Journal Of Anthropological And Archaeological Sciences

Research Article

Southern Patagonian archaeologicalsites (47°-49 S; 72° W, Argentina)as pollen Records: Pollen Preservation Analysis Considerations for Accurate Palaeoenvironmental Reconstructions

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Received: March 16, 2020 **Published:** May 28, 2020

Abstract

Fossil pollen records from archaeological sites have been largely used to reconstruct past vegetation dynamics and environmental conditions in relation to anthropogenic factors. In this paper we examine the degree of pollen preservation from four archaeological sequences in the west of Santa Cruz Province, Argentina (47-49° S; 72°W) to determine different types of pollen damage and their associated causes. Sites located in the Pueyrredón Lake basin (47° S; 72° W, CMN1 and CMN2) and in the San Martín Lake basin (49° S; 72° W, CPD and B10q) were selected due to differences in morphology, topographic position and orientation but with similar surrounding vegetation composition. Pollen deterioration indices were calculated considering differences in pollen taphonomy mainly related to both biochemical (degraded grains) and mechanical (broken and crumpled grains) damage. The results show that different factors (sedimentological, environmental and anthropogenic) could affect pollen preservation. The preservation problems of CMN1, CPD and B10q were not significant. However, pollen assemblage of CMN2 presented remarkable mechanical and biochemical damage. The main causes of problems of pollen preservation detected were: exposure to high temperatures, oxidation, pH effect, abrasion of the sediments that caused mechanical damage and the presence of fungi associated with biochemical damage.

Keywords: Pollen preservation; Biochemical and Mechanical deterioration indices; Archaeological sites; Pollen; taphonomy; Palaeoenvironmental reconstruction; Patagonia

Introduction

Palynology is an essential tool to estimate the vegetation composition as well as to reconstruct vegetation history. The importance of pollen analysis as a method of palaeoenvironmental reconstruction lies in the fact that: (a) pollen grains are extremely resistant, which allows them to be preserved in deposits where other types of fossils have been destroyed by diagenetic processes, and (b) pollen grains are produced in large quantities and have distinctive characteristics at family level, and some at the level of genus or species [1]. Pollen grains are affected by various processes from the time of dehiscence to the time of recovery and analysis. The understanding of the preservation problems and pollen-vegetation relationships can be used to interpret and reconstruct past vegetation [2-7]. Also, the quantification of pollen preservation is an approach that can improve the confidence of such interpretation [8].

Numerous reports of pollen degradation types were performed [9-14]. According to Cushing [11] six preservation types were differentiated: (1) corroded, (2) degraded, (3) crumpled and exine thinned, (4) crumpled but exine normal, (5) broken, and (6) well-preserved. Various taphonomic processes occur in aerial/subaerial mineral deposits such as soils bearing archaeological evidences where they can potentially distort the original pollen content [15]. In pollen assemblages where deterioration is marked, the less robust taxa may first decay, while the more resistant types will appear to accumulate owing to the disappearance of less resistant grains [16]. Several studies have discussed factors that can cause mechanical and / or biochemical damage through experimental studies or from the depositional environment [7,8,15,17-21]. While damaged grains may indicate a degraded assemblage, they may also indicate an assemblage augmented by reworked grains. Moreover, recent studies considered the role that pollen preservation quantitative analysis can provide as a tool to help understand and interpret the palynological records [8,21].

In arid and semiarid regions the fossil pollen sequences from archaeological sites have been largely used to reconstruct past vegetation communities and environmental conditions where other deposits for pollen and macrofossil analysis are not common [22-27]. The archaeological sequences from Patagonia (Santa Cruz Province, Argentina) have been an important source of palaeoecological information because of their chronological record and abundance compared to other non-archaeological sites. However, inferences based on the interpretation of fossil pollen associations in archaeological sites have been widely questioned due to potential biases related to anthropogenic and/or biotic origins [28,29]. In this paper, we xamine the degree of pollen preservation of four archaeological sites (caves and rock-shelter) from Patagonia to determine different types of pollen damage. In this sense, we discuss the occurrence of evidence in the archaeological layers of different factors that could influence in the pollen preservation.

Archaeological and Palaeoenvironmental Background

In the northwest of Santa Cruz Province the first palynological studies in archaeological sequences were conducted by Mancini and Trivi de Mandri [30]; Mancini et al. [31]; Mancini [32] and Trivi de Mandri et al. [33]. Horta et al. [34] reconstructed the vegetation history and contextualized the different human occupations in the landscape from other archaeological sequence of the Pueyrredón Lake area (47° S). In the southwest, Mancini [31] provided valuable information to reconstruct the vegetation history during the Holocene. In the San Martín Lake area (49°S) Bamonte [35] and Bamonte et al.[36], studied pollen content from two archaeological sites being the first studies of palaeoenvironmental reconstruction from this type of records. To the east, in the Central Plateau, several sites were studied with the aim of reconstructing palaeoenvironmental conditions in relation with human occupations in more arid conditions since c.a. 11000 yr BP [37-40]. The first ccupations, subsequent expansions and eventual decline of human groups have been the result of a complex set of factors [41]. Thus, numerous archaeological and anthropological studies have discussed how the life of populations was determined by the environmental characteristics and availability of resources [42-44]. Ariztegui et al. [41] have postulated that the human-environment interaction was much higher in climatically sensitive areas and near watershed areas. In this sense, the role and influence of climatic conditions may have been a decisive factor in decisions made by populations [45,46]. This human influence together with environmental interactions should be considered carefully to present reliable vegetation and palaeoenvironmental reconstruction [24, 47,48] while working in an archaeological context.

Study area

The considered area covers latitude from 47° to 49°S and is characterized by an arid and semi-arid climatic regime. The climate is influenced by the South Pacific anticyclone. Southern westerlies are an important component of the climate system at hemispheric and global scale since they influence large-scale atmospheric circulation and precipitation patterns in the Southern Hemisphere [49-51]. The uplift of the westerly air masses over the western slopes of the Andes in Patagonia Argentina produces therefore the orographic precipitation with a maximum precipitation of 1000 mm/yr, whereas the subsidence over the eastern side of the Andes, in the Central Plateau produces dry conditions with a precipitation lower than 200 mm/yr. The Andes topography and the potential advection of eastern moisture are the main forces that generate changes in precipitation and, consequently, in the west– east Patagonian vegetation gradient [52-54].

