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## Phytolith assemblages as a promising tool for reconstructing Mediterranean Holocene vegetation

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### Abstract

The reliability of phytolith assemblage analysis for characterizing Mediterranean vegetation is investigated in this study. Phytolith assemblages are extracted from modern and buried Holocene soils from the middle Rhône valley (France). The relation between modern phytolith assemblages and the surrounding vegetation, as well as between fossil assemblages and contemporaneous vegetation, already reconstructed through other proxies, is discussed. We demonstrate that the main northwestern Mediterranean biomes are well distinguished by soil phytolith assemblage analysis. In particular, the density of pine and nonconiferous trees (densities expressed relatively to the grass cover) and the overall degree of opening of the vegetation appear well recorded by three phytolith indexes. North Mediterranean vegetation changes during the Holocene period, mainly tree line shifts, pine wood development and deforestation are poorly documented, due to the scarcity of proxy-preserving sites. Phytolith assemblage analysis of soils, buried soils, and sediments appears to be a promising technique to fill this gap.

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**Keywords:** Phytolith; Pine; Paleoenvironment; Mediterranean area; Holocene, Buried soils

### Introduction

The Mediterranean area is a transitional bioclimatic zone between temperate and tropical zones or between humid and arid zones. It is characterized by a staged vegetation (Ozenda, 1964) adapted to summer drought and cool winters. The Rhône valley is located on the French northern side of the Mediterranean basin, below the northern limit of the Mediterranean climatic zone, and at the boundaries of the Medio-European and Alpine climatic influences. This location makes the Rhône valley vegetation highly reactive to climatic changes and anthropic pressure. Moreover, a lot of archeological sites have recently been found in the Rhône valley, during excavations preparing the construction of the

new “TGV-Méditerranée” fast railway line, providing new environmental and archeological data.

The main vegetation changes in the northwestern Mediterranean area during the Holocene period, have been regionally traced through pollen and charcoal studies (De Beaulieu, 1977; Triat-Laval, 1978; Jalut, 1974; Vernet and Thiébault, 1987; Quezel, 1999). However, usual paleobotanical tools reach their limits in these sites.

The occurrence of pine in the Mediterranean vegetation is a marker of natural and anthropic stress. *Pinus sylvestris* is a species often considered as characteristic of mountain stage (Rameau et al., 1994), but its occurrence in southern areas (Tricastin, south Drôme) shows that edaphic factors are probably as determinant as climatic factors in its distribution. In case of weak human pressure, the pine timberline goes down when the climate gets cooler and when soils deteriorate. This colonizing species is a heliophyte and a

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pyrophyte, its spread being favored by fire, whether of natural or human origin (Pons and Thinon, 1987; Trabaud, 1989). As pine scatters great quantities of pollen, its variations in pollen spectra have often only regional scale significance and local variations under punctual pressure are not distinguished. Retracing more precisely the history of pine in this area would mean retracing fire history, in which both climate and human action have a great part to play (Barbero et al., 1990; Bradshaw, 1993; Berger and Thiébaud, 2002).

Pollen-preserving sites are scarce, especially for recent Holocene times (Argant, 1990; Argant in Berger, 1996). Since they only trace woody plants, charcoal records show limitation in detecting forest/grassland shifts in response to human or natural stresses. Moreover, charcoal fragments from archeological sites allow a determination of collected fuel species, but they give no information about the spatial distribution of the various vegetal associations.

These limitations argue for the use of phytolith assemblage analysis as a complementary proxy. Phytolith assemblages, which are well preserved in oxidizing environments such as soils and buried soils, provide information on grass subfamilies (Twiss, 1992) and tree cover density (Alexandre et al., 1997, 1999; Barboni et al., 1999). Phytolith assemblage analysis has been used to trace with accuracy Holocene grassland dynamics and forest–grassland shifts in America, tropical Africa, and Asia (Kurman, 1985; Piperno, 1988; Tsutsuki et al., 1993; Inoue and Sase, 1996; Fredlund and Tieszen, 1997a,b; Alexandre et al., 1997, 1999; Barboni et al., 1999). Moreover, some studies have shown that pine species produce distinctive leaf phytoliths (Klein and Geis, 1978). At last, phytolith density (2.3), size (2–50  $\mu\text{m}$  for morphotypes with taxonomic value), and deposition patterns (they simply fall on the ground without any anemophile or entomophile transport) would argue in favor of distance transportation shorter than that for pollen.

Except for preliminary studies showing that phytoliths are well-represented in alluvial and colluvial deposits as well as in buried soils of the Rhône valley (Alexandre and Meunier, 1997; Delhon, 1998; Verdin et al., 2001), phytolith assemblage analysis has never been applied to the northern Mediterranean area.

Before attempting a reconstruction of vegetation changes, it is essential to investigate first the reliability of modern phytolith assemblages for the recording of vegetation sources. This preliminary study, even if dealing with a reduced number of samples, is the first step toward large paleoenvironmental phytolith-based reconstruction in the French Mediterranean area. Phytolith assemblages are extracted from modern soils and buried Holocene soils from the middle Rhône valley. The relationship between modern phytolith assemblages and the surrounding vegetation, or fossil phytolith assemblages and the contemporaneous regional vegetation, already reconstructed through other proxies, is discussed.

## 1. The middle Rhône valley and the Valdaine basin

The middle Rhône valley (Fig. 1) is located in southeastern France, between the Alps and the Massif Central. The average annual rainfall ranges from 800 to 1200 mm. Precipitation is concentrated mainly over spring and autumn, while summer is affected by typical Mediterranean dryness. While the pluvio-nival river Rhône flows continuously, its tributaries dry out in summer.

The basins of the Roubion and Jabron rivers together form the Valdaine basin (Fig. 2), mainly composed of Quaternary alluvial formations in the center, of marls in the foothills, and of limestone and sandstone on the periphery (Berger, 1996).

Because of three climatic influences (Mediterranean, Medio-European and Alpine), of microclimatic conditions related to various topographies (plains, slopes, hills and terraces), and of strong and ancient human pressure, the middle Rhône valley landscape shows a mosaic of vegetation typical for the supra-Mediterranean stage (Ozenda, 1964, 1994). Forests are oak coppices, dominated by *Quercus pubescens* and pine woods (*Pinus sylvestris*). They usually contain an undergrowth of trees and grasses. Pine woods are the pioneer formations after fire in the area, when oak groves settle only secondly (Pons and Thinon, 1987; Trabaud, 1989). The grasslands result mainly from grazing on the slopes. They consist of grasses belonging to the Festucoideae subfamily of Poaceae and of grazing-resistant dicotyledons. Riverbanks are covered by dense but small riparian formations dominated by *Alnus* and *Fraxinus* in the plains and *Populus* on the foothills.

