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## Article

# The Mediterranean Old-Growth Forests: Anomalies or Relicts? The Contribution of Soil Charcoal Analysis

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**Abstract:** Old-growth forests are of high interest for biodiversity conservation, especially in the disturbance-prone Mediterranean landscapes. However, it remains unclear whether the survival of patches of old-growth forest in the degraded Mediterranean landscapes results from local anomalies or from past, larger forests. Therefore, in this study, we assessed (1) the origin, (2) the long-term ecological trajectory, and (3) the mechanism(s) that explain the survival of a Mediterranean old-growth forest, the Sainte-Baume forest. To achieve this, we used soil charcoal analysis. We opened fifteen soil profiles in the forest and five in its surrounding areas for soil description and sampling. The soil descriptions enabled us to highlight in situ soil horizon and colluvial layers. A total of 1656 charcoal pieces from different soil samples were taxonomically identified to characterize the composition of past forests. Selected charcoal pieces ( $n = 34$ ) were dated to obtain chronological data. Our investigations indicate that the survival of the Mediterranean old-growth forest, in the context of the semi-open/open Mediterranean landscapes, is the result of a combination of biotic and abiotic factors, which reduced the influence of past forest disturbances. Thus, the resistance and resilience of the forest areas are preserved over a long-term ecological trajectory. Therefore, the potential of Mediterranean old-growth forests as baseline reference points for the conservation of biodiversity is related to the identification and maintenance of the local biotic and abiotic factors which allowed the survival of the old-growth forest.

**Keywords:** forest conservation; ecological trajectory; fire; anthracology; forest composition; resilience; resistance



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## 1. Introduction

Old-growth forests are of high interest in terms of both biodiversity conservation [1–3] and climate mitigation [4–6], due to their specific structure, function and composition. This is especially true in disturbance-prone landscapes [7–9]. This is particularly the case in the Mediterranean basin, in which the joint effects of ancient and significant human activities and climatic constraints have caused the widespread presence of semi-open to open ecological systems, with too-short disturbance return intervals to allow the development of mature forest [10–13]. Nevertheless, in this area, several isolated woodland patches have been recognized that present features of old-growth forest [3,10,14–16].

To date, it remains unclear whether the survival of these old-growth forest patches in the degraded Mediterranean landscape results from local anomalies, or whether they are the relict patches of forests that had a larger spatial distribution in the past and which have been affected to achieve degraded forest status. This is a key question, since, in the latter case, the old-growth forests might play a significant role for forest conservation at the Mediterranean basin scale, as a baseline reference. Therefore, it is of great importance that the origin and long-term trajectories of the Mediterranean old-growth forests are assessed.

However, despite a large number of paleoecological and biogeographical investigations in the area, the issue remains poorly understood. This is probably because, in the Mediterranean basin, old-growth forests are rare, isolated and restricted to small areas [3,10]. As a consequence, their past dynamics are blurred compared with the insights garnered from the larger-scale sites studied in most paleoecological investigations. In addition, the insights on such patches of old-growth forest are also limited in terms of spatial resolution because, in the context of the Mediterranean region, archives of records of classical paleo-indicators, such as pollen grains, are rare. Therefore, to investigate the origin and the long-term trajectory of Mediterranean old-growth forests, and the mechanisms that have enabled them to survive until today, it was necessary for us to apply an approach that permitted the investigation of the forest's long-term history at a local scale. To this end, we used soil charcoal records that are particularly relevant at a stand scale [17–19] and which provide local information about the past fire regime and forest composition [20–22]. By conducting this investigation in an area of the Sainte-Baume forest with a clear distinction between a patch of old-growth forest and a matrix of degraded forest states (i.e., secondary successional forest states), a comparison was enabled of three sharply demarcated general ecological units, with the patch of old-growth forest surrounded by semi-open/open ecosystems.

Within that study area, soil samples were taken from each ecological unit for soil charcoal analysis, in order to assess (1) the origin, (2) the long-term trajectory, and (3) the mechanism(s) operating to explain the survival of the old-growth forest in the Mediterranean context of an open/semi-open landscape.

## 2. Materials and Methods

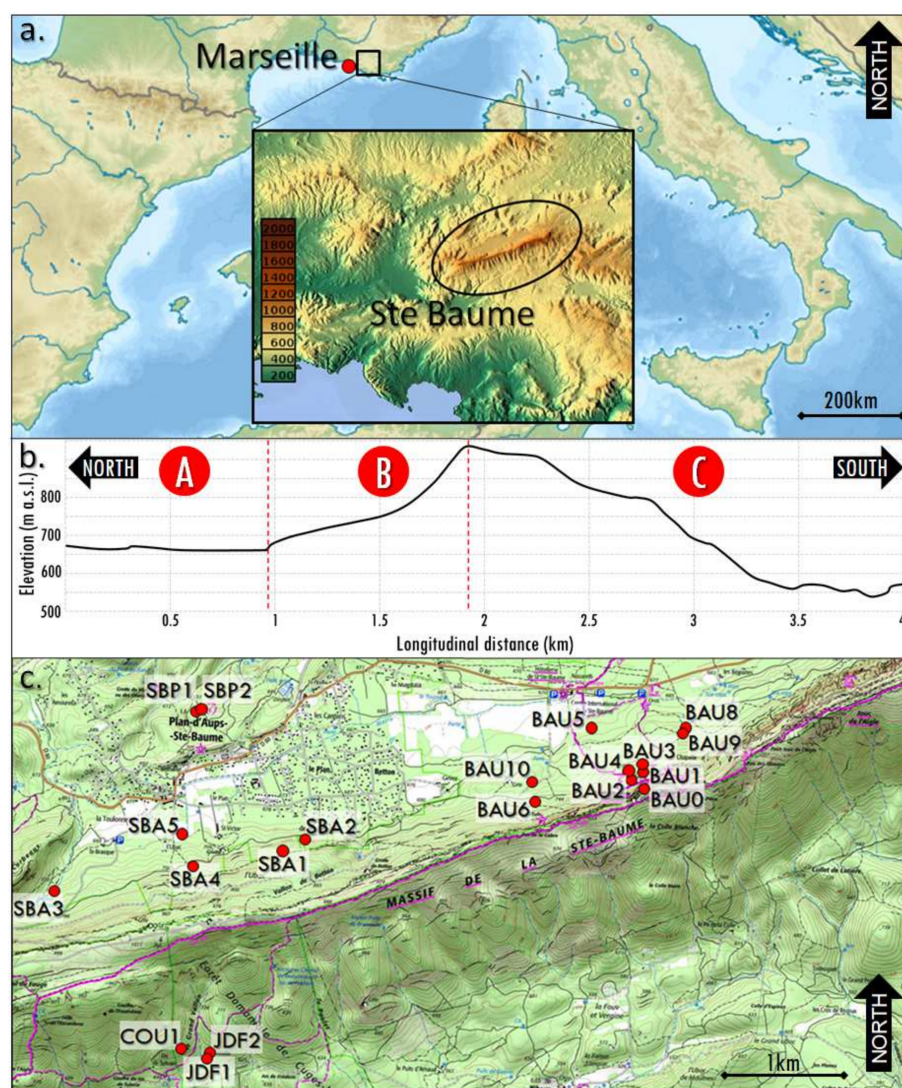
### 2.1. The Study Area

The study area is located on the northwestern side of the Mediterranean basin, in southern France (Figure 1a), in the calcareous Provence region [23]. The geological sub-stratum consists of limestones, from hard and compact to soft and easily erodible (i.e., marls; [24]), with karstic systems [25,26].