Vegetation heterogeneity at a regional scale (Figure 1) reflects the constraints imposed by the climatic, topographic, and edaphic features [55,56]. The vegetation distribution is determined by a strong precipitation gradient reflected in the communities represented by dense forest to open forest and steppes to the east [57-59].

Archaeological case studies

Pueyrredón Lake area. Two archaeological sites (cave and rock-shelter) located in the Pueyrredón Lake area (Figure 1.A).

Milodon Norte 1 Cave (CMN1; 318 m a.s.l.), located on the south shore of Pueyrredón Lake , is a cave of 3 m high and 5 m deep with a NW orientation. The excavations carried out at the site made it possible to identify a layer with a tephra from the Hudson volcano eruption (6800 ± 100 year BP). Human occupations have been recorded above and below the tephra, suggesting a long sequence of occupation of the cave [60]. The oldest occupation registered for this site is dated to 7790 ± 30 ^{14}C yr BP [34]. Milodon Norte 2 Cave (CMN2; 306 m a.s.l.) is a rock-shelter of 2 m high and 4 m deep. Its orientation is N and it is located 1.5 km from Pueyrredón Lake. The geological setting is comparable to CMN1. Lacustrine sediments constitute the basal part of the filling of the rock-shelter. In CMN2, the evidence of shelter use is confined to a restricted space. The radiocarbon dates show that the cave was occupied during the late Holocene [34].

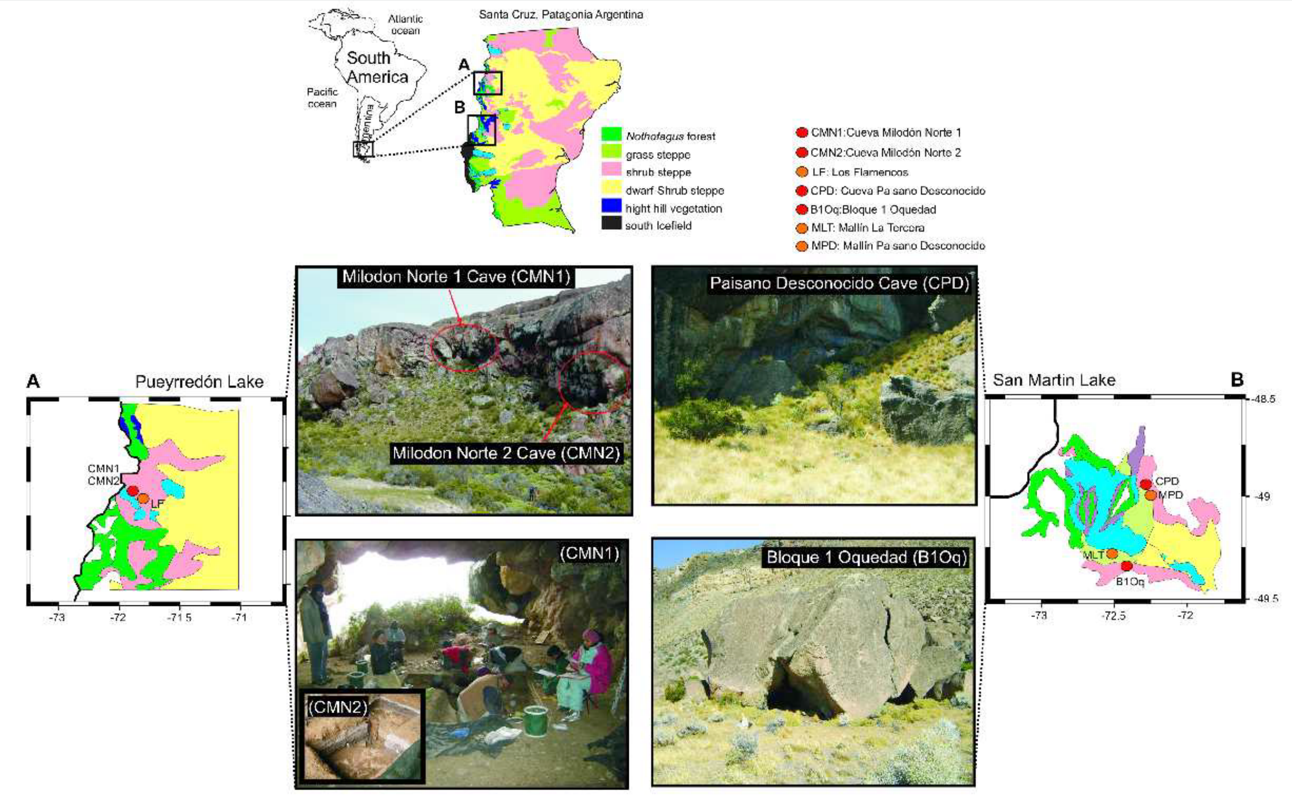

Figure 1: Study area location. Patagonia, Argentina maps showing the main vegetation units. A) Pueyrredón Lake basin B) and San Martín Lake basin with archaeological sites location (CMN1, CMN2, CPD, B1Oq), non-archaeological sequences (LF, LT) and vegetation distribution respectively. CMN1: Cueva Milodón Norte 1, CMN2: Cueva Milodón Norte 2, CPD: Cueva Paisano Desconocido, B1Oq: Bloque 1 Oquedad, LF: Los Flamencos, LT: La Tercera.

This area is relevant as it provides important topographic, lithologic, floristic and faunal resources which have led to much archaeological research [61-65]. In this sense, geoarchaeological studies [66] postulate that the sector would have been free of ice (late Pleistocene) and the height lake level was lower, which would have exposed coastal strips and topographic resources attractive to populations. The direction of these human movements would probably have been east- west, with a possible intensification in the use of subsistence resources, as part of a process of fission and spatial constriction of the original population. Among the raw materials of local origin, the presence of obsidian, in addition to raw materials used for making of scrapers and swords, was observed. A non-local raw material, black obsidian, was also found. The interaction between populations was associated with the rupestrian art of the area. The existence of bones in CMN1 indicated the use for consumption of *Lama guanicoide*, *Felis concolor*, *Zaedyus pichiy* and a variety of birds. Also, the different patterns of skeletal remains of guanaco corresponding to all the stages of processing of this prey indicate: places of slaughter, disarticulation and dismemberment and sites of preparation and final consumption. The presence of rodents in the archaeological layers is due, in most cases, to post-depositional processes [62].