## 2. Material and methods

### 2.1. Modern samples and local vegetation

As a selective dissolution of phytoliths occurs in litter and soil, reference classification must be based on modern phytolith assemblages from soil humic horizons. Therefore, five soil samples were collected from five spots located under main supra-Mediterranean vegetation formations (Fig. 2).

Sample 1 comes from a sandy-loam alluvial soil (mollic fluvisol according to the American “Soil Taxonomy,” Soil Survey Staff, 1975) located on the bank of the Vermon river. This soil developed under a riparian forest dominated by *Alnus* and *Fraxinus*, with mainly Festucoideae grasses. This forest has been in place since the beginning of the 20th century which is the last period of deep incisions of riverbeds in the area.

Sample 2 comes from a loamy-clay brown to red calcic soil (luvic calcisol) located on a gravely Würmian terrace of the Roubion river. This soil developed under a bicentennial oak grove (*Quercus pubescens*) with a clear grassy undergrowth layer (Festucoideae).

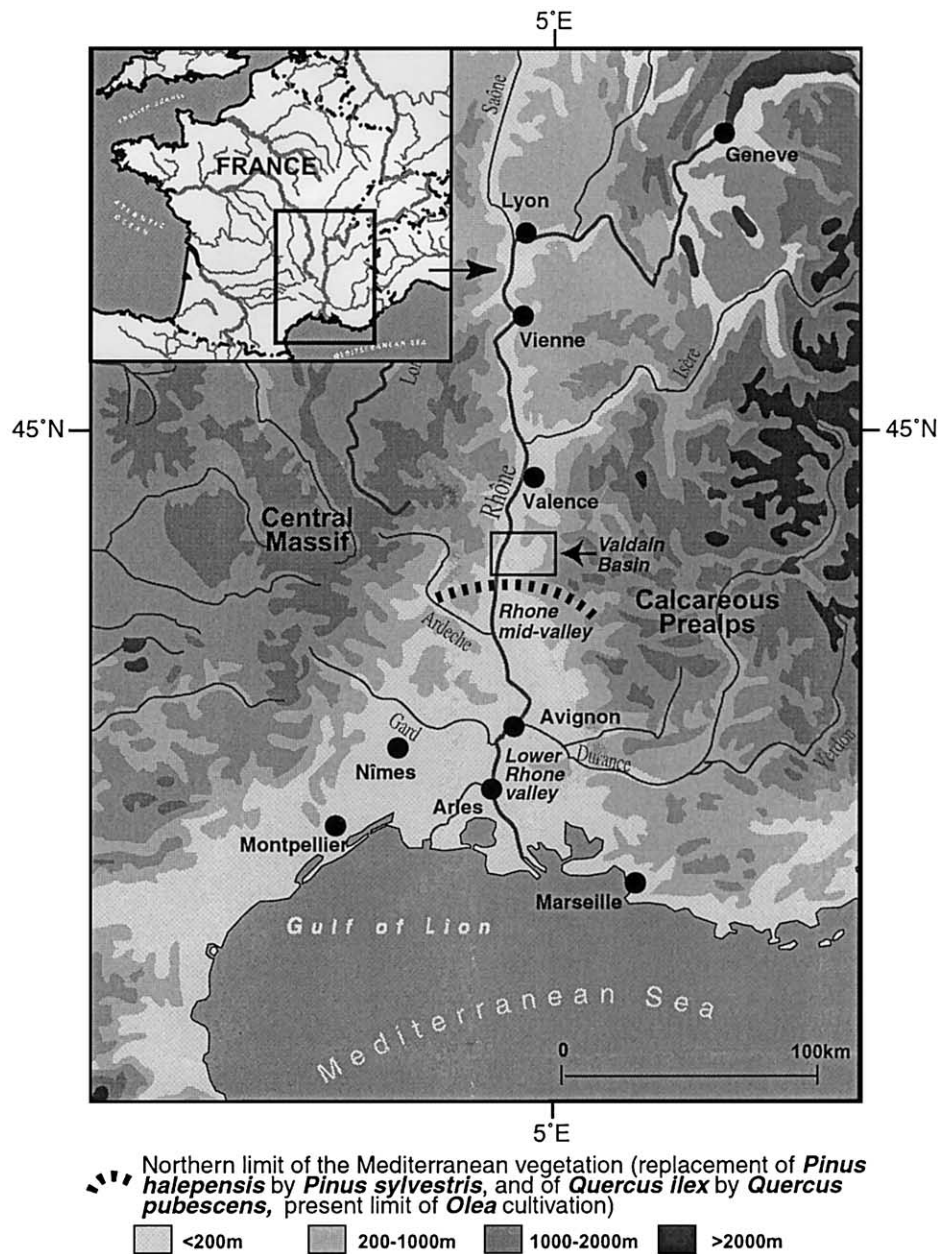


Fig. 1. Location of the middle Rhône valley: the Valdaine Basin and the North limit of the Mediterranean vegetation according to Ozenda (1994).

Sample 3 comes from a calcareous colluvial soil (rendzic leptosol) developed on recently stabilized Bad Lands on the marly south-orientated slopes of the Roynac hill. This soil developed under a grassland (mainly Festucoideae grasses), with scattered pines (*Pinus sylvestris*), that is not currently grazed. This soil should be younger than 100 years as photographs from the beginning of the 20th century show that rills widely marked the slopes, preventing soil development.

Sample 4 comes from a clay regosol (calcaric regosol) located on the marly south-orientated slopes of the Roynac hill. This soil developed under a pine wood (*P. sylvestris*) including some juniper (*Juniperus communis*) and a dense undergrowth grass layer (Festucoideae).

Sample 5 comes from a loamy and deep alluvial soil (eutritic fluvisol) located in the very low alluvial plain at the confluence of the Rhône and one of its tributaries, the Aygues river. This soil developed under a reed (*Phragmites communis*) and grass cover (Festucoideae) around a drainage ditch. As the last sediment deposition occurred during the 19th century, soil and herbaceous vegetation cannot be older than 150 years.

## 2.2. Fossil samples and contemporaneous regional vegetation

Six fossil samples are studied. They come from dated levels of pedosedimentary sequences of three different sites.