The climate in the study area corresponds to a climate typical of Mediterranean mountains, with relatively cool and moist winters, and warm, dry summers [27]. The local meteorological station records an average annual rainfall of 950 mm and a mean annual temperature of 10 °C [24]. However, considering the Sainte-Baume area and its surroundings included in the study area, the climatic conditions may vary greatly, due to the heterogeneity of the physical factors. Indeed, the study area is composed of three different ecological units (Figure 1b) based on variations in elevation, exposure, topography, vegetation distribution and soil type.

First, to the north of the Sainte-Baume Forest is a plateau between 650 and 700 m above sea level (a.s.l.). It is covered mainly by pioneer coniferous woodland dominated by pine (*Pinus sylvestris*) and juniper (*Juniperus communis* and *Juniperus oxycedrus*). Local soils are skeletal soils, rich in calcareous stones of various sizes, corresponding to skeletal rendzic regosols.

In the center of the study area is the old-growth forest of Sainte-Baume, between 700 and 850 m a.s.l., on a small site at the bottom of a calcareous cliff about 300 m high. This is a mix of deciduous broadleaf and coniferous forest with mesophilic traits [24]. More precisely, at the bottom of the cliff is a first belt of forest several tens of meters wide, dominated by beech (*Fagus sylvatica*) and yew (*Taxus baccata*), with a few silver fir trees (*Abies alba*; [28]). In this part of the old-growth forest, *Fagus* trees have been dated by dendroecological measurements to be about 200 years old (i.e., among 45 trees, the oldest is 249 years and the mean age is 182 years [29]).



**Figure 1.** Presentation of the study area: (a) location of the Sainte-Baume forest area; (b) topographical profile of the Sainte-Baume Massif on the north–south axis passing through the so-called “hotellerie”, the Saint-Pilon Pass and the Vallon de Trébuquet. The red borders mark the three ecological units in the study area (A: northern plateau; B: Sainte-Baume forest; and C: southern exposure); (c) location of the sampled soil profile in the study area (red dots) (used maps under GNU General Public Licenses).

Farther from the cliff, toward the northern plateau, is a second forest belt dominated by downy oaks (*Quercus pubescens*) with maple (*Acer* spp.) and lime trees (*Tilia* spp.; [24]). The local soils here are mostly deep, rich in organic matter and corresponding to cambisols.

Finally, on the southern side of the cliff, is an area of gently to locally steeply sloping land, from 900 to 650 m a.s.l. There, the vegetation is a typical calcareous Mediterranean scrubland with Aleppo pine (*Pinus halepensis*), evergreen kermes and holm oaks (*Quercus coccifera* and *Quercus ilex*, respectively), junipers (*Juniperus* spp.), gray-leaved cistus (*Cistus albidus*), rosemary (*Rosmarinus officinalis*) and strawberry tree (*Arbutus unedo*) [24,30–33]. Local soils here are comparable with those present on the northern plateau of the study area, i.e., rendzic regosols soils, but also locally with deep sedimentary accumulation (possibly greater than 1 m), in small stream/valley bottoms (colluvic soils), whereas, on relief ridges, soils are absent, and the calcareous substrate is directly on the surface (skeletal soils).

## 2.2. Local History

Little is known about pre-historical presence in the Sainte-Baume area. However, in the soundings of the study area, several archeological sites indicate the ancient presence of humans, such as the Neolithic site of the “Clos des Roques” c. 30 km northeast of the Sainte-Baume, or the Mesolithic grave of Cuges-les-Pins on the west of the southern side of the Sainte-Baume cliff [34]. Other studies have shown the presence of Roman road networks, which connected the Huveaune Valley, north of the investigated plateau of the Sainte-Baume, to the ancient city of Marseille [35]. At the regional level, very dense occurrences of Roman structures have been found and studied, such as villas or oppidum [36].

During the XIII century, a monastery was constructed in the Sainte-Baume forest [37], which gave a sacred statute to the forest. Therefore, from that period, the forest was protected [24]. Hunting, grazing and wood harvesting were prohibited [38]. Only after the French Revolution, when clergy’s land ownership was given to the local population, was the forest exploited [37–40]. This continued until 1973, when the forest became a biological reserve [33]. Since 2017, the area has been included as a nature regional park, and since 2018, the forest area has been recognized nationally as “exceptional woodland”.

Finally, it is interesting to note that, during the XVII and XVIII centuries, the area surrounding the Sainte-Baume Forest was an important source of ice (in stone coolers), lime for lime ovens and charcoal production, with sites that are still visible today in the field [41].

## 2.3. Sampling Strategy and Soil Description

The sampling strategy was designed to take account of the three ecological units of the study area (Figure 1). However, because our research focus was on the issue of the old-growth forest, the main effort of sampling was carried out in the Sainte-Baume forest, where 15 soil profiles were sampled along an east–west axis. To investigate the surroundings of the current distribution area of the old-growth forest, two soil profiles on the northern plateau and three soil profiles on the southern exposure of the cliff were sampled (Figure 1c).

All the soil profiles were opened manually to a length of 1–2 m and down to the bedrock. Each soil profile was cleaned and documented with scale drawings. Soil horizons and colluvial layers were distinguished and described according to the properties observable in the field (i.e., color, texture, structure, etc. [42–44]). Once the soil profile had been documented, c. 10 L of soil/soil sediment was taken for charcoal extraction. The samples were taken from the base to the top of the horizon, following the identified sediment layers or soil horizons, with a maximum height of 10 to 20 cm per sample to maintain a fine vertical resolution of sampling [22].

## 2.4. Soil Charcoal Identification

Taxonomic charcoal analysis was carried out on charcoal pieces larger than 1 mm which were extracted from the soil samples. We focused on these “mega-charcoal” pieces because they were large enough to be identifiable taxonomically [45], and because they were of local resolution, according to the catchment topography [17,18,22].

The extraction of the charcoal pieces from the soil samples was carried out using the protocol described by [46,47]. First, the samples were wet-sieved to separate the mineral fraction from the organic matter, the latter containing most of the charcoal pieces, which are low in density, floating readily. The mineral fraction was rinsed several times until the floating charcoal pieces were extracted and added to the organic fraction. Finally, the extracted organic fraction was dry-sieved using three different mesh sizes: 1–2 mm, 2–5 mm and >5 mm.

The taxonomic identification of the charcoal pieces was performed using wood anatomy criteria, because wood anatomy is fixed by carbonization [22]. The identification of both the genus and species level is possible if the charcoal pieces are not too dirty (e.g., covered in loamy sediment) and/or over-transformed (vitrified charcoal [48]).

Identification keys, wood anatomy atlases [49–51] and the charcoal reference collections of the LIEC-University of Lorraine and of the IMBE-Aix-Marseille University were used to achieve taxonomic identification. The criteria were assessed under a stereoscopic microscope at 10× to 75× magnification and an episcopic microscope at 200×, 500× and 1000× magnifications, for up to 90 charcoal pieces per sample, taken at random, equal proportions in each of the three size fractions.

