.05$ ;  $** p < 0.01$ ) are reported. Summary statistics also are reported for each model ( $F$  statistics and  $p$  values).

Type		Estimate	Std. Error	$t$ Value	Summary Statistics
Meristematic	(Intercept)	0.055	0.131	0.419	RSE: 0.272 (71 df)
	Unlogged stand	0.119	0.077	1.537	Multiple $R^2$ : 0.033
	Delta fluo	-0.129	0.452	-0.285	Adjusted $R^2$ : -0.008
	Delta Chl content	-0.077	0.124	-0.621	$F$ : 0.809 (3 and 71 df)
					$p$ -value: 0.493
Non-meristematic	(Intercept)	0.184	0.099	1.854	RSE: 0.229 (78 df)
	Unlogged stand	0.142	0.067	<b>2.109 *</b>	Multiple $R^2$ : 0.227
	Delta fluo	0.592	0.193	<b>3.063 **</b>	Adjusted $R^2$ : 0.197
	Delta Chl content	-0.060	0.093	-0.650	$F$ : 7.628 (3 and 78 df)
					$p$ -value: <0.001



**Figure 5.** Fitted modeled relationships between growth rate and delta fluo, according to the linear models of Table 2, for meristematic (left;  $p > 0.05$ ) and non-meristematic fragments (right;  $p < 0.01$ ). Bands represent 95% confidence intervals. Solid line = logged, dashed line = unlogged.

#### 4. Discussion

After logging, forest lichens are exposed to a sudden increase in solar radiation and dry conditions, which, if in excess of their ecological range, may negatively affect their photosynthetic activity and, hence, their overall vitality [54]. A transplantation experiment with *L. pulmonaria* beginning during a sunny and dry period induced extensive and irreversible bleaching after only 40 days [32]. These effects, mostly studied in boreal forests (e.g., [62,63]), can be exacerbated in Mediterranean oak forests [48], where high light and temperature (especially during summer), together with the decoupling of moisture and light availability across an annual cycle, are supposed to influence the generation time of *L. pulmonaria* in the long-term [33]. The results of our transplant experiment outlines that the exposure in a logged stand has negative effects on the growth capacity of non-meristematic fragments. Regarding the case of meristematic fragments, higher photosynthetic performances and growth are found irrespective of forest management, suggesting the potential for “meristematic tissues” under suitable growth conditions (north side of the trunk, at about 100 cm from the ground) for maintaining

vital thalli of *L. pulmonaria* after logging. The positive correlation found between chlorophyll content (as well as  $F_V/F_M$ ) and the growth of our transplants fits with the indications of Gauslaa et al. [55]. They reported a positive correlation between chlorophyll content and growth of *L. pulmonaria* in boreal forests and a negative relationship between total chlorophyll content (as well as  $F_V/F_M$ ) and prolonged high-light exposures in clear cuts, suggesting that excess high light induced chlorophyll degradation and affected lichen growth [55]. Concerning the case of Mediterranean oak forests, a negative impact by logging on the vitality and growth of *L. pulmonaria* is expected for the thalli exposed to high light, such as on the south side of the boles (unpublished data). Comparative studies on growth rates of *L. pulmonaria* (e.g., [55,64] and references therein) reveal a wide variability depending on several factors, including micro- and macro-climatic parameters, forest structure and management. Further, our results point to the importance of the regenerative capacity of “meristematic tissues” (and, hence, of young healthy thalli) for maintaining vital populations of *L. pulmonaria* in relation to forest management.

Generally, the populations of *L. pulmonaria* are subject to sudden changes of environmental conditions after coppicing events and, as a consequence of logging, lichen thalli can have different fates:

- They can be directly destroyed/lost due to the logging of the trunks for timber production, as already occurred in the investigated oak forest (loss of 40% of *L. pulmonaria* biomass) [48].
- They can remain attached to the bark of retained trees and face a gradient of more or less stressing conditions according to their cardinal exposure, position on the trunk, and distance among the trees.

Referring to the investigated forests, the fate of the thalli left after logging likely falls in one of the following situations:

- (a) Some of the thalli show visible symptoms of damage consisting of discoloration and bleaching of the surfaces. Curling can occur as a strategy to limit the damage [65]. The thalli can experience a significant reduction of photosynthetic performance (decreased vitality) and the absence of growth. They become thinner due to the significant reduction (by 35%) of the algal layer [49]. Most of them likely will be lost in the long term.
- (b) Some of the thalli try to acclimate to ongoing stresses by melanization of the fungal cortex and reduction of the photosynthetic activity. To a certain extent, forest lichens are able to produce melanin to screen excess solar radiation, they increase their thickness in situations with more light, as well as their water holding capacity to acclimate for limited water availability and rare occurrences of hydration events [56,66]. However, in the long term, they still can be at risk.
- (c) Some of the thalli are not negatively affected; they maintain their vitality and growth rates thanks to suitable microclimatic conditions that locally persist, even after logging. They can be preserved in the long term. This is also the case for our transplant experiment (in particular for meristematic fragments, likely due to their situation and the north side of the trunk).