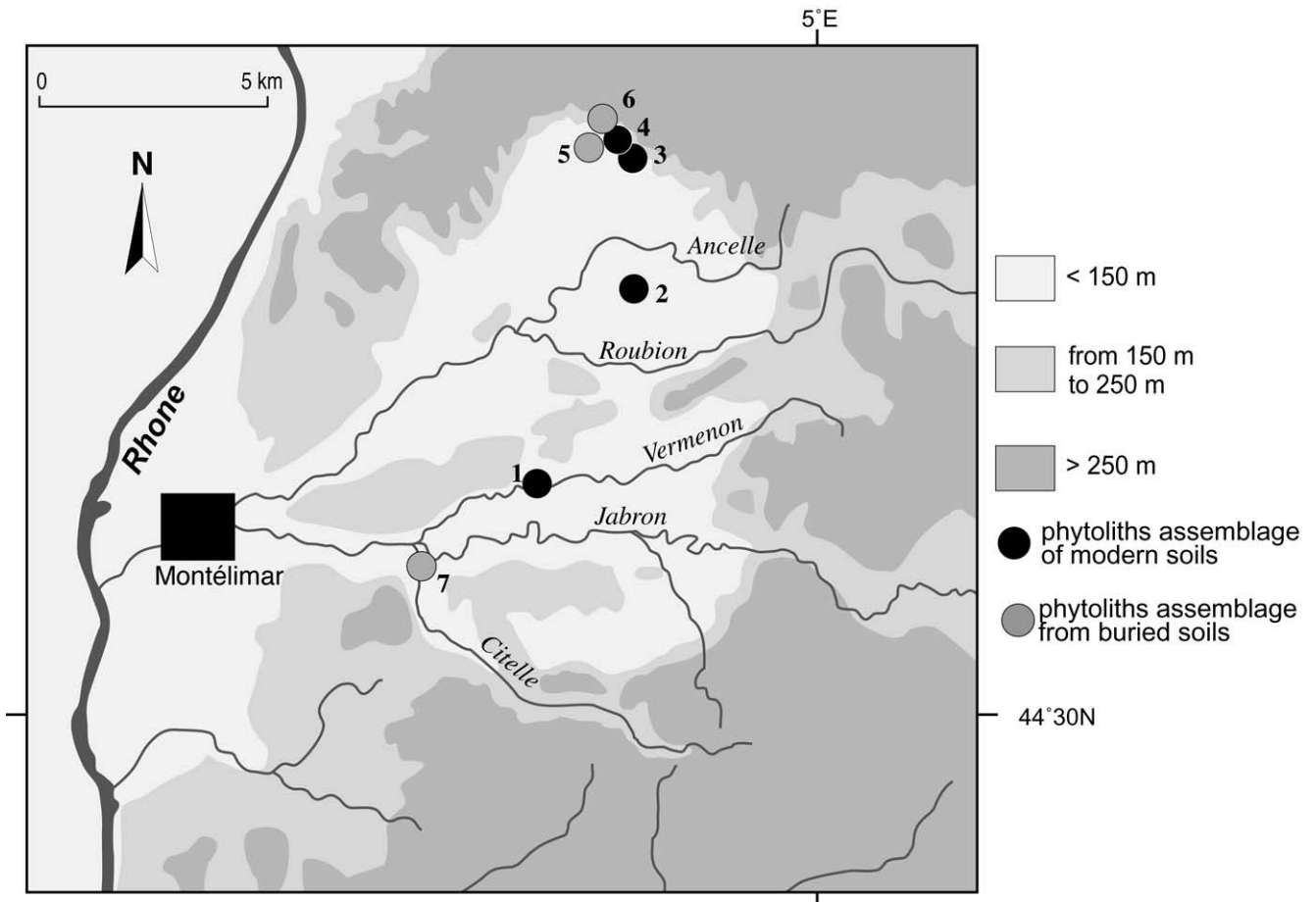


Fig. 2. The Valdaine Basin. Location of the main sampling sites: (1) river forest of Vermenon River at “les feuilles” site, (2) “Andran” forest on gravely Würmian terrace, (3, 4) south slope of marly hills of Roynac, (5) prehistoric settlement of Roynac-le-Serre, (6) prehistoric settlement of Roynac-les-Roches, and (7) prehistoric settlement of Espeluche-Lalo.

The levels formed during Holocene vegetation and morpho-climatic regional phases that are well documented (e.g., Triat-Laval, 1978; Vernet and Thiébault, 1987; Thiébault, 1988; Leveau and Provansal, 1993; Berger, 1996).

Sample 6 comes from a buried soil collected at Roynac-le-Serre (Fig. 2), on the northern slope of the Valdaine basin. It developed ca. 5685  $^{14}\text{C}$  yr B.P. (Table 1), during

the Neolithic period of the area (around 6500 to 3800 yr B.P.). This period is characterized by the development of agro-pastoral practices that have since contributed to the extension of the opening of Atlantic oak forests.

Samples 7a and 7b come from a black buried soil and an alluvial deposit located at Roynac-les-Roches (Fig. 2). They postdate an early Holocene layer ( $9845 \pm 90$   $^{14}\text{C}$  yr B.P./

Table 1  
Radiocarbon dates cited in the text

Site	Reference	Structure	Phase	Material dated	Matrix/context	$^{14}\text{C}$ yr B.P.	cal yr B.C.: 2 sigmas
Lalo	AA 32644	St. 8	17		Pit sediment	$6220 \pm 60$	5320–4990
	AA32639	St.73	10		Pit sediment	$6420 \pm 65$	5490–5260
	AA 24897	42	9b	Charcoal layer	Channel silt	$6705 \pm 60$	5720–5480
	ETH 14840	Drilling	6b	Charcoal layer	Alluvial palaeosoil	$6930 \pm 70$	5990–5660
	AA 24898	64b	5	Isolated charcoal	Alluvial palaeosoil	$7535 \pm 65$	6470–6230
Roynac-les-Roches	ETH15401	C5, 2,2	Ensemble 26	Charcoal layer	Alluvial silt	$9785 \pm 80$	9500–8605
	ETH1502	C25, d2	Ensemble 26	Charcoal layer	Alluvial silt	$9845 \pm 90$	9710–8735
Roynac-le-Serre	ARC 1443	St.A, soil S9	Phase I2b	Charcoal cluster (hearth)	Alluvial silt	$5685 \pm 100$	4775–4360

9785 ± 80 <sup>14</sup>C yr B.P., Table 1). They are related to the end of charcoal zonation phase 1 (from 11,000 to 8000 yr B.P., Heinz and Thiébault, 1998), characterized by the dominance of *P. sylvestris* and *Juniperus* and to the end of Preboreal pollen subdivision (from 10,300 to 8900 yr B.P., Triat-Laval, 1978), characterized by the dominance of pine pollen. Moreover, pine microcharcoal has been identified in sample 7b (Thiébault, unpublished data). This pine-dominant open vegetation could be linked to cool and dry climatic conditions (Heinz and Thiébault, 1998). Pedological studies show a concentration of microcharcoal and secondary carbonate precipitation in these samples, which indicates a repetition of fire events and high evapotranspiration values (J.-L. Brochier, unpublished data). Epipalaeolithic and Mesolithic lithic items are associated with these pe-dosedimentary formations.