### 2.5. Charcoal Data Handling

Because of the mixing of soil from different layers over time [52–54], the results of the taxonomic analysis in this study are expressed as a proportion of taxon occurrence (i.e., taxon frequency) from the extracted charcoal assemblages per soil profile (with all depths cumulated). The absolute quantities of charcoal per sampled layer of sediment or soil horizon were not considered in this study. The only analysis related to the depth of sampling was used to compare the charcoal richness in colluvial layers vs. in situ soil horizons. For these latter data, the dry weight of the charcoal fraction (at 60 for at least 72 h), after extraction from the samples, was obtained and soil charcoal concentration was expressed relative to the dry weight of the corresponding sample, cumulated per colluvial layer and per in situ soil horizon [22].

The analysis of the taxonomical data was carried out through multivariate analysis. First, we used a cluster analysis to look at the frequency of every identified taxa per sampled soil profile. This cluster analysis was based on Euclidean similarity measures, proceeded on ©PAST software [55]. Then, a correspondence analysis was performed of the frequencies of taxa according to sampled soil profiles for all three ecological units of sampling, related to the successional/autecological groups defined after the cluster analysis. This correspondence analysis was carried out using ©C2 software [56].

### 2.6. Chronological Analysis

Several single charcoal pieces, previously taxonomically identified, were selected for radiocarbon dating. The selection of the charcoal pieces to achieve <sup>14</sup>C dating was performed according to (1) their significance to the overall charcoal taxonomic spectrum, and (2) the context of sampling from which they were obtained, to provide chronological data about the soil erosion history (i.e., the ages of the erosion events; [57–59]). The carbon-14 (<sup>14</sup>C) dating was carried out by accelerator mass spectrometry (AMS) at the Laboratory of Radiocarbon Measurement of Trondheim University in Norway (lab reference: Tra) and by the Poznań Radiocarbon Laboratory in Poland (lab reference: Poz). The radiocarbon ages were calibrated with a two-standard-deviation (2σ) 95% confidence interval on the OxCal program [60], using the IntCal20 dataset [61].

## 3. Results

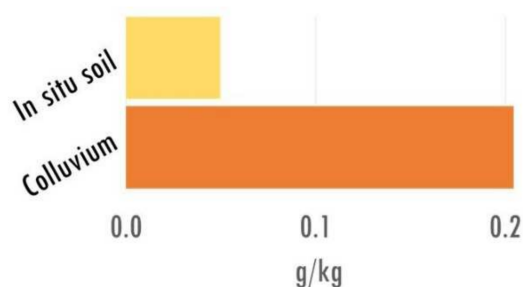
### 3.1. Soil Description and Charcoal Richness

Only seven of the 20 sampled soil profiles contained in situ soil horizons, with one or two distinguishable horizons, Ah and/or Bw, covered by colluvial layers. The 13 remaining soil profiles sampled contained only colluvial layers of varying thicknesses (from a few centimeters to several tens of centimeters; Table 1).

Consequently, more samples have been taken from colluvial layers (i.e., 56 samples, providing a total of 281 kg of sediment) than from in situ soil horizons (i.e., 17 samples, providing a total of 70 kg of soil). Nevertheless, the charcoal record, expressed in concentrations (i.e., mg of charcoal >1 mm per kg of sampled soil or sediment), enabled us to compare the charcoal richness of samples by the type of archive (e.g., soil versus soil sediment). Thus, it can be seen that the total charcoal concentration in the colluvial layers was more than four times the charcoal concentration in the in situ soil horizons (Figure 2). This observation was consistent for the three ecological units sampled in the study area.

**Table 1.** Overview of the material from sampled soil profiles (\* soil classification and soil qualifiers according IUSS Working Group WRB 2015: sk: skeletal, rz: rendzic, co: colluvic, RG: regosols, CM: cambisol).

Units of Investigation	Profile	Type of Soil *	Type of Archive		Max Depth (cm)	Nbr Samples
			Coll.	In Situ		
Northern plateau	SBP1	sk-rz-RG	x	x	50	3
	SBP2		x	x	55	3
Sainte-Baume forest	SBA1	co-CM	x	x	60	5
	SBA2		x	x	70	5
	SBA3		x	-	85	4
	SBA4		x	x	70	4
	SBA5		x	x	140	4
	BAU0		x	-	30	2
	BAU1		x	-	30	2
	BAU2		x	-	30	2
	BAU3		x	-	30	2
	BAU4		x	-	30	2
	BAU5		x	-	30	2
	BAU6		x	-	50	5
	BAU8		x	-	50	5
	BAU9		x	-	50	5
BAU10	x	-	50	5		
Southern exposure	JDF1	co-rz-RG	x	-	50	5
	JDF2	co-rz-RG	x	x	145	6
	COU1	sk-rz-RG	x	-	50	2



**Figure 2.** Total charcoal concentration by type of archive (all samples cumulated).

The absolute quantities of charcoal per sampled layer of sediment or soil horizon were not considered in this study because of the considerable biotic and abiotic heterogeneities of the sampling areas. These heterogeneities may have resulted in large variation in the previous fire regime, possibly strongly influencing the charcoal record (e.g., the type and amount of fuel, topography, wind exposure, etc. [62]). Moreover, the charcoal record was not related to the depth of sampling because soils are not chronologically stratified paleoarchives. Both physical (e.g., uprooting; [54,63]) and biological processes (e.g., bioturbation; [52,64]) are potential sources of soil mixing. This appears to be particularly relevant in an area with marked relief [53], where the interpretation of chronological data from charcoal records related to depth of sampling should be subject to considerable caution [65].

### 3.2. Taxonomical Analysis

A total of 1656 charcoal pieces, from 20 soil profiles, were identified according to tree species, representing 18 different taxa. Deciduous *Quercus* was, by far, the most commonly identified taxon, representing 42% of the entire charcoal assemblages, followed by *Fagus* at 18%, *Taxus* and *Acer* at 8% each and *Juniperus* at 7%. All the other taxa identified were recorded at frequencies of less than 1% of the whole charcoal assemblages (Table 2).

**Table 2.** Frequencies of identified taxa in the charcoal assemblages per sampled soil profile.

Units of Investigation	Profile of Sampling	Nbr of Ident.	<i>Fagus</i>	<i>Juniperus</i>	<i>Acer</i>	<i>Taxus</i>	Dec. <i>Quercus</i>	Everg. <i>Quercus</i>	<i>Ulmus</i>	<i>Ilex</i>	<i>Sorbus</i>	<i>Tilia</i>	<i>Cistus</i>	<i>Pinus</i>	<i>Erica</i>	<i>Phillyrea</i>	<i>Crataegus</i>	<i>Rosmarinus</i>	<i>Arbutus</i>	<i>Pistacia</i>	
Northern plateau	SPB1	23	-	17	39	-	30	-	-	-	-	-	-	13	-	-	-	-	-	-	
	SPB2	22	-	14	-	-	50	-	-	-	-	-	-	36	-	-	-	-	-	-	
Sainte-Baume forest	SBA1	100	21	-	11	9	47	-	-	3	9	-	-	-	-	-	-	-	-	-	
	SBA2	178	21	-	5	-	65	-	3	2	1	3	-	-	1	-	-	-	-	-	
	SBA3	67	28	-	6	8	58	-	-	-	-	-	-	-	-	-	-	-	-	-	
	SBA4	98	33	-	9	-	44	-	3	2	-	9	-	-	-	-	-	-	-	-	
	SBA5	114	25	-	5	11	51	-	4	-	-	-	-	4	-	-	-	-	-	-	
	B0	29	26	39	26	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	B1	58	55	-	-	45	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	B2	56	41	-	-	22	5	31	-	2	-	-	-	-	-	-	-	-	-	-	-
	B3	89	-	-	-	98	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	B4	41	24	-	-	13	33	26	-	-	5	-	-	-	-	-	-	-	-	-	-
	B5	18	-	-	-	21	-	42	-	12	12	-	-	-	-	-	-	-	-	-	-
B6	127	22	-	-	-	-	78	-	-	-	-	-	-	-	-	-	-	-	-	-	
B8	121	3	-	-	-	-	96	-	-	1	-	-	-	-	-	-	-	-	-	-	
B9	105	70	2	-	-	1	1	-	-	2	-	25	-	-	-	-	-	-	-	-	
B10	107	2	-	-	2	-	96	-	-	-	-	-	-	-	-	-	-	-	-	-	
Southern exposure	JDF1	139	-	7	-	-	48	12	-	-	-	-	4	-	4	6	5	4	9	3	
	JDF2	143	-	15	-	-	50	8	-	-	-	-	5	-	1	3	6	-	6	6	
	COU1	21	-	52	-	-	24	-	-	-	-	-	-	-	10	-	-	14	-	-	