According to palaeoenvironmental studies it is estimated that in the last 2500 ^{14}C yr BP there was a decreasing tendency of humidity in the region. This would have generated new environmental scenarios and the incorporation of new spaces, reaching its highest point with great droughts around 900 ^{14}C yr BP, in the period of the Medieval Climatic Anomaly [63]. The reduction in the availability of water sources and the decrease of the water level in Pueyrredón-Posadas and Salitroso lakes, generated the availability of new spaces around the coasts.

San Martín Lake area. In the San Martín Lake area, two pollen fossil records from archaeological sites (cave and rock- shelter) (Figure 1.B).

Paisano Desconocido Cave (CPD; 500 m a.s.l.) is a site located in the rocky hillside in the northeastern shore of the San Martín lake. In this zone three rocky sites with archaeological material in the surface were registered. The site presents an entrance of 14 m, 16.9 m deep and a maximun height of 2.1 m and is oriented to the west where *Nothofagus* forest develops. The coluvial sediment dominates with aeolian deposits and sand-clay layers. Radiocarbon dates were performed on *L. guanicoe* bones showing anthropogenic modifications [68]. The oldest date from the site corresponds to early Holocene (8000 ± 40 ^{14}C yr BP) and the subsequent radiocarbon dates are 6930 ± 40 ^{14}C yr BP, 4040 ± 30 ^{14}C yr BP and 3030 ± 30 ^{14}C yr BP. According to Espinosa et al. [68] the archaeological record shows low intensity of human use of this space. The archaeological record of CPD is similar to the one found in the southern shore, showing logistic use of the site as intermediate camps before accessing the forest.

Bloque 1-Oquedad (B1Oq; 354 m a.s.l.) is a rock-shelter located in the southern shore of the San Martín Lake in a blocks field produced by gravitational processes that expanded east to west along 1400 meters. In this area eight repair blocks were registered, one of them is B1Oq. It is a broken block oriented to the north and protected from the wind; its surface is 10.5 m x 2.8 m. The sedimentology of the site is represented by aeolian sediments on a layer of till. The chronology highlights a first occupation level around 9700 ^{14}C yr BP, which was found over till level and that continues until around 1000 ^{14}C yr BP [69]. Radiocarbon dates were realized on bones of *Lama guanicoe* showing anthropogenic modifications. The first occupation moment was at 9760 ± 60 ^{14}C yr BP and the rest radiocarbon dates correspond to 2270 ± 50 ^{14}C yr BP, 1040 ± 50 ^{14}C yr BP and 1030 ± 50 ^{14}C yr BP. The artefactual and archaeofaunistic sequence suggest a relative stability in the human activities along the time. The precedent information suggests that the southern shore of the lake was used as a logistic base and was of redundant form with low use intensity. The archaeological evidence showed that the site was used for activities related with final activities in lithic manufacturing process and artifact discard [70].

Materials and Methods

Archaeological sites pollen sampling

Four archaeological sequences (CMN1, CMN2, CPD and B1Oq) were used to determine different types of pollen damage and their associated causes. Milodón Norte Cave 1 and 2 (CMN1, CMN2), Paisano Desconocido Cave (CPD) and Bloque 1 Oquedad (B1Oq), were selected to perform the present study due to their differences in morphology, topographic position and orientation, but similar surrounding plant communities [34-36,71].

Both CMN1 and CMN2 archaeological sites were excavated next to the cave entrance and following natural stratigraphic layers. CMN1 is bigger than CMN2 considering square meters and also has a longer sequence, both in stratigraphic terms and in chronological depth [72]. A section of this area was selected for excavation, providing a hearth overlapping up to the basal lacustrine sediment (Figure 1.A). They were approached in order to identify various activity areas within the site and to evaluate possible changes in their use over a chronological sequence [72-74]. The pollen samples were obtained from sectors that are distant from the burning cores of the bonfires archaeologically identified. On the other hand, in CMN2, the archaeological excavation was focused on the sector that showed hearth overlays and its surface has not expanded far [34].

In CPD, the excavation was performed next to the lateral right near the cave entrance and samples were obtained for pollen analysis. Pollen content was analyzed from10 sediment samples [36]. In B1Oq and CPD, the samples were taken each artificial level of 5 cm from profile of 1m2 excavation [68]. The excavation in B1Oq was carried out in the middle of the site close to the wall whereas in CPD it was carried out next to the right side near the cave entrance[66,68,75]. Fifteen sediment samples for pollen analysis were taken in B1Oq from each artificial level (Figure1.B).

The pollen content of 10 archaeological layers from CMN1, 8 from CMN2, 10 from CPD and from15 B1Oq was analyzed.

Pollen extraction

Sediment samples (between 10 and 20 gr) were processed according to standard palynological techniques. Three tablets of *Lycopodium clavatum* spores were added as markers prior to pollen extraction and in order to estimate representative pollen sums and pollen concentration. The standard procedures include: hot KOH 10 % to remove clays and humid acids, HCl 10 % to remove carbonates, heavy liquid with $ZnCl_2$ (δ=2) to separate the mineral fraction by flotation, HF to remove silicates and acetolysis to eliminate organic matter [16,76,77]. The residues were mounted in glycerine and analyzed under microscope (1000 x) and pollen grains identification and preservation problems were performed by using the pollen reference collection of the Laboratory of Paleoecology and Palynology (UNMdP) (Figure. 1-Appendice).

Pollen diagrams were plotted using TILIAGRAPH (TGView, 2.0.2, Grimm, 2004). Palynological richness was calculated using the rarefaction technique in order to better understand vegetation dynamics and correlated with preservation indices. The analysis was performed with Psimpoll 4.10 [78] .