Samples 8a and 8b were collected in the low alluvial plain of the Citelle river, in the southern part of the Valdaine basin, at Espeluche-Lalo (Fig. 2). Sample 8a comes from a clayey-silted buried soil on the bank of a paleochannel. It is located between two sedimentary levels: the lower dated to 6705 ± 60 <sup>14</sup>C yr B.P. (Table 1), and the upper is attributed to an early neolithic settlement (6220 ± 60 <sup>14</sup>C yr B.P., Table 1). The studied part of this sequence was abruptly buried by sediments, due to slope instability ca. 6000 <sup>14</sup>C yr B.P. (after 6930 ± 70 <sup>14</sup>C yr B.P. and between 6420 ± 65 and 6220 ± 60 <sup>14</sup>C yr B.P., Table 1). Microcharcoal fragments extracted from this sample were dicotyledons and belong mainly to *Quercus* sp. (Delhon, 1998). Sample 8b comes from a rubified buried soil developed on late glacial silts deposited on the Wurmian terrace of the Citelle river. It developed contemporaneously with a black buried soil dated to 7535 ± 65 <sup>14</sup>C yr B.P. (Table 1). Microcharcoal fragments found in this sample have been identified mainly as monocotyledons and oak (Delhon, 1998). Sample 8c comes from an alluvial level without pedological features, on the bank of the Citelle river. Multiplicity of its possible vegetation sources will be taken into account when discussing its phytolith assemblage. These three samples are attributed to the pollen Atlantic subdivision (from ca. 8000 to 5000 yr B.P.) and to the charcoal zonation phase 2 (from ca. 8000 to 6000 yr B.P.), both characterized by a dominance of deciduous oak forest (Triat-Laval, 1978; Vernet and Thiébault, 1987; Heinz and Thiébault, 1998). These charcoal and pollen zonations point out the main vegetation trends, but the dominance of deciduous forests does not exclude the persistence of open or less densely wooded areas.

### 2.3. Methods

About 10 g of dry material was analyzed. Phytoliths were extracted from the 2- to 50- $\mu$ m fraction after calcium carbonate removal using HCl, organic matter oxidation using H<sub>2</sub>O<sub>2</sub>, particle size fractionation, and densimetric separation in a ZnBr<sub>2</sub> solution with a density of 2.35 (Kelly, 1990; Fredlund and Tieszen, 1994). Phytoliths were mounted on

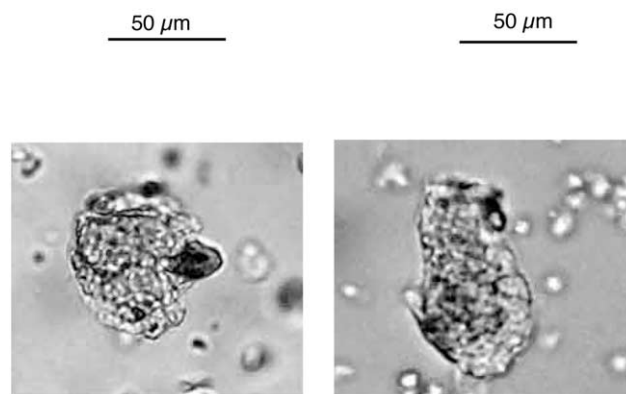


Fig. 3. Microphotographs of modern (left) and fossil (right) spherical-with-sockets pine morphotype. Bar is 50  $\mu$ m.

microscope slides in glycerol, which allows a three-dimensional observation. For each assemblage, more than 200 phytoliths of diameter greater than 5  $\mu$ m were counted, at 500 $\times$  magnification.

Phytoliths were classified into nine morphotypes previously described (Twiss, 1992; Alexandre et al., 1997; Barboni et al., 1999; Runge, 1999): elongate, fan-shaped, point-shaped, rectangle-and-rondel, cross-and-dumbbell, smooth-spherical, rough-spherical, cork-cell like, and silicified vessel. A tenth morphotype was observed. Since it has not been previously described, we named it “spherical-with-sockets” (Fig. 3).

According to Twiss taxonomic classification of grass phytoliths (Twiss et al., 1969; Twiss, 1992), which was later improved by Mulholland (1992), elongate, point-shaped, and fan-shaped morphotypes are produced by the whole Poaceae family. Rectangle and rondel morphotypes are produced in great amount by the Festucoideae subfamily, while cross-and-dumbbell morphotypes are produced mainly by the Panicoideae subfamily. The main exceptions to this pattern, in the Mediterranean area, is the production by *Stipa* (Festucoideae) and *Arundo donax* (Arundinoideae, an introduced humid soils tall grass) of a small amount of cross-and-dumbbell morphotypes.

The rough-spherical morphotype is produced in dicotyledon wood (Geis, 1973; Bozarth, 1992; Scurfield et al., 1974; Laroche, 1976; Piperno, 1988). Some studies called it “circular rugose” (Piperno, 1988; Alexandre et al., 1997, 1999; Barboni et al., 1999), others “rough sphere” or “rough-spherical” (Kealhofer and Penny, 1998; Runge, 1999). In order to emphasize its three dimensional-shape, we opt for the latter nomenclature. Previous studies have shown that the ratio D/P equals to dicotyledon rough- and smooth-spherical morphotypes (D) versus Poaceae morphotypes (P) in a phytolith assemblage was a reliable proxy for the tree cover density in intertropical areas (Alexandre et al., 1997, 1999; Barboni et al., 1999). Nevertheless, the dicotyledon tissue from which smooth-spherical phytoliths originate is still unknown. Silicified vessels are produced by

dicotyledons (Piperno, 1988) but are scarcely found in soil assemblages (Alexandre et al., 1997). This is the reason why this morphotype is not taken into account when calculating D/P ratio. The cork-cell-like morphotype is not used here for ecological interpretation as it is produced by numerous families (Ellis, 1979; Alexandre et al., 1997; Barboni et al., 1999). The unclassified group is composed of phytoliths showing no identifiable shape.