In the northern plateau, only 45 charcoal pieces were identified, from two soil profiles. These represented four taxa: deciduous *Quercus* at a frequency of 40%, *Pinus* at 25%, *Acer* at 20% and *Juniperus* at 16%. In the Sainte-Baume forest, from the charcoal assemblages of 15 soil profiles, the two most frequent taxa, of the 11 identified, were *Fagus* at 25% and *Taxus* at 15%. Less frequently occurring taxa were *Acer* at 8%, and other taxa such as *Tilia*, *Sorbus* and *Pinus*, each present at less than 3%. Finally, from the charcoal assemblages of the three soil profiles sampled on the southern exposure, the most frequent taxa, among the ten identified, were deciduous *Quercus* at 40% and *Juniperus* at 25%. Less frequently observed taxa were evergreen *Quercus* at 7%, *Rosmarinus* at 6%, *Erica* and *Arbutus* each at 5%, *Crataegus* at 4% and several other taxa at frequencies below 3%.

Cluster analysis of the taxonomic record for each sampled soil profile showed that the soil profiles JDF1 and JDF2 on the southern exposure were the first level of connection, with the third sampled profile on the southern exposure, COU1. Then, the soil profiles on the southern exposure were connected to those from the northern plateau, while only after occurred the connection to the soil profiles of the Sainte-Baume forest (Figure 3). Cluster analysis also indicated that the identified taxa presented variable levels of similarity to one another. Thus, based on these similarities and the autecological characteristics of the identified taxa, successional groups could be formed. A first group, corresponding to the scrub (evergreen) vegetation, included *Crataegus*, *Cistus*, *Pistacia*, *Phillyrea*, evergreen *Quercus* and *Arbutus*, along with *Rosmarinus*, *Erica* and *Ilex*. A second group, corresponding to the pioneer taxa, included *Juniperus* and *Pinus*. A last group, corresponding to the mesophilic taxa, included *Sorbus*, *Tilia*, *Acer*, *Ulmus* and *Taxus*. The only taxa distinct from these groups were *Fagus* and deciduous *Quercus*, which represented most of the charcoal records.

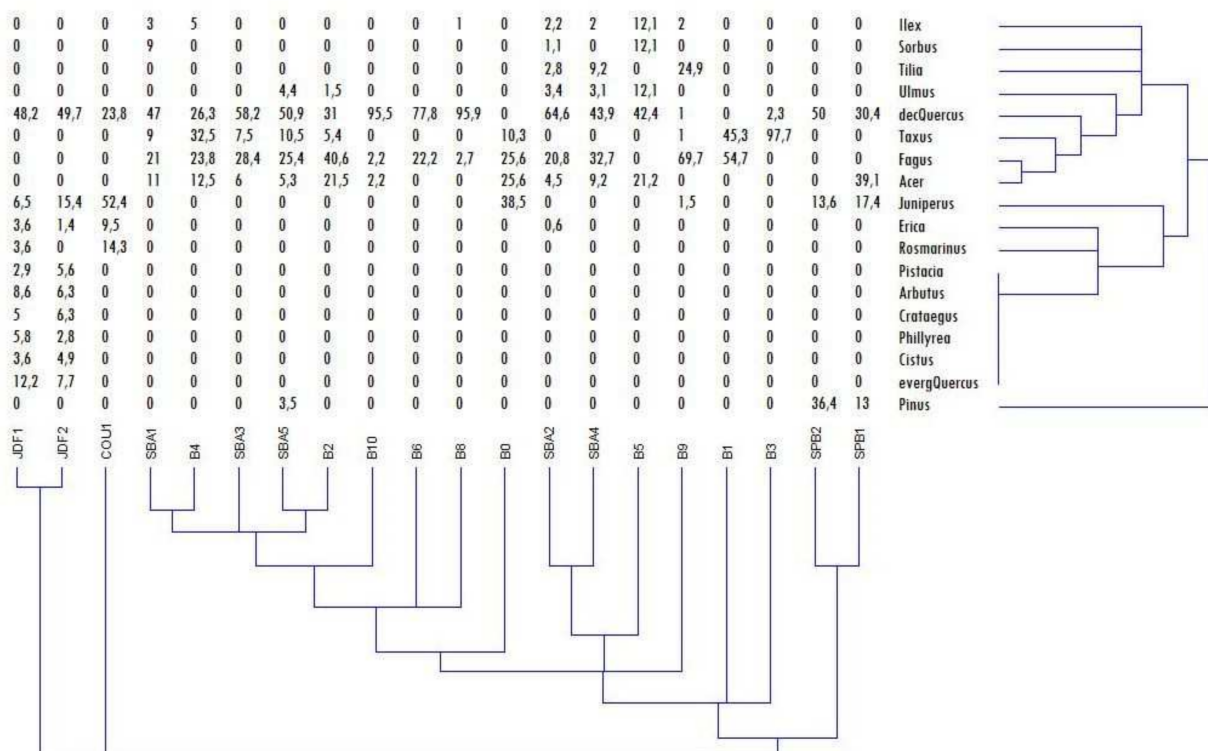
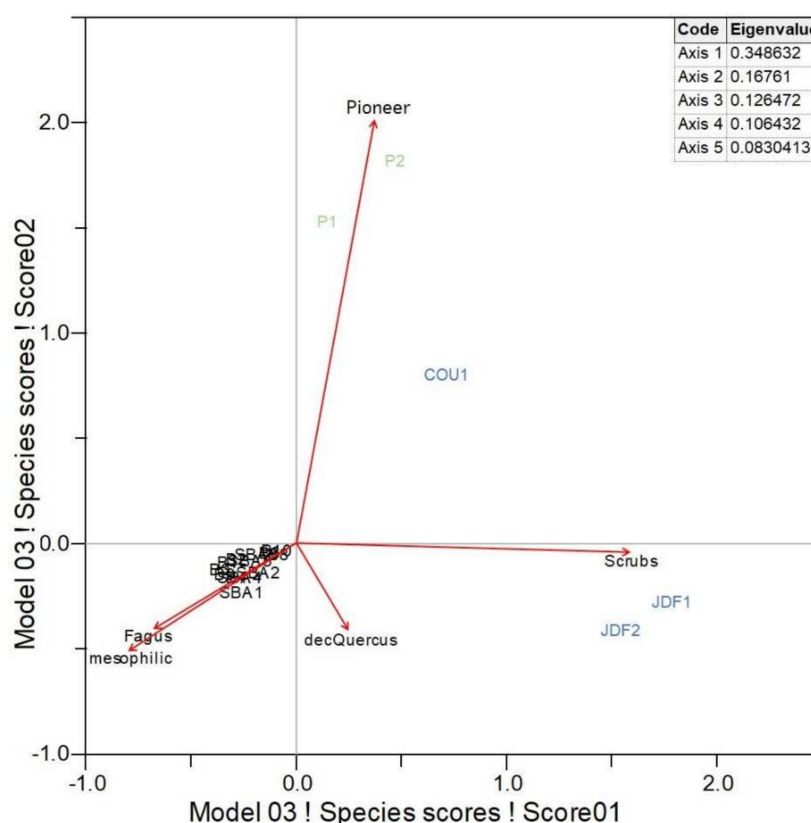


Figure 3. Cluster analysis based on Euclidean similarity measures.