Differential preservation evaluation

In order to evaluate the different types of pollen deterioration and their possible associated causes (environmental factors, anthropogenic, sedimentological processes, among others) we determined pollen deterioration indices. Pollen damage characteristics considered are:

Biochemical damage: Corrosion causes the pollen exine to be pitted, etched or perforated and results from biochemical oxidation. Degradation causes general thinning of the exine and loss of structural elements, and results from chemical oxidation within aerial and subaerial environments. Ultimately the exine may deteriorate until only an outline of the grain is left.

Mechanical damage includes: Breaking or splitting grains, and crumpling grains, which can result from physical transport prior to deposition and/or from post- depositional sediment compaction.

In the present study we calculated four indices to recognize different types of pollen deterioration. *Well preserved* includes grains without observable deterioration. *Biochemical deterioration* includes degraded grains and this index was calculated as *Total degraded pollen grains/ Total pollen grains.* The *Mechanical* *deterioration* includes broken and crumpled grains. The index was calculated as *Total (crumpled + broken) pollen grains/ Total pollen grains* (Table 1). The pollen categories were determined by comparing the fossil pollen grains with perfectly preserved pollen reference collection from the Laboratory of Paleoecology and Palynology (UNMDP) (Figure 1- Appendice).

Table 1: Preservation categories used for identified pollen grains modified of Jones et al. (2007) according to our case study and calculation of mechanical and biochemical index.

	Deterioration type	Description	Category	Index
Well preserved	No observable deterioration		Well presevated	
Biochemical deterioration	Degraded	Exinethinned and/or structural features fused and ind	Degraded	No degraded grains/No Total grains
Mechanical deterioration	Broken	Grain split or fragmented	Broken	No (broken + crumpled) grains/ No Total
	Crumpled	Grain squashed	Crumpled	

Figure 1 Appendice: Examples of pollen reference collection of the Laboratory of Paleoecology and Palynology (UNMDP). a,b) *Mulinum*; c,d) Asteraceae subf. Asteroideae; e) Poaceae; f, g) *Schinus*; h, i) *Nassauvia*; j, k) Cariophyllaceae; l, m) *Nothofagus*; n,o) *Berberis*; p, q) *Empetrum*; r,s) Rubiaceae; t, u) Cyperaceae; v) *Ephedra*; w, x) *Colliguaja*; y) *Euphorbia*.

The values of the indices varied between 0 and 1. If all pollen had been well preserved, both indices would have been 0; the higher the indices, the poorer the preservation. The variation of values from the biochemical and mechanical deterioration index from the archaeological layer were tested with the Wilcoxon analysis. The p-values were obtained for each sample, and all those that were lower than the 0.05 significance level, were significantly different from the data set. Table 1-Appendice summarizes the indices calculated for all archaeological layers recorded.

Table 1- Appendice: Biochemical and Mechanical deterioration index. The variation of values weretested with the Wilcoxon analysis. p-values were obtained for each sample, and all those that were minor than the 0.05 significance level, were significantly different from the data set. * indicates significantly well preserved and ** indicates significantly bad preserved.

Site	Archaeological Layer	Biochemical deterioration index	p value	Mechanical deterioration index	p-value
CMN1	0	0,0063	0,941	0,0095	0,652
	1	0,0027	0,429	0,0108	0,73
	2	0,0000	0,062	0,0000*	0,015
	3	0,0000	0,062	0,0137	0,9
	4	0,0000	0,062	0,0000*	0,015
	5	0,0373	0,132	0,0821	0,125
	6	0,1000**	0,003	0,7000**	0,003
	7-1	0,0223	0,156	0,0318	0,56
	7-2	0,0000	0,062	0,0000*	0,015
	8	0,0000	0,062	0,0023	0,054
CMN2	1	0,0000*	0,015	0,0330	0,31
	2	0,4286**	0,015	0,8429**	0,015
	3	0,2625	0,46	0,8625**	0,01
	4	0,1786	0,68	0,8607**	0,031
	5	0,3883**	0,031	0,2524	0,46
	6	0,1818	0,81	0,0682	0,078
	7	0,1410	0,375	0,4231**	0,037
	8	0,0822*	0,046	0,0329*	0,015
CPD	1	0,0582	0,3	0,4514**	0,09
	2	0,1982*	0,003	0,1036*	0,019
	3	0,1053	0,3	0,0965*	0,011
	4	0,1400*	0,027	0,1700	0,12
	5	0,0362	0,08	0,4768**	0,003
	6	0,0280*	0,019	0,4411**	0,007
	7	0,0351	0,054	0,4737**	0,049
	8	0,0000*	0,003	0,5714**	0,044
	9	0,1275	0,054	0,1863	0,16
	10	0,0847	0,73	0,0424*	0,033
B1Oq	1	0,0683*	0,001	0,3253**	0,001
	2	0,1628	0,36	0,2849**	0,001
	3	0,0500*	0,0009	0,1133	0,22
	4	0,1585	0,24	0,0563	0,22
	5	0,1615	0,3	0,1354	0,14
	6	0,3913**	0,001	0,1652*	0,04
	7	0,4490**	0,001	0,0816	0,9
	8	0,2384	0,17	0,0000*	0,001
	9	0,1674	0,77	0,0362*	0,02
	10	0,2637**	0,047	0,0386*	0,03
	11	0,1485	0,064	0,0330	0,01

	12	0,2468	0,14	0,0865	0,72
	13	0,1659	0,68	0,0088*	0,001
	14	0,2620	0,055	0,0655	0,5
	15	0,0636*	0,001	0,0231*	0,005

Also, Spearman correlation analysis was carried out to evaluate the relationship between pollen concentration, palynological richness, biochemical deterioration index and mechanical deterioration index (Table 2). All these analyses were performed using the PAST 2.16 software [79]. The Figure 2 shows examples of preservation differences in pollen grains of all sites analyzed.