### 3. Results

Both modern and fossil phytolith assemblages show a significant proportion of unclassified phytoliths (from 16.5 to 53.2% of the phytolith sum). Classified morphotypes are similar in modern and fossil samples (except silicified vessels found in only one fossil assemblage, 7b) and are expressed (Table 2 and Fig. 4) as percentages of the sum of classified phytoliths. Confidence intervals have been calculated for each percentage. They can be broad (due to the great quantity of unclassified phytoliths in some assemblages), showing that low percentages must be cautiously discussed. A common point to all assemblages is the strong dominance of rectangle-and-rondel morphotypes, mainly produced by the Festucoideae subfamily, among the grass phytoliths with subfamily significance, while the cross-and-dumbbell morphotypes never surpass 2.3% of the classified phytoliths, which is not an amount significantly different from zero.

#### 3.1. Phytolith assemblages from modern samples (Table 2, Fig. 4)

The grassland phytolith assemblage is characterized by the absence of dicotyledon phytoliths (rough- and smooth-spherical) and the strong dominance of grass morphotypes (93%). Among the latter, the only subfamily significance is borne by the rectangle-and-rondel morphotypes. The spherical-with-sockets morphotype accounts for 6.3%.

The pine-wood phytolith assemblage shows the highest amount of spherical-with-sockets morphotype (20%) among modern assemblages. Grass phytoliths are again abundant (78%), while dicotyledon phytoliths, represented by the rough-spherical morphotype, do not exceed 1% (not significantly different of 0). This assemblage gives a D/P ratio of 0.01.

The oak grove phytolith assemblage presents a high amount of dicotyledon phytoliths: 13.8%, consisting of 12.5 and 1.4% of rough- and smooth-spherical morphotypes, respectively. However, grass phytoliths are still abundant (85.7%). This assemblage gives a D/P ratio of 0.16. Even if the cross-and-dumbbell morphotype reaches its maximum value in this assemblage, its occurrence remains nonsignificant with regard to the confidence interval. No spherical-with-sockets phytoliths were counted in this assemblage.

The riparian-forest phytolith assemblage presents a pro-

portion of dicotyledon phytoliths that is even higher than the previous assemblage (14.5%), and the rough-spherical morphotype is better represented (8.3%) than the smooth-spherical morphotype (6.2%). Grass phytoliths are still abundant (78.2%). The D/P ratio reaches 0.19. The spherical-with-sockets morphotype does not reach 1%.

As for the grassland assemblage, the phytolith assemblage from the reed formation is characterized by a strong dominance of grass phytoliths (97%). Some cross-and-dumbbell morphotypes are represented, but not in statistically representative amounts (1.5%). Dicotyledon phytoliths (here smooth-spherical morphotype) reach only 2.5%. Thus, this assemblage shows a D/P ratio of 0.03 only. The spherical-with-sockets morphotype is not represented.

#### 3.2. Phytolith assemblages from archeological samples

The phytolith assemblages of samples 7a and 7b were produced and deposited contemporaneously with the end of postglacial pine dominance. Both samples are characterized by the two highest proportions of the spherical-with-sockets morphotype (29.8 and 25.9%, respectively). Grass phytoliths account for 59.5 and 52.8%, respectively, and their spherical dicotyledon phytoliths for 1.37 and 0. However, sample 7b is the only one showing a significant proportion of dicotyledon silicified vessels (7.5%).

The phytolith assemblage of sample 6 was deposited during a period of expansion of agro-pastoral practices, whose effects on the vegetal landscape are still under discussion (Beeching et al., 2000). Grass phytoliths are numerous (87%); dicotyledon phytoliths account for 5%, equally divided among rough- and smooth-spherical morphotypes. All the grass phytoliths with subfamily significance come from the Festucoideae subfamily. No spherical-with-sockets phytoliths have been encountered.

Phytolith assemblages 8a, 8b, and 8c were deposited during the Atlantic period of oak forest development. Assemblages 8a and 8b show the two highest D/P ratios: 0.29 and 0.44, respectively. Dicotyledon phytoliths account for 21.47 and 28.48%, respectively, including both rough- and smooth-spherical morphotypes. Grass phytoliths represent 72.8 and 65.2%, respectively, and are dominated by rectangles-and-rondels. Cross-and-dumbbells morphotypes can be due either to the presence of *Stipa* (Festucoideae) or of some Panicoidae (e.g., *Setaria* sp.). The spherical-with-sockets morphotype is absent from assemblage 8a and accounts for 3.5% in assemblage 8b.

Assemblage 8c is extracted from an alluvial layer. The difference of this assemblage compared to 8a and 8b assemblages lies in a far lower D/P ratio (0.10). Dicotyledon phytoliths account for 8.3%, including smooth-spherical (2.9%) and rough-spherical (5.4%) morphotypes. Grass phytoliths account for 84.3%. The spherical-with-sockets morphotype represents 3.9%.

Table 2  
Abundance of the different phytolith morphotypes in modern and fossil assemblages

Hypothesis on taxonomic significance:	Non used			Pinaceae	Dicots			Poaceae			Ratios		
	Morphotypes:	Unclassified <sup>a</sup>	Cork-cells like phytoliths <sup>b</sup>		Silicified vessels <sup>b</sup>	Spherical with sockets <sup>b</sup>	Smooth spherical <sup>b</sup>	Rough spherical <sup>b</sup>	Elongate, point and fan-shaped <sup>b</sup>	Rectangle and rondel <sup>b</sup>	Cross and dumbbell <sup>b</sup>	D/P <sup>c</sup>	Pi/P <sup>d</sup>
Modern samples													
Riparian forest	1	35	6.2	0	0.5	6.2	8.3	19.7	58.6	0	0.19	0	0.19
Oak grove	2	16.5	0	0	0	12.4	1.4	45.6	37.8	2.3	0.16	0	0.16
Grassland	3	41.1	0.5	0	6.3	0	0	14.9	78.4	0	0	0.07	0.07
Pine wood	4	35.6	1	0	20	1	0	5.4	72.7	0	0.01	0.26	0.27
Reed formation	5	36.7	0.5	0	0	2.5	0	20	75.5	1.5	0.03	0	0.03
Fossil samples													
Roynac-le-Serre (grassland expansion)	6	80	8	0	0	2.5	2.5	71	16	0	0.06	0	0.06
Roynac-les-Roches (pine forest expansion)	7a	27.4	5.2	0	29.8	0	1.4	42.1	17.4	0	0.02	0.50	0.52
	7b	53.2	13.9	7.5	25.9	0	0	45.3	7.5	0	0	0.49	0.49
Espeluche-Lalo (oak wood expansion)	8a	46.5	4.7	0	0	5	16.4	34.6	38.3	0	0.29	0	0.29
	8b	41.7	2	0	3.5	25.9	3	29.3	35.3	0.5	0.44	0.05	0.50
	8c	37	3.4	0	3.9	2.9	5.4	16.7	67.7	0	0.10	0.05	0.15

<sup>a</sup> In percentage of the phytolith sum (all the phytoliths observed are taken into account).