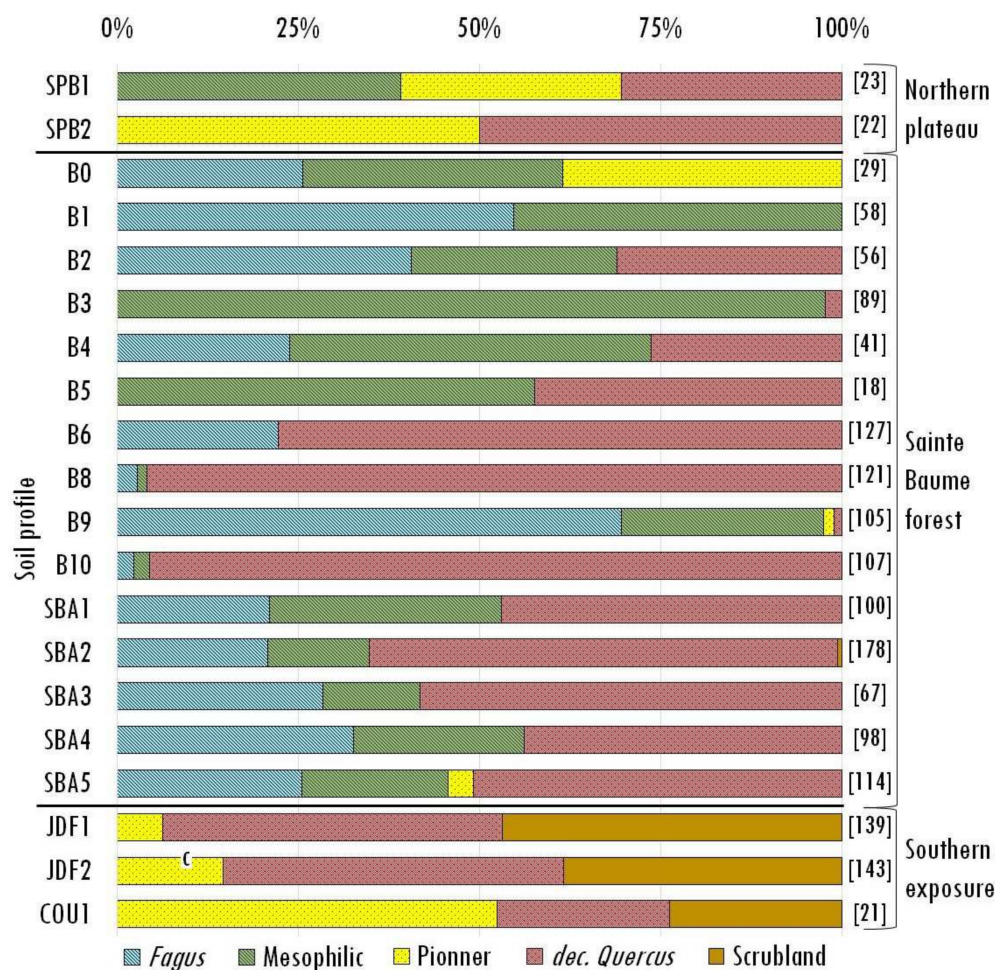
The correspondence analysis of the taxonomic frequencies grouped by successional groups and by *Fagus* and deciduous *Quercus* show a clear pattern for each sampled soil profile (Figure 4). On axis 1 are the sampled profiles that represent taxonomic assemblages with mesophilic affinities, plus *Fagus*, which correspond to those taxa from the Sainte-

Baume forest, distinct from communities representing scrubland vegetation, i.e., those from the soil profiles sampled on the southern exposure. On axis 2 are the sampled profiles that represent taxonomic assemblages with mesophilic characteristics, as well as *Fagus*, which correspond to those taxa from the Sainte-Baume forest, compared to communities representing pioneer taxa from the northern plateau. Moreover, the eigenvalues of the correspondence analysis were relatively low. The axis 1 results explain about 35% of the variability in the database, whereas the axis 2 results explain about 17% of the variability in the database. This appears to be in agreement with the fact that the two different set of soil profiles, those from the northern plateau and those from the southern exposure, each represented by only a few samples, constitute the major part of the data heterogeneity, compared with the large set of soil profiles from the Sainte-Baume forest.



**Figure 4.** Correspondence analysis of the frequencies of taxa according to sampled soil profiles for all three ecological units (blue: southern exposure; black: Sainte-Baume forest; green: northern plateau) related to the successional groups (pioneer: *Juniperus* and *Pinus*; scrubland: *Ilex*, *Rosmarinus*, *Crataegus*, *Cistus*, *Pistacia*, *Phillyrea*, evergreen *Quercus*, *Arbutus* and *Erica*; mesophilic: *Sorbus*, *Tilia*, *Acer*, *Ulmus* and *Taxus*) and the two most frequent taxa (*Fagus* and deciduous *Quercus*).

Finally, we can observe that the taxa, even when grouped into successional/ecological groups, vary in their frequency depending on the ecological unit under consideration. *Fagus* is recorded exclusively in the samples from the Sainte-Baume forest, whereas the other mesophilic taxa are present in the Sainte-Baume forest and a little on the northern plateau. The pioneer taxa are mainly present on the northern plateau and the southern exposure, with only a low frequency in the Sainte-Baume forest. The scrubland vegetation is present only on the southern exposure, whereas, in contrast, deciduous *Quercus* is present in significant quantities in all three ecological units investigated (Figure 5).



**Figure 5.** Taxon frequency in the identified soil charcoal assemblages, represented by taxonomic groups with different successional stages (pioneer: *Juniperus* and *Pinus*; scrubland: *Ilex*, *Rosmarinus*, *Crataegus*, *Cistus*, *Pistacia*, *Phillyrea*, evergreen *Quercus*, *Arbutus* and *Erica*; mesophilic: *Sorbus*, *Tilia*, *Acer*, *Ulmus* and *Taxus*), except that the two most frequent taxa are represented separately (*Fagus* and deciduous *Quercus*).