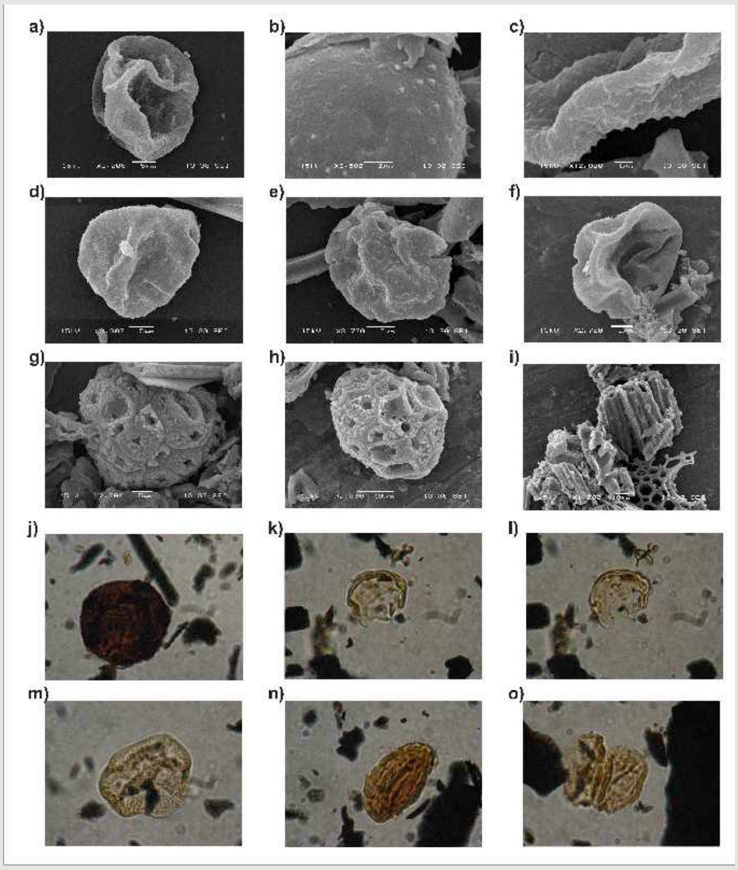

Figure 2: Examples of pollen grains showing different types of deterioration. Electronic microscopy: a) Partly degraded Asteraceae subf. Asteroideae; b, c) Partly degraded Asteraceae subf. Asteroideae (detail of spine); d- f) Partly degraded *Nothofagus*; g, h) Partly degraded Cariophyllaceae; i) Charcoal detail. *Optic microscopy* (all pollen grains photographed at x1000 magnification): j) Exine very dark of *Nothofagus*; k, l) Partly broken grains of *Nothofagus*; m) Partly degraded Cariophyllaceae; n, o) Partly degraded Asteraceae subf. Asteroideae.

Table 2: Spearman's correlation coefficient between pollen concentration, palynological richness, biochemical deterioration index and physical deterioration.

		Pollen concentration	Richness palynological
Biochemical deterioration index	CMN1	-0,38	-0,23
	CMN2	-0,52	-0,54
	CPD	-0,59*	-0,35
	B10q	-0,43	-0,62**
Mechanical deterioration index	CMN1 CMN2 CPD	-0,49	-0,05
		-0,90***	-0,66**
		-0,44	-0,57*
	B10q	0,35	0,17

*moderate correlation
**strong correlation

***very strong correlation

Additionally, in order to identify possible causes of deterioration in our discussion of results we consider the following:

a. The pollen concentration of the samples analyzed, since it can be associated with cultural activities.

b. Presence /absence of tephra layers and / or bonfire in the archaeological layers analyzed.

c. Representativeness of anemophilous pollen types regarding to the geographical orientation of the cave (e.g. long distance types such as *Nothofagus* sp. and *Podocarpus* sp.)

d. Representation of exotic pollen types related to cultural activities in archaeological layers.

Electronic and optical microscopy

Additionally, photos in the electronic and optical microscopes were taken to exemplify differences in the grains preservation (Figure 2). For scanning electron microscopy (SEM) observations the sub-samples were dehydrated using ethanol 99%, mounted on exposed film, attached to studs, and coated with gold in Mar del Plata University (UNMDP).

Results

According to the calculated indices, although some layers of the archaeological sites analyzed presented some problems of preservation, the general results indicate that there was good pollen preservation. Total taxa, percentages of indeterminate, crumpled and broken pollen grains, concentration and pollen sum, and mechanical and biochemical indices calculated are shown in Figure 3. The results are described below.

Pueyrredón Lake area

Milodon Norte Cave (CMN1): In the samples of this cave good preservation is seen in both ornamental structures and walls of the pollen grains compared with the grains of the reference collection (Fig. 1-Appendice). The total pollen taxa oscillated between 5-24 and the percentage of indeterminate grains varied between 0-1 %. Only layer 6 of CMN1 was considered barren because the pollen count was of 20 grains. According to these results the mechanical and biochemical indices for this layer presents high and moderate values respectively (Figure 3. A, Table 1- Appendice).

The pollen types with preservation problems (degraded, broken and crumpled grains) were *Poaceae, Asteraceae subf. Asteroideae* and *Mulinum* (Table 3).

The pollen information for this cave showed an environmental scenario dominated by grass steppe associated with dwarf-shrubs and shrubs (high values of Poaceae, low values of dwarf- shrubs such as, *Empetrum* and *Mulinum*) during the early Holocene until the establishment of shrub steppe in the middle-late Holocene (distinctive pollen types are *Mulinum*, *Berberis*, *Asteraceae subf. Asteroideae*, *Poaceae* and *Nothofagus*) [71].

Milodon Norte Cave (CMN2): Remarkable preservation problems were found in CMN2 with very low pollen sum in layers 2, 3, 4, 5, 6 and 7 which were also considered barren due to low pollen sum and low pollen concentration. A low number of total taxa were found in this site. The degraded and crumpled grains varied between 10-40% and 5-40% in broken grains, with the highest percentages in the layers previously mentioned (Figure 3. B). The mechanical deterioration was statistically significant in layer 2, 3, 4 and 7 while the layer 5 was statistically significant in biochemical deterioration (Table 1-Appendice). The pollen types mainly affected in their preservation were *Nothofagus, Poaceae, Mulinum, Asteraceae subf. Asteroideae* and *Euphorbiaceae* (Table 3). Also, all grains observed under optical microscope in this site presented a very dark coloration.