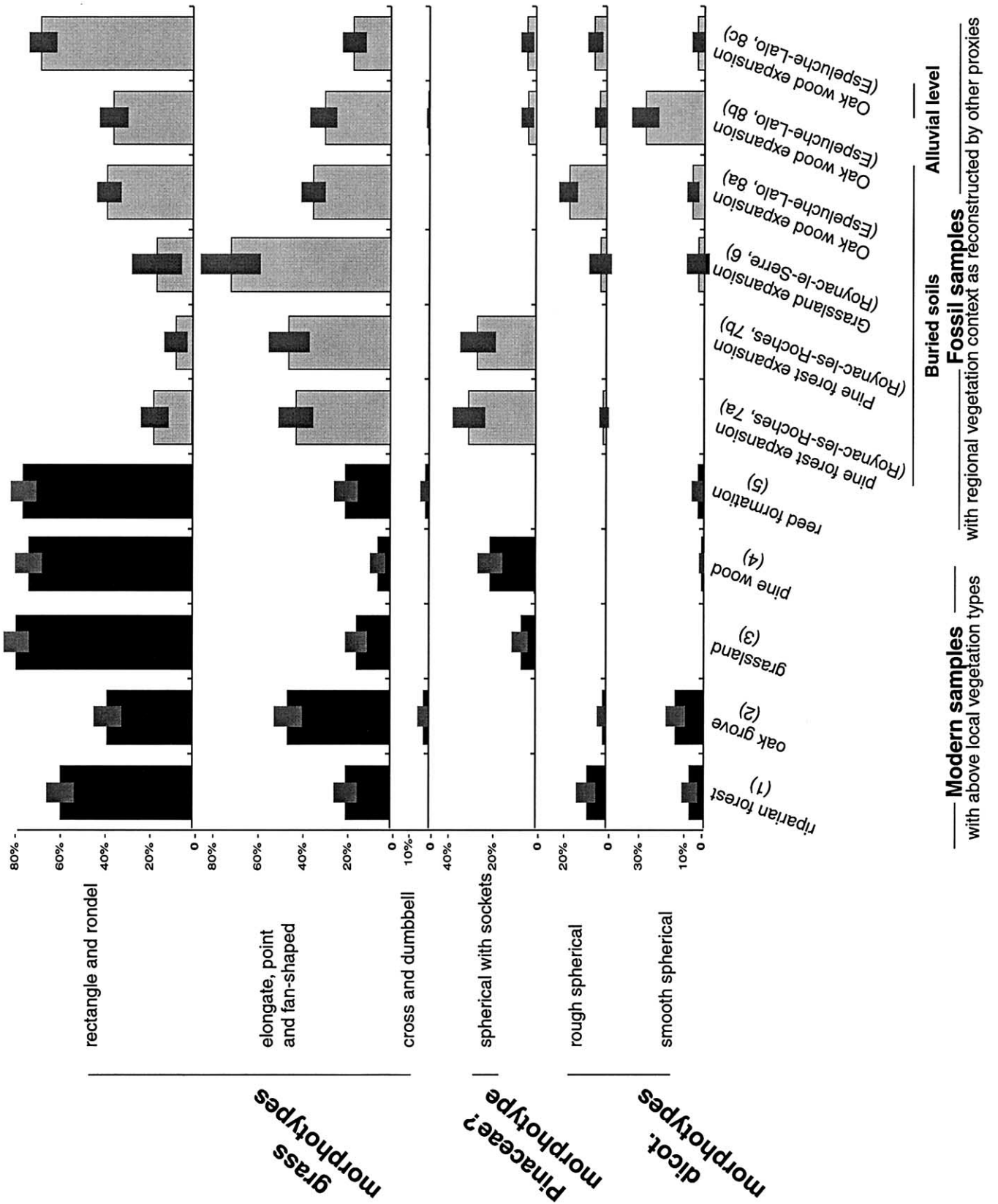
<sup>b</sup> In percentage of the classified phytolith sum (only significant morphotypes are taken into account).

<sup>c</sup> Ratio of Dicotyledon *versus* Poaceae phytoliths: D/P

<sup>d</sup> Ratio of Pinaceae *versus* Poaceae phytoliths Pi/P

<sup>e</sup> Ratio of Tree *versus* Poaceae phytoliths: T/P





## 4. Discussion

### 4.1. Toward a new phytolith index tracing broad-leaved tree cover density for the northwest Mediterranean area

The D/P ratio equal to dicotyledon rough- and smooth-spherical morphotypes (D) versus Poaceae morphotypes (P) proved to be a reliable proxy of the tree cover density in tropical areas (Alexandre et al., 1997, 1999; Barboni et al., 1999).

In the present case, where gymnosperms can be well represented, investigation of relationships between D/P ratios and vegetation sources demonstrate that this phytolith index is a proxy of the broad-leaved tree cover density. Two types of assemblages can be distinguished by their D/P ratio (Table 1 and Fig. 5): The first shows D/P ratios higher than 0.1. It includes modern assemblages 1 and 2 collected under dicotyledon forests and fossil assemblages 8a and 8b contemporaneous with oak forest development. Thus, high D/P ratios accurately record high broad-leaved tree cover density.

The second group shows D/P ratios far lower than 0.1. It includes modern assemblage 3 produced by grassland, modern assemblage 4 produced by pine wood, modern assemblage 5 related to reed formations, fossil assemblage 6 related to the Neolithic period, and fossil assemblage 7a related to the pine forest expansion phase. Low D/P ratios accurately trace a low representation of dicotyledon trees. Four assemblages among the five showing low D/P ratios contain statistically nonreliable proportions of dicotyledon phytoliths, which prevent more precise comparisons between them. Fossil assemblage 8c, recovered from an alluvial level contemporaneous with the oak forest development, shows a D/P ratio of 0.10. Because it has not been extracted from a buried soil, this assemblage is discussed below.