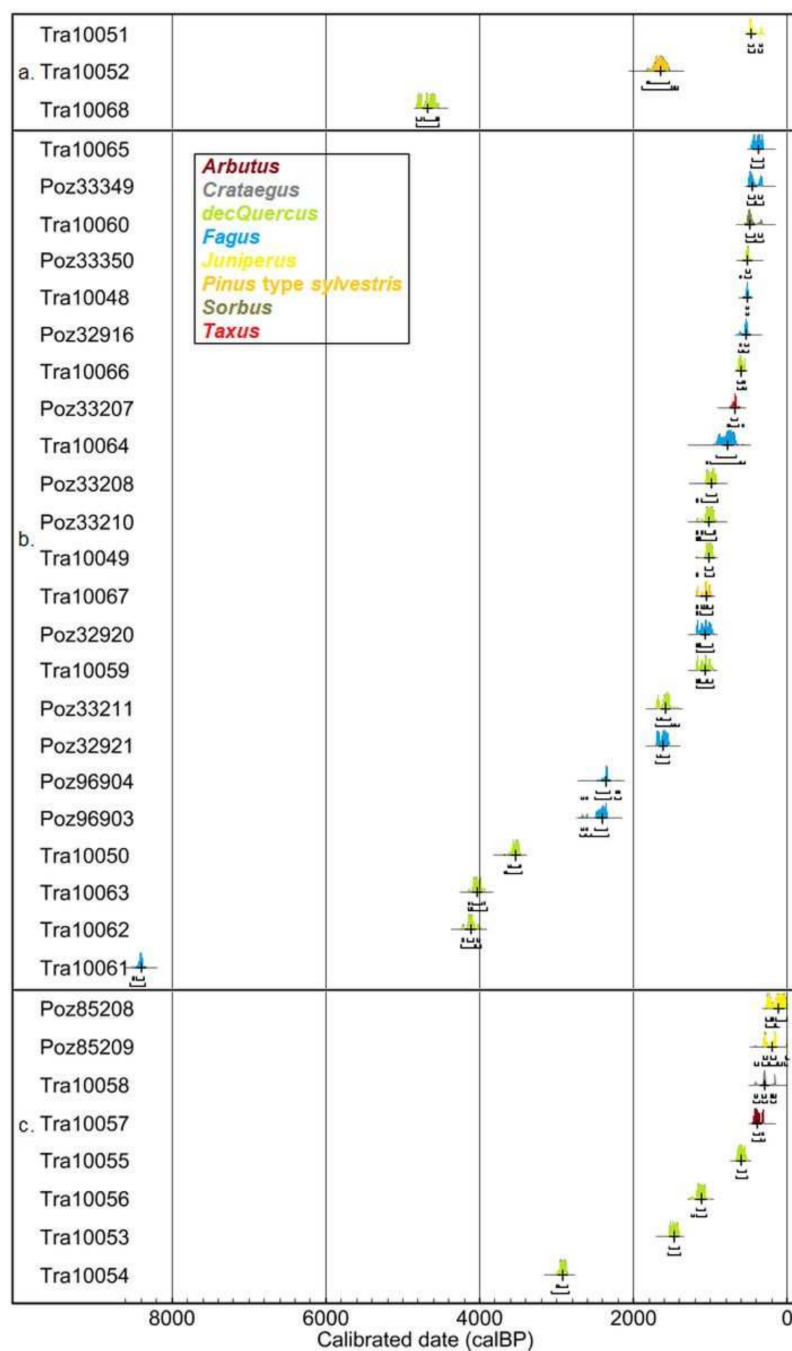
### 3.3. Chronological Analysis

A total of 34 charcoal pieces were dated by the AMS radiocarbon method. These charcoal pieces were selected from 15 of the 20 soil profiles sampled from the study area. This C dating includes three dates from two soil profiles from the northern plateau, twenty-three dates from eleven soil profiles from the Sainte-Baume Forest and eight dates from three soil profiles on the southern exposure. These dated charcoal pieces represented 15 deciduous *Quercus*, ten *Fagus*, three *Juniperus*, two *Pinus*, one *Taxus*, one *Sorbus*, one *Crataegus* and one *Arbutus* (Table 3).

**Table 3.** Dates of charcoal pieces obtained by  $^{14}\text{C}$  accelerator mass spectrometry (AMS) calibrated with a two-standard-deviation ( $2\sigma$ ) 95% confidence interval on the OxCal program [60] with the IntCal20 dataset [61].

Units of Investig.	Lab. Ref.	Profile	Taxa	Conv. Age BP $\pm$ Error	Cal. Age BP	Cal. Age BCE/CE
Northern plateau	Tra10051	SBP1	<i>Juniperus</i>	389 $\pm$ 16	504-334	1446-1616 CE
	Tra10052	SBP1	<i>Pinus</i> type <i>sylvestris</i>	1770 $\pm$ 59	1825-1553	125-398 CE
	Tra10068	PDA1	deciduous <i>Quercus</i>	4129 $\pm$ 21	4815-4549	BCE 2866-2600
Sainte-Baume forest	Tra10060	SBA1	<i>Sorbus</i>	426 $\pm$ 40	534-325	1416-1625 CE
	Tra10059	SBA1	deciduous <i>Quercus</i>	1162 $\pm$ 26	1177-987	774-963 CE
	Tra10062	SBA1	deciduous <i>Quercus</i>	3755 $\pm$ 20	4224-4000	BCE 2275-2051
	Tra10063	SBA1	deciduous <i>Quercus</i>	3692 $\pm$ 20	4090-3973	BCE 2141-2024
	Tra10061	SBA1	<i>Fagus</i>	7620 $\pm$ 31	8509-8372	BCE 6560-6423
	Tra10065	SBA3	<i>Fagus</i>	333 $\pm$ 18	465-311	1486-1639 CE
	Tra10066	SBA3	deciduous <i>Quercus</i>	600 $\pm$ 15	646-548	1304-1403 CE
	Tra10064	SBA3	<i>Fagus</i>	851 $\pm$ 85	930-666	1021-1285 CE
	Tra10067	SBA3	<i>Pinus</i> type <i>sylvestris</i>	1155 $\pm$ 14	1174-987	776-963 CE
	Tra10048	SBA4	<i>Fagus</i>	494 $\pm$ 16	537-510	1414-1441 CE
	Tra10049	SBA4	deciduous <i>Quercus</i>	1126 $\pm$ 15	1062-979	889-971 CE
	Tra10050	SBA4	deciduous <i>Quercus</i>	3329 $\pm$ 23	3632-3480	BCE 1683-1531
	Poz32916	BAU1	<i>Fagus</i>	525 $\pm$ 30	629-509	1322-1442 CE
	Poz33207	BAU1	<i>Taxus</i>	750 $\pm$ 35	733-658	1217-1292 CE
	Poz33208	BAU2	deciduous <i>Quercus</i>	1085 $\pm$ 35	1060-932	891-1019 CE
	Poz32920	BAU2	<i>Fagus</i>	1160 $\pm$ 30	1177-983	773-968 CE
	Poz33210	BAU3	deciduous <i>Quercus</i>	1115 $\pm$ 35	1173-935	778-1015 CE
	Poz32921	BAU6	<i>Fagus</i>	1725 $\pm$ 30	1706-1562	245-389 CE
	Poz96903	BAU7	<i>Fagus</i>	2385 $\pm$ 35	2680-2342	BCE 731-393
	Poz33349	BAU8	<i>Fagus</i>	390 $\pm$ 30	510-320	1441-1631 CE
Poz33350	BAU8	deciduous <i>Quercus</i>	480 $\pm$ 30	543-498	1408-1452 CE	
Poz33211	BAU8	deciduous <i>Quercus</i>	1690 $\pm$ 35	1698-1531	253-419 CE	
Poz96904	BAU10	<i>Fagus</i>	2340 $\pm$ 35	2485-2213	BCE 536-264	
Southern exposure	Tra10058	JDF1	<i>Crataegus</i>	249 $\pm$ 26	426-0	1524-0 CE
	Tra10057	JDF1	<i>Arbutus</i>	309 $\pm$ 14	432-306	1519-1645 CE
	Tra10055	JDF1	deciduous <i>Quercus</i>	599 $\pm$ 39	655-539	1295-1411 CE
	Tra10056	JDF1	deciduous <i>Quercus</i>	1208 $\pm$ 18	1219-1066	732-884 CE
	Poz85209	JDF2	<i>Juniperus</i>	225 $\pm$ 30	310-0	1640-0 CE
	Tra10053	JDF2	deciduous <i>Quercus</i>	1608 $\pm$ 23	1553-1415	397-536 CE
	Tra10054	JDF2	deciduous <i>Quercus</i>	2821 $\pm$ 21	2976-2861	BCE 1027-912
	Poz85208	COU1	<i>Juniperus</i>	120 $\pm$ 30	272-11	1679-1940 CE