This rock-shelter represents a temporary window of the last thousand years; the palynological information is scarce due to low pollen sum and bad preservation of grains. The main pollen types present are *Mulinum,* Asteraceae subf. Asteroideae and others shrubs, which can be associated to a shrub steppe [71]. Although pollen assemblages are consistent with CMN1 for the same period, CMN2 contains five archaeological layers in which important preservation problem have been found.

In this paper, summary pollen diagrams of CMN1 and CMN2 are presented, detailed information in the pollen results and their respective palaeoenvironmental reconstruction can be found in Horta et al. [34].

San Martín Lake area

Paisano Desconocido Cave (CPD): This cave presents some archaeological layers with degraded, crumpled and /or broken pollen grains. The total pollen taxa varied between 10 and19 and the indeterminate pollen ranged between 1 and 5%. Layers 1, 5, 6, and 7 presented high percentages of crumpled grains (5-80%) while percentages of degraded and broken grains were moderate (1-20%) and low (1-7%), respectively. This observation was made using the perfectly preserved pollen reference collection from Laboratory of Paleoecology and Palynology (UNMDP) as reference, see Figure 1-Appendice. The number of indeterminate grains was low because although many grains were crumpled, it was possible to distinguish their morphology. The pollen types mainly affected were Poaceae and Asteraceae subf. Asteroideae (Figure 2 and Table 3).

Table 3: Summary of information of the archaeological layers with some type of deterioration (Biochemical and/or Mechanical), main affected pollen types and possible associated causes.

Site	Archaeological layers	Type of deterioration	Degree of preservation	Affected pollentypes	Possible causes
CMN1	6	Biochemical and Mechanical	Poor	Poaceae Asteraceae Subf. Asteroideae	Presence of fungi that could affect the integrityof thepollenwallsand ornamental structures. Sandy sediments: Abrasion and removal of sediments that could cause grain breakage.
CMN2	2,3,4,5,7	Mechanical	Very Poor	Nothofagus Poaceae Asteraceae Subf. Asteroideae Mulinum Euphorbiaceae	High amount of charcoal in the sediments. Indicator of bonfires as cultural practises. High temperatures can cause oxidation damage. The structural elements of the grains appear partially fused and the pollen preservation is extremely low.
CPD	1,5,6,7,8	Mechanical	Moderate	Poaceae Asteraceae Subf. Asteroideae	Process of erosion and moisture in the rock, accumulation of salts, pH changes in sediments.
B1Oq	1,2	Mechanical	Moderate	Poaceae Asteraceae	Abrasion between the sediments.
	6,7,10,15	Biochemical	Moderate	Subf. Asteroideae Mulinum Nothofagus	Oxidation could be the main cause of the biochemical deterioration.

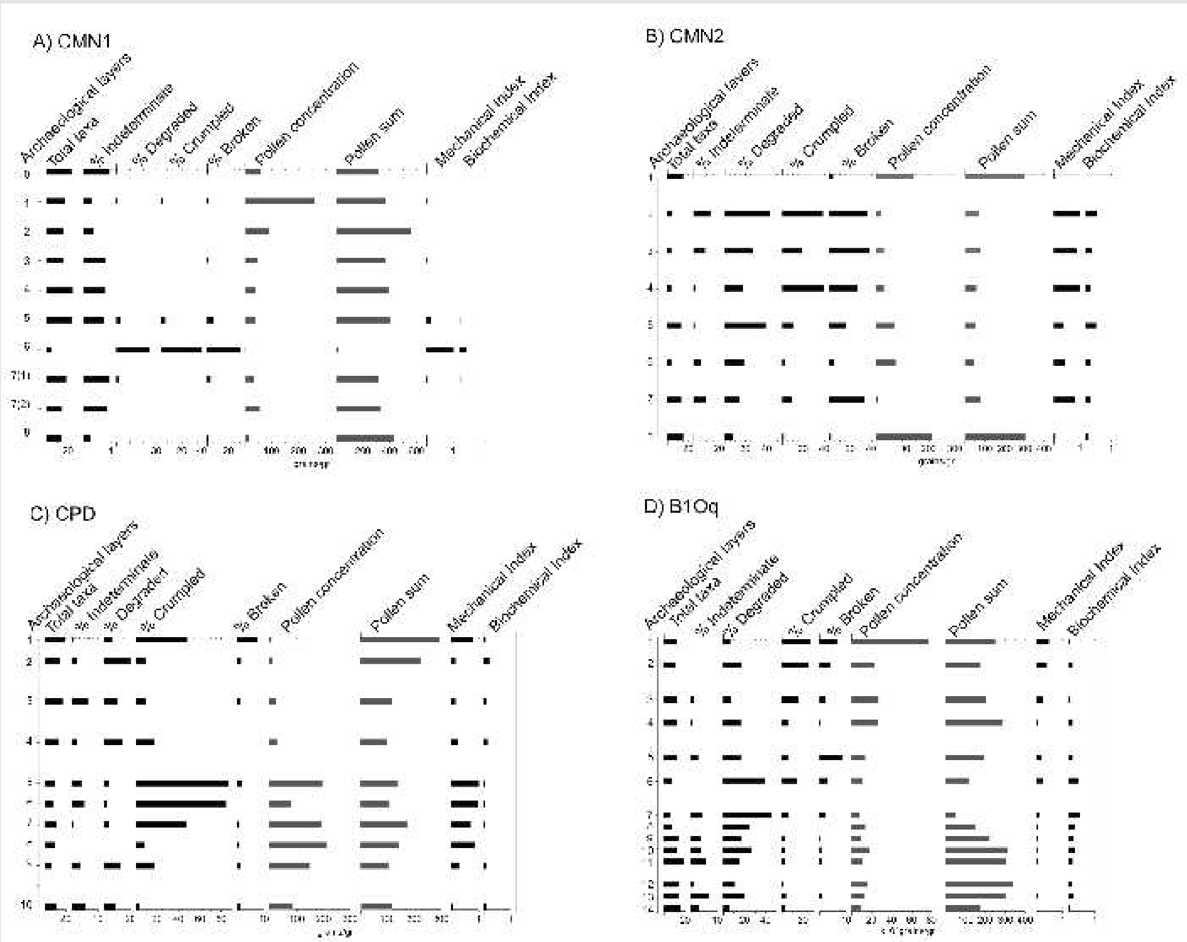

Figure 3: Diagram with percentage of poorly preserved grains, sums and pollen concentration and biochemical and mechanical indices of the archaeological sites studied: A- CMN1, B- CMN2, C- CPD and D- B1Oq.