The D/P value of 0.1, used here as a lower limit to distinguish dicotyledon forest records from other records, is far lower than the previously published values of 7.0, 0.6, and 1.5, respectively, related to a semi-evergreen forest in Congo (Alexandre et al., 1997), a riparian forest in Ethiopia (Barboni et al., 1999), and a transition zone between a tree savana and a mesophytic forest in Brazil (Alexandre et al., 1999). Tropical broad-leaved forests are denser than Mediterranean forests and the D/P ratio appears accurate enough to express this difference.

### 4.2. Toward a new phytolith index of pine cover density for the northwest Mediterranean area

Phytolith assemblages deposited under modern pine woods and during the Holocene pine expansion (assem-

blages 7a and 7b) are characterized by a high representation of the spherical-with-sockets morphotype, while this morphotype is absent or scarcely represented in other assemblages. The following discussion assesses the reliability of this morphotype for tracing pine cover.

Pine phytoliths have already been observed, in situ, in needles (Klein and Geis, 1978; Bartoli, 1981). However, observation scales varied and the spherical-with-sockets morphotype could not be surely recognized from published pictures. Our investigation of gymnosperm needle phytoliths from the CEPAM-CNRS (Sophia Antipolis, France) reference collection showed that a Pinaceae, *Picea abies*, produces phytoliths presenting a spherical shape and bearing a special honeycombed ornamentation, similar to the spherical-with-sockets morphotype present in our modern and fossil assemblages (Fig. 3). *Picea abies* is not represented in the area, but pine is a close relative. However, redundancy is inherent to phytolith production and there is no evidence that gymnosperms other than Pinaceae may not produce the same spherical-with-sockets morphotype. In the northwest Mediterranean area, pine (especially *P. sylvestris* for the supra-Mediterranean stage) and juniper (*J. communis*) have dominated the gymnosperm population since the beginning of Holocene. We investigated needles of *J. communis*, but no typical phytoliths were found. Since past and modern formations dominated by pine produce high proportions of the spherical-with-sockets morphotype, we conclude that this morphotype reveals the presence of pine in Holocene past and modern vegetation of the northwest Mediterranean area.

The Pi/P ratio of Pinaceae spherical-with-sockets phytoliths (Pi) versus Poaceae phytoliths (P) reaches values higher than 0.26 for assemblages produced by modern pine woods and during the Holocene pine expansion phase, while it does not reach 0.1 for the other assemblages. Thus, the Pi/P ratio appears to be a new and reliable proxy of pine cover density for Holocene vegetation reconstruction in the Rhône valley.

In order to apply this new pine proxy to other areas, further investigations of phytolith production of various pine species and other gymnosperms are needed. This can be achieved by analyzing phytolith assemblages from soils of various modern gymnosperm formations.

### 4.3. Toward a new phytolith index tracing tree cover density for the north-west Mediterranean area

The northwestern Mediterranean area has experienced phases of forest opening since the beginning of Holocene, due either to climatic conditions or to human pressure, as shown by charcoal analysis (Vernet and Thiébaud, 1987; Heinz and Thiébaud, 1998). However, charcoal analysis is

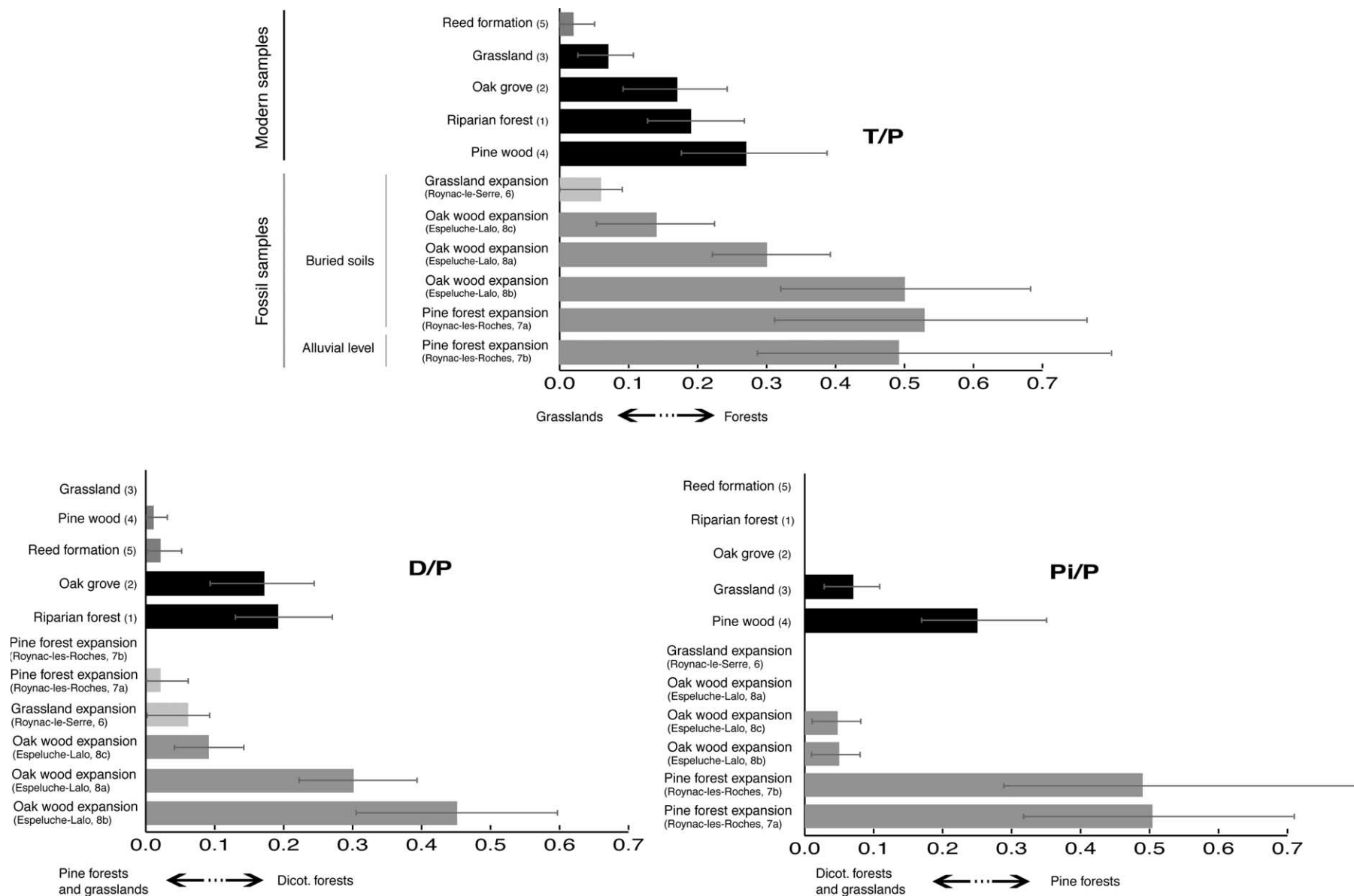


Fig. 5. Graphic representation of T/P (Tree phytoliths versus Poaceae phytoliths) and of its two components, D/P (Dicotyledon phytoliths versus Poaceae phytoliths) and Pi/P (*Pinaceae* phytoliths versus Poaceae phytoliths) ratios, showing the boundaries between forests and grasslands, between pine forests and other formations, and between dicotyledon forests and other formations. Modern samples are represented with black bars and fossil samples with grey bars; in these two pools of samples, the ratios calculated from nonsignificant percentages are represented with lighter bars (T/P, sample 6 and reed formation; D/P, samples 6, 7a, pine wood, and reed formation)