*Fagus* was the oldest dated taxon, with a date from the Mesolithic period, while other samples were dated at different periods during the late Holocene. Deciduous *Quercus* was dated during the late Neolithic period/Bronze Age and the Middle Ages/Modern times, but with a gap from the late Bronze Age to Antiquity. The evergreen taxa (i.e., *Pinus*, *Juniperus* and *Arbutus*), as well as the pioneer taxa (i.e., *Crataegus* and *Sorbus*) were dated only from the late Holocene, with an increasing frequency from the Middle Ages to Modern times. Finally, the one date obtained for *Taxus* was from the late Middle Ages (Figure 6).



**Figure 6.** Age distribution of the dated charcoal pieces from the study area for each ecological unit sampled (a) northern plateau; (b) Sainte-Baume forest; and (c) southern exposure) according to dates obtained from  $^{14}\text{C}$  accelerated mass spectrometry (AMS) calibrated with a two-standard-deviation ( $2\sigma$ ) 95% confidence interval on the OxCal program [60] with the IntCal20 dataset [61].

## 4. Discussion

### 4.1. Origin of the Sainte-Baume Forest

Our chronological database indicates that the oldest local record of vegetation is for *Fagus* at c. 8000 years before present (BP) (Table 3). This record proves that *Fagus* had been present in the Sainte-Baume forest (dated from soil profile SBA1; Figure 1c) since the end of the early Holocene. At a regional level, *Fagus* is reported at a low frequency during the early Holocene [66–68]. Nevertheless, some evidence for its ancient presence has been identified locally [69–71], supporting its record at the Sainte-Baume forest at

around 8000 years BP. Such an ancient presence of *Fagus*, locally, might result from its early spread from cryptic glacial refuges in western Europe [72–75]. Subsequently, no more chronological evidence was recorded for *Fagus* until the late Holocene. This might indicate that *Fagus* was not locally present anymore because of forest disturbances, possibly due to human activities, as is the case for most of the forests of the Mediterranean basin in the mid-Holocene [10–12]. However, regional records suggest that *Fagus* might have remained present in the long-term. Furthermore, we observed a gap without any dates for any taxa in our chronological database covering nearly the entire mid-Holocene (Figure 6). Thus, *Fagus* has probably remained locally present in the Sainte-Baume forest since the end of the early Holocene, but no fire disturbances occurred to provide charcoal records of this taxon between c. 8000 and c. 2500 years BP.

In addition to *Fagus*, several other taxa with mesophilic affinities were dated in this study, namely the records of two charcoal pieces of *Taxus* and one of *Sorbus*, all from the ecological unit of the Sainte-Baume forest. These charcoal pieces were dated back to the late Holocene (Table 3). The presence of *Taxus* supports several additional sources of evidence for this taxon in the region over the same period, related notably to late Neolithic settlements [76–78]. These late Holocene records are in accordance with our record of *Fagus* from the same period. Taken together, these records indicate the presence of a mesophilic mixed forest since at least the late Holocene on the site of the Sainte-Baume forest.

Thus, according to our chronological evidence, the origin of the Sainte-Baume forest goes back to Roman development during Antiquity or to the establishment of a monastery on the area during the High Middle Ages (AD 1000 to 1250), as had been postulated previously [24,79–81].

Deciduous *Quercus* were also commonly present in the charcoal assemblages from all the sampled ecological units (Figure 5). This deciduous oak is very likely to be *Quercus pubescens*, since this is the only Mediterranean deciduous oak species present in the calcareous soils of Provence. Its presence at a regional level in the Mediterranean landscapes has been attested to since at least the mid-Holocene [69,71,82]. This is in agreement with our record for this taxon, which has been locally present since c. 4500 years BP on the northern plateau, since c. 4000 years BP in the Sainte-Baume forest and since c. 3500 years BP on the southern exposure, with relatively frequent occurrences subsequently until Modern times (Figure 6). Therefore, we postulate that deciduous *Quercus*, most likely *Q. pubescens*, was present, since the mid Holocene, in the Sainte-Baume forest, possibly in combination with *Fagus* and other taxa with mesophilic affinities. Moreover, deciduous *Quercus* was also present in the areas surrounding the Sainte-Baume forest, as confirmed by the dates we obtained for this taxon from charcoal samples from the northern plateau and the southern exposure of the Sainte-Baume forest, dating from the mid- to the late Holocene (Figure 6, Table 3).

#### 4.2. The Long-Term Trajectory of the Sainte-Baume Forest and Its Surroundings

Analysis of the charcoal assemblages from the study area clearly indicates different taxa comprising the vegetation from the various ecological units sampled (Figures 3 and 4).

The samples from the Sainte-Baume forest show taxonomic groupings closely resembling the present-day vegetation in the area, of a mesophilic mixed forest. Thus, it seems that the ecological trajectory of the Sainte-Baume forest did not change in the long term, or did not change sufficiently to induce significant changes in forest composition. Indeed, the presence of pioneer taxa in the local charcoal assemblages, such as *Juniperus* and *Pinus* (Figure 5), indicates the occurrence of on-site fire disturbances. This is supported by the frequent presence of colluvial layers in the soil profiles of the Sainte-Baume forest, which contained most of the charcoal records (Figure 2), and indicate the well-recognized coupled mechanisms of fire and post-fire erosion [57,59,83]. However, these disturbance events did not generate large forest openings, thereby preventing large-scale soil erosion and subsequent significant changes in the long-term forest trajectory.

The charcoal assemblages of the samples from the northern plateau show little evidence of mesophilic taxa but more pioneer taxa (Figure 5). This finding is consistent with regional forest openings and crop development since the Neolithic period in the Mediterranean regions [12,84,85]. However, the number of sampled soil profiles in the northern plateau is too low to exclude the possible occurrences of other vegetation types in that area. For the more recent past, written sources assert that forest harvesting occurred on the northernmost belt of the Sainte-Baume forest during the French Revolution [24,37]. These forest openings might explain the shift from the mesophilic forest to the pioneer forest on the northern plateau, resulting today in a mixed deciduous *Quercus/Pinus* forest. However, the only mesophilic taxon found on the northern plateau is *Acer* spp. This taxonomic record might correspond to a Mediterranean *Acer* such as *Acer monspessulanum*, which is present on-site today. The post-pioneer autecology of *Acer* spp. [86] might also explain its presence on the northern plateau, in pioneer-dominated charcoal collections.