Also, the pollen sum varied between 100 and 292 grains, some of the lowest sums were found in layers with preservation problems. According with values of crumpled and broken grains, layers 1, 5, 6, 7 and 8 were statistically significant in the mechanical index (Table 1-Supplementary material). Very low values are present in biochemical index (Figure 3. C).

The cave CPD represents a temporary window between 8000 ± 40 14C yr BP and 3030 ± 30 14C yr BP. The pollen assemblage showed a grass steppe with high values of Poaceae and accompanying herbs in the early Holocene while in the middle Holocene a change is observed towards a shrub steppe (increase of *Mulinum, Asteraceae subf. Asteroideae* and others shrubs) [36].

Bloque 1 Oquedad (B1Oq): The rock-shelter B1Oq presents in general moderate pollen preservation. However, archaeological layers 1, 2, 6, 7, 10 and 15 showed some type of preservation problem statistically significant (Table 1-Appendice).

The number of total taxa varied between 5 and 13 and the pollen sum oscillated between 49 and 339 grains. Regarding these results, the lower pollen sums corresponds to archaeological layer 7 with preservation problem (45% degraded grains). The percentages of degraded and crumpled grains in layer 6 are 40% and 15% respectively. Moderate-low values of crumpled, degraded and broken grains are present in layers 1 and 2. Layers 1 and 2 showed mechanical deterioration (mechanical index: 0.32-0.28) while layers 6, 7 and 10 exhibited biochemical deterioration (biochemical index: 0.39-0.449- 0.26 respectively) (Figure 3. D and Table 1- Appendice). The total pollen taxa varied between 5 and13 and the indeterminate pollen range between 1% and 7%. The pollen types with preservation problems are *Poaceae, Asteraceae subf. Asteraceae*, *Mulinum* and *Nothofagus* (Table 3).

For 2270 ± 50 14C yr BP the pollen assemblage of B1Oq showed a vegetation dominated by Asteraceae subf. Asteroideae that changed to a vegetation represented by grass, herbs, *Asteraceae subf. Asteroideae* and *Mulinum* mainly [36] (Figure 3).

Discussion

Taphonomic processes and pollen preservation and concentration: sedimentation, erosion, exposition to fire, anemophilous pollen dispersal and antropogenic influence

Pueyrredón Lake area: Milodon Norte 1 Cave (CMN1): *relation with erosion and anemophilous pollen dispersal* In cave CMN1, samples conservation was good in all layers except in layer 6 (Figure 3).

The main cause of deterioration in this layer was due to mechanical damage (statistical significance 0.70- Table 1-Appendice). In addition, a low pollen concentration was observed and the main deteriorated pollen types were *Poaceae, Asteraceae subf. Asteroideae* and *Mulinum*. López Sáez et al. [29] suggest that one of the main agents that destroys pollen grains is mechanical degradation. During the transport and sedimentation phases, some pollen grains are more easily deteriorated than others. The pollen type Poaceae presented broken grains, which can be associated with the thin pollen wall more vulnerable to breakage due to the abrasion that could be exerted by loose particles such as sand in sediment deposition (pollen-clasts collisions). *Asteraceae subf. Asteroideae* and *Mulinum* presented degraded and crumpled but not broken grains. This may be due to the fact that the walls of these grains are relatively thick which prevents breakage by abrasion when compared to the characteristics of Poaceae. In the case of degraded grains (biochemical index: 0.10) it can be explained by the moderate presence of fungi in the sediments, which, although they did not exert a marked deterioration in the walls, damaged the ornamental structures. In these grains, the sculptural and structural details were determined with difficulty during the observation of the microscope. In relation to sedimentology, layer 6 is composed of sandy and carbonaceous sediments, which may be related to the poor amount and preservation of pollen grains present. According to Havinga [80] chemical compounds of a basic nature, such as carbonate, can act as degradation agents of the pollen wall. Another point considered in this cave was the presence of anemophilous pollen types, mainly *Nothofagus* as a transport pathway. In CMN1, *Nothofagus* presented high concentration possibly associated with an overrepresentation of the taxon. The orientation and height of the cave entrap the pollen grains carried by the winds from the west.

Milodon Norte 2 Cave (CMN2): an example of exposition to fire and antropogenic influence The archaeological layers of CMN2, presented an abundant amount of charcoal particles, which is an indicator of the presence of bonfires associated with cultural practises by hunter- gatherer groups. According to the archaeological data, this sequence has successive levels of bonfires, charcoal particles and the presence of bone fragments burned, mainly present in archaeological layers 2, 3, 4, 5 and 7. The high temperatures to which the pollen grains were exposed may be the main cause of conservation problems. In these grains, the structural elements seem to be partially fused, as if the exine were molded by some soft substance that had come together under the influence of pressure or heat. The high temperatures related to fire, such as the bonfire, are an extreme case of oxidation, which damages the wall quickly when oxygen is available.

In all the layers with pollen preservation problems it was observed that *Nothofagus*, Poaceae, Asteraceae subf. Asteroideae, *Mulinum* and *Euphorbiaceae* were the most affected pollen types. Havinga [13] has shown that the *sporopollenin* content of pollen grains is variable, and as *sporopollenin* is apparently the most oxidation-resistant component of the pollen grain, its abundance in the exine strongly affects the susceptibility of the grain to oxidative destruction. *Poaceae* and *Nothofagus* present walls with characteristics psilate and microechinate that may be more likely to be damaged and / or broken by mechanical factors such as the above mentioned.