unsuitable for detecting grass cover. Quantifying the degree of opening of the vegetation cover appears possible, using a new phytolith index, T/P, corresponding to the ratio of characteristic tree phytoliths, including angiosperm and gymnosperm species (T), versus Poaceae phytoliths (P):  $T/P = D/P + Pi/P$ .

Among our assemblages two groups are distinguished (Table 1, Fig. 5). Assemblages produced by forests show a T/P ratio higher than 0.15 (pine wood (4), 0.27; oak grove (2), 0.16; riparian forest (1), 0.19; Preboreal pine expansion (7a and 7b), 0.52 and 0.49; Atlantic oak wood expansion (8a and 8b), 0.29 and 0.50), while assemblages produced by grasslands show a T/P ratio lower than 0.1 (grassland (3), 0.07; reed formation (5), 0.03; local forest regression (6), 0.06). The vegetation density variations appear well-mirrored by this T/P ratio. Even if more modern and fossil samples must be processed in order to validate the boundary values proposed here for each ratio, this demonstrates that the T/P phytolith index can be used as a quantitative proxy of the opening of the northwestern Mediterranean vegetation.

All of the three indexes proposed here have shown accuracy in describing Mediterranean vegetation, but further studies are still needed in order to increase the number of assemblages analyzed and to decrease confidence intervals.

#### 4.4. Space scale representation of phytolith assemblages from buried soils and alluvial deposit

The Pi/P, D/P, and T/P ratios from the five modern phytolith assemblages studied here reflect the pine representation, the broad-leaved tree representation, and the opening degree of the top vegetation formations. Each related vegetation patch is not older than 200 years and does not extend over large areas. Thus, the five phytolith assemblages, extracted from soil humic horizons, record with accuracy the modern local vegetation. This result corroborates the local representativeness of phytolith assemblages from soils, as already suggested by Bartoli and Guillet (1977).

Charcoal and pollen records from distant sites (Triat-Laval, 1978; Planchais, 1985; Vernet and Thiébault, 1987; Heinz and Thiébault, 1998) show that the Mediterranean vegetation has always had a mosaic pattern, even if a strong increase in heterogeneity, due to agro-pastoral practices, is recorded from around 6000  $^{14}\text{C}$  yr B.P. (Vernet et al., 1987). Phytolith assemblages from buried soils from similar or close (about 10 m) locations and dated to the same regional vegetation phase 7a and 7b and 8a and 8b give close phytolith indexes, in agreement with regional vegetation trends (pine forest development and oak forest development, respectively). However, assemblage 7b also shows a high amount of silicified vessels produced by dicotyledons that were never found in other assemblages. This can be explained by a local vegetation feature, as the local presence of dicotyledons within the regional pine formations. Assem-

blage 7b comes from an alluvial level, and the vegetation heterogeneity is thus better recorded in this assemblage than in soil samples. Assemblages 6 and 8, both dated to the Atlantic period, record a grassland settlement and a broad-leaved forest, respectively. While assemblages 8a and 8b have been collected in the very low Citelle River alluvial plain, assemblage 6 comes from the slopes of the Roynac hill. The topographic heterogeneity may explain the heterogeneity of the vegetation's responses to natural fires and anthropic stress. This shows that phytolith assemblage analysis is accurate for reconstructing local variations of the vegetation, due either to local instability of the ecosystem or to local pressure (especially human), inside the regional pattern given by pollen and charcoal studies.

Assemblage 8c, also contemporaneous with the Atlantic oak forest development, shows a middle D/P ratio and significant amounts of pine and grass phytoliths. Since it is recovered from an alluvial level, it includes phytoliths originating from all the different patches of vegetation covering the Citelle basin at this time. Oak forest, scattered pine stands remaining from the previous phase or developed after fire, and opening areas linked to human disturbance could cohabit on the slopes, while the riverbanks would have been covered by riparian formations.

These results suggest that fossil phytolith assemblages extracted from buried soils record the vegetation features at a local scale, while phytolith assemblages from sedimentary layers are likely to reflect the regional mosaic of vegetation.

## Conclusion

Phytolith assemblage analysis is applied to a north Mediterranean environment. Although the number of processed samples is low, our results show that phytolith assemblage analysis is accurate for tracing local vegetation features otherwise unrecognizable by other proxies. Unlike pollen, phytolith assemblages are well preserved in soils and buried soils and give a local scale record. Unlike charcoal, phytolith assemblages give quantitative information on the degree of opening of the recorded vegetation. Phytolith assemblage analysis is consequently complementary to pollen and charcoal studies.

Three phytolith indexes have been displayed as accurate tracers of the Rhône valley vegetation features. D/P ratio, corresponding to the ratio of dicotyledon spherical morphotypes (D) versus Poaceae phytoliths (P), traces broad-leaved tree cover density. Pi/P ratio corresponding to the ratio of pine spherical with sockets morphotype (Pi) versus Poaceae phytoliths (P), records pine cover density. T/P ratio, which is the sum of Pi/P and D/P ratios, records the overall tree (T) cover density or opening degree of the vegetation. The use of these three ratios in fossil phytolith sequences would greatly improve reconstruction of pine timberline shifts, oak forest development, and spatial and temporal variations of

the forest's opening in the northwestern Mediterranean region.

The main changes of the northwest Mediterranean vegetation during the Holocene period were due to climatic stress and human disturbance. Distinguishing their respective influence during the late Prehistory is still one of the main challenges for Mediterranean paleoenvironmental research. These first results show that phytolith assemblage analysis is a promising tool in this field.

However, in order to extend these results to other Mediterranean areas, correlation between values of soil phytolith indexes and both floristic and physiognomic data of the various vegetation sources are required.

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