The record of deciduous *Quercus* (cf. *Q. pubescens*) in the charcoal assemblages on the northern plateau, as well as in those from the Sainte-Baume forest and the southern exposure, indicates the great importance of this tree over the entire study area. This agrees with the report that *Q. pubescens*-dominated woodlands constituted a considerable part of the Mediterranean landscapes in the past [10–12], at least until they became heavily degraded by human activities, such as pastoralism, combined with anthropogenic fires, since the Neolithic, and especially during the Roman Age and onward [13,84,87,88]. Therefore, it is particularly interesting to note that our chronological database and our charcoal taxonomic analysis assemblages indicate that deciduous *Quercus/Q. pubescens* remains present in the long-term forest trajectory of the Sainte-Baume forest and its surroundings until today. Deciduous *Quercus/Q. pubescens* is, however, absent today from the southern exposure of the Sainte-Baume forest. Nevertheless, it is notably present in the historical local charcoal collections (Figure 5), with the chronological data indicating its presence on-site at least c. 500 years ago, and far back as c. 3500 years BP (Table 3). The latter, more ancient, record for this taxon on the southern exposure appears later than those of the Sainte-Baume forest and the northern plateau (Table 3), and even than the Holocene dynamics of this taxon [69,71,82]. The locally strong erosive processes associated with local past land use and steep slopes (Figure 1) probably erased the early and mid-Holocene communities at the sampling locations. In addition, the number of sampled soil profiles was low on the southern exposure of the Sainte-Baume forest. Thus, it does not allow the exclusion of other, possibly older, vegetation records. Nevertheless, on this latter ecological unit, the taxonomic composition of the charcoal assemblages was very similar to the current local vegetation, except for deciduous *Quercus*. This is a clear, semi-open woodland, with xeric, pioneer/post-pioneer vegetation, with, in the recent past (c. 500 years ago), the presence of deciduous *Quercus/Q. pubescens*. This fits with the “classical” Mediterranean long-term vegetation dynamics following land use, soil erosion and climate peoration [13,84,87,88].

#### 4.3. The Mechanism(s) of Survival of the Old-Growth Sainte-Baume Forest

Our investigations point out that the origin of the Sainte-Baume forest and its long-term trajectory show significant locally specific features, in contrast with the vegetation trajectory of the surrounding area (Figure 1b). Indeed, the charcoal assemblages and their chronology are significantly different from those from the northern plateau and the southern exposure, with the presence of taxa with mesophilic affinities in the Sainte-Baume forest (Figures 5 and 6). The plant communities from the northern plateau and the southern exposure show some similarities in vegetation composition, although they also present some differences, with mostly pioneer species on the northern plateau and evergreen scrub on the southern exposure (Table 2 and Figure 3). This finding is supported by the eigenvalues of the correspondence analysis, which are relatively low (Figure 4). This is in agreement with the fact that the charcoal assemblages from the soil samples from the northern plateau and the southern exposure constitute the major part of the data heterogeneity, even though both sites are represented by only a few samples, compared

with the large set of soil profiles obtained from the Sainte-Baume forest. Therefore, the past vegetation composition and dynamics of the Sainte-Baume forest, since its origin at least c. 3000 years BP and its subsequent long-term trajectory, has been very local, as it is today, as a patch of temperate forest in a matrix of semi-open/open ecosystems.

To explain the survival of this forest patch, several hypotheses have been postulated, related to local abiotic factors or anthropological influences [24,40]. Our investigations suggest that, indeed, local abiotic factors and anthropogenic influences played a significant role in the survival of the Sainte-Baume forest over a long-term ecological trajectory until the present day. It appears that the origin of the forest and its development until the mid-Holocene were probably closely related to the appropriate local abiotic conditions caused by the presence of the cliff. This specific factor was, and still is, favorable for the occurrence of temperate environmental conditions, in contrast to the southern exposure of the Sainte-Baume forest, on which the forest vegetation seems to have been degraded early in time to create open/semi-open states, as has been demonstrated in many areas of the Mediterranean region [10–13].

While the establishment of the monastery during the Middle Ages does not explain the origin of the Sainte-Baume forest, its long-term survival and development is probably a result of the sacred statute that limited the use of forest resources [38]. The existence of a sacred statute permitted the maintenance of the ancient, natural, mesophilic Sainte-Baume forest as the remarkable old-growth forest it is today.

However, the charcoal record and colluvial layers from within the Sainte-Baume forest (Table 1 and Figure 2) attest to the fact that the forest was subjected to regular local fires and soil erosion events. The chronology of these local events of forest disturbances (Table 3 and Figure 6) fit well with the strong phase of human impact on the Mediterranean ecosystems which occurred during the late Holocene [87–90]. However, in the Sainte-Baume forest, these forest disturbances did not cause the opening-up of the forest and subsequent soil degradation, and thus did not cause changes in vegetation composition in the long term. Therefore, we postulate that the past forest disturbances in the Sainte-Baume forest were only local (i.e., at stand scale) and occurred with a high return interval. This was due probably to the fact that the Sainte-Baume forest exhibited (1) a high ecological resistance to disturbances, at a large scale, thanks to the protection from the monastery, and (2) a high resilience at the local scale thanks to the favorable local environmental conditions caused by the presence of the cliff.

## 5. Conclusions

The paleoecological approach, based on local indicators, applied to the current study permitted the identification of the origin of the Sainte-Baume Forest as being in the mid-Holocene, at least. Moreover, in the same time window, we show that the surroundings of the forest area, at least the northern plateau and the southern exposure of the Sainte-Baume forest, were in very different states in terms of vegetation composition, corresponding to degraded forest landscapes. Thus, it is clear that, if the modern old-growth Saint-Baume forest is a relict patch of forest with mesophilic affinities, its origin is related to very old natural vegetation dynamics, and its establishment from the mid-Holocene to the late Holocene is probably related to a local specific factor, namely the influence of the cliff. This local relief also played a role in the long-term survival of the Sainte-Baume forest, as a mixed deciduous broadleaf–conifer forest with mesophilic traits. Indeed, our approach allowed us to highlight forest fire disturbances, and the resistance and the resilience of the forest system, which limited the impact of these disturbances on the local scale and over short time periods, without change in the forest state in the long term and on a large scale. In the end, the mechanisms that have conferred such resistance and resilience to the Sainte-Baume forest have been identified as (1) the forest protection thanks to the presence of a monastery since the early Middle Ages that conferred a sacred statute to the forest, and (2) the local temperate environmental conditions on the north side of the cliff.



To conclude, the investigations conducted on this case study of the Sainte-Baume old-growth forest indicate that the survival of the Mediterranean old-growth forests, in the context of the semi-open/open landscapes of the Mediterranean basin, results from a combination of favorable biotic and abiotic factors. Although this combination of factors probably differs locally, it plays a role by limiting the influence of the disturbances which might impact on the forest area, possibly on different temporal scales. In this way, the resistance and the resilience of the forest areas are preserved on a long-term ecological trajectory. Therefore, if it is true that the Mediterranean old-growth forests are of prime importance for the conservation of forest biodiversity, their potential as a baseline reference state is strongly limited by the spatial scale of the biotic and abiotic factors involved as a mechanism to explain the survival of the old-growth forests. Therefore, the potential of the Mediterranean old-growth forests to act as a reference is related to the identification and maintenance of the local biotic and abiotic factors which permit the survival of the old-growth forests.

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