Logging may affect the vitality of *L. pulmonaria* left on isolated oak trees much more than on retained forest patches and unlogged oak stands [49]. Specifically, in a previous study [48] carried out one year after logging, the thalli showed visible changes in 46% of the isolated trees. Such changes consisted of melanization (in this case this could be seen rather as an acclimation than a stress response to the new environment), or in the worst case (14%) evident discoloration, bleaching, up to extensive necrosis in *L. pulmonaria*. Conversely, the remaining fraction (54%) still consisted of healthy thalli, with a dominant greenish color and absence of discolorations and necrotic parts [48]. These observations (based on native thalli) are consistent with the results of our transplant experiment, that the growth of the transplants in the logged stand was lower than in the unlogged stand. Furthermore, despite our not measuring melanin production, our field observations indicate that several fragments exposed in the logged stand (irrespective if meristematic or non-meristematic) had a darker appearance when dry compared to those in the unlogged area, as similarly reported by Coxson and Stevenson [64]

in boreal forests subjected to partial cuts. Noteworthy, *L. pulmonaria* transplanted into clear-cuts in boreal forests showed that logging in winter was less harmful than logging in summer, being associated with higher growth rates of the species than during the latter one [63] and that, in most cases, such healthy thalli were N oriented [63,67] well reflecting the delicate balance between humidity, light availability, and prevention of desiccation risk that influences the ecophysiology and distribution of *L. pulmonaria* [55]. Except for one case, we did not find new regeneration lobules along the cut edges of meristematic fragments (after one year), as the micro-transplants likely included only a narrow apical zone. Considering intact *Lobaria* lobes, this rather thin portion is not attached to the bark and undergoes curling during drying that contributes to protect it from photoinhibitory damage [65]. Conversely, in another experiment, we observed the presence of small regeneration lobules, already after one year, in transplants of large thallus fragments saved from the logged area and exposed for conservation purposes in three oak forests within nature reservations in Tuscany [44].

*Lobaria pulmonaria* is a sub-oceanic species strongly dependent on macro- and micro-climatic conditions for its dispersal and establishment [34,68]. Concerning Italy, it meets its optimal climatic suitability in areas characterized by small variations in seasonal temperature, high atmospheric humidity, intermediate conditions of diffused light, and low or negligible air pollution [69]. Considering a local scale, these microclimatic conditions could be altered by forest management, thus putting at risk the health of the populations, and compromising their probability of survival [49,55]. Taking this perspective, recent works also estimated that, due to the simultaneous loss of climatic suitability and habitat availability, the distribution range of *L. pulmonaria* in Italy will decrease by 80% by 2060 [35,43]. A partial reduction of the spatial overlap between the climatic niche of *L. pulmonaria* and that of its host tree species in the Mediterranean region, as well as the invasion of native woods by alien species (e.g., black locust) are expected to further threaten *L. pulmonaria* populations [35]. Considering such a complex risk framework, it becomes essential to understand the ecology of the species and its microscale dynamics to be able to tailor and convey conservation strategies more effectively at all stages of population development [34]. Taking a broader perspective, the conservation of suitable habitats by maintaining tree species diversity in mixed stands and increasing the proportion of deciduous trees, maintaining large trees and regeneration layers, and allowing heterogeneous light conditions within the stands [36] appears important.

Our results support the view that effective conservation-oriented management for this species should be tailored at the habitat-level and, especially, at the tree-level [30,33,34]. Indeed, the results reveal that the probability of survival of the species is influenced by an interaction between abiotic and biotic factors whose effects may vary during the life cycle of the lichen. Recent results indicated that in oak (*Quercus* sp.pl.)-dominated forests the effect of habitat was significant only for adult thalli of *L. pulmonaria*, while the early life stages of the lichen were habitat-independent and were strictly associated with tree-level factors [34]. Meristematic fragments have more chance to establish new individuals and survive in suitable conditions, such as the northern side of the trunk and at an adequate height (e.g., about 100 cm), likely reflecting their high requirement for adequate solar radiation (under humid conditions) to sustain photosynthesis. Under the same suitable conditions, non-meristematic fragments did not show the same performances, likely due to a limited regenerative capacity of “adult tissues” (compared to meristematic ones) to face the variation of environmental conditions that suddenly occur after forest logging.

## 5. Conclusions

The vitality and growth of *L. pulmonaria* in relation to forest management were tested in view of possible conservation strategies of this sensitive and threatened species in Mediterranean oak forests. Comparing transplants in logged and unlogged stands, the results pinpointed a lower growth of *L. pulmonaria* in the logged stand with respect to the unlogged stand, with non-meristematic fragments being more sensitive to the effects of forest management. It is suggested that a conservation-oriented management should be tailored at the habitat-level and, especially, at the tree-level. The probability of

survival may vary during the life cycle of the lichen, with meristematic fragments having more chance under suitable microclimatic conditions after logging.

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