These grains have a very thin exine that can disappear from the sediments by breaking up into pieces too small to be recovered or identified. In this cave the damage is mainly generated by broken or crumpled grains. Moreover, layer 2 presents an ash level that can alter the thermal properties and permeability of soils causing damage to the walls of the grains [10].

Also, this layers present moderate biochemical deterioration (Figure 3. B and Table 1- Appendice) that also affects the pollen preservation. Regarding the Spearman correlation coefficient, there is a strong correlation between mechanical deterioration produced by charcoal and the pollen concentration and between palynological richness and mechanical deterioration (Table 2). These results indicate that in CMN2 the lowest values of pollen concentration are associated with layers which have the highest index of mechanical deterioration (Table 2). The high charcoal proportion is an indication that the high temperatures of the bonfire not only affected the walls of the grains but also, in some cases, caused the total destruction of the grains. Furthermore, these layers showed grains with dark colors (Figure 2. j) and very low pollen sum (Figure 3. B). According with Campbell [10] the thermal alteration is principally observable as a progressive darkening of the grain, from yellow through orange-brown to red-brown, brown, and black. In CMN2, this depends on the site of the cave where was the bonfire and heat range in sediments. In addition, the few pollen types preserved were those with more robust exine.

On the other hand, pollen types such as Euphorbiaceae and Mutisieae are present at high percentages in this cave [34]. The pollination type of these taxa is entomophilous, so an underrepresentation is normally expected. The high values found may be due to the fact that these ornamental flowers could have been brought to caves by hunter-gatherer groups [34]. However, it is important to notice that the pollen representation variation could be related not only to bias in the preservation but also to differences in the anthropogenic use of archaeological sites.

San Martín Lake Area: Paisano Desconocido Cave (CPD) and Bloque 1 Oquedad (B1Oq): sedimentation, erosion and oxidation

The values of biochemical and mechanical deterioration index of CPD suggest that mechanical damage prevails in this site compared to the biochemical one (Figure 3. C, Table 1 - Appendice). The archaeofaunal records indicate the predominance of *Lama guanicoe* with signs of anthropogenic processing. In this sense, the archaeological record (manufactured artifacts and debitage) indicates the logistic use of this space, but temporarily. This site may have functioned as operational camps, where humans, hunter gatherers inhabitants could have articulated logistic items (catching prey and obtaining rocks and / or pigments) [68]. Therefore, the development of these activities does not seem to be the main factor that could have affected the physical preservation of the grains. The CPD site mainly presents colluvial-eolian laminated sediments with sandy-clayey lenses [68]. The accumulation of material of different size but homogeneous lithology included in a sandy-clayey matrix could have been transported by gravity and be one of the causes of the crumpled pollen grains by sediment transport. According to Súnico (personal communication) in the upper part of the cave, there is a process of erosion and moisture in the rock, mainly evident in the contact areas between the rock and the cave floor together with the strong accumulation of salts, which results in the slabs on the floor of the cave being released from the ceiling and pH changes in sediments. The presence of saline sediments may be related to crumpled pollen grains, mainly in 5, 6 and 7 layers. In Campbell [10], a tendency to rupture in distiller water and a to pucker inwards in stronger salinities in pollen grains were showed. Causes such as pH change in sediments, wet-dry cycles, may have favored the biochemical deterioration of pollen grains by oxidation. Campbell [10] and Havinga [13] have shown that the pollen is perhaps oxidized more readily than it is damaged by most other means. Oxidation is likely mediated in part by pH, particularly in soil. The microbial attack proceeds more readily on a pollen grain that has firts been somewhat oxidized, if only by prolonged exposure to air. In addition, the effect of repeated wet-dry cycles was shown to control the mechanical destruction of pollen grains since, it causes flexion with repeated shrinking and swelling [17,18]. In this study, according to the Spearman rank correlation coefficient (Table 2), biochemical deterioration has affected the pollen concentration to a moderate degree (-0.59). Given the calculation of the mechanical and biochemical deterioration indices for B1Oq, layer 6 seems to divide the sequence into two general deterioration patterns. In the upper part the mechanical deterioration prevails over the biochemical and in the lower part the opposite pattern is observed. The B1Oq site is composed of relatively homogeneous aeolian sediment without clear evidence of variation in composition and consolidation [69,70]. The abrasion between the sediments could have had an influence on the mechanical deterioration of the pollen grains (Figure 3. D, Table 3 and Table 1-Appendice). Although, there is a strong anthropogenic influence on the site for the late Holocene (guanaco bones with anthropogenic modifications and other archaeological signal). The intense use of the site and the increase in human activities in c.a. 1000 yr BP does not seem to be a determining factor in the mechanical damage found in pollen grains. Towards the end of the deposit (upper block) post-depositation processes are observed possibly by the action of the rodents; nevertheless this does not seem to be the determinant factor of the mechanical deterioration of the grains of pollen. There is a strong correlation between the rate of biochemical degradation and the palynological richness (Table 2). In this case, oxidation could also be the main cause of the biochemical deterioration found. However, there is no direct evidence of a factor that could be causing this biochemical deterioration.

In many environments of Patagonia there are not continuous records, so archaeological sites are an important deposit for palaeoenvironmental reconstructions [26,32,34,36]. However, it should be considered that the information provided represents temporal windows and may present some anthropogenic bias, so it is important to study the potentiality of the sequences through preservation analysis. On the other hand, it is important to compare the information provided by archaeological sequences with the palaeoenvironmental information provided by continuous sequences. In this sense, the archaeological sites considered in this paper (CMN1, CMN2, CPD and B1Oq) were compared with continuous sequences (LF and LT) published in Bamonte y Mancini [81]; Bamonte et al. [36]; Marcos et al. [82]. This comparison allowed the detection of biases in the pollen information of archaeological sites and complemented palaeoenvironmental interpretations. In this paper summary a schematic model of vegetation changes from the archaeological sites (CMN1, CMN2, CPD B1Oq) and non-archaeological sites (LF and LT) are presented; the details of the paleoenvironmental reconstructions of sites were published in Marcos et al. [71], Marcos et al. [82], Bamonte et al. [34, 84] (Fiure 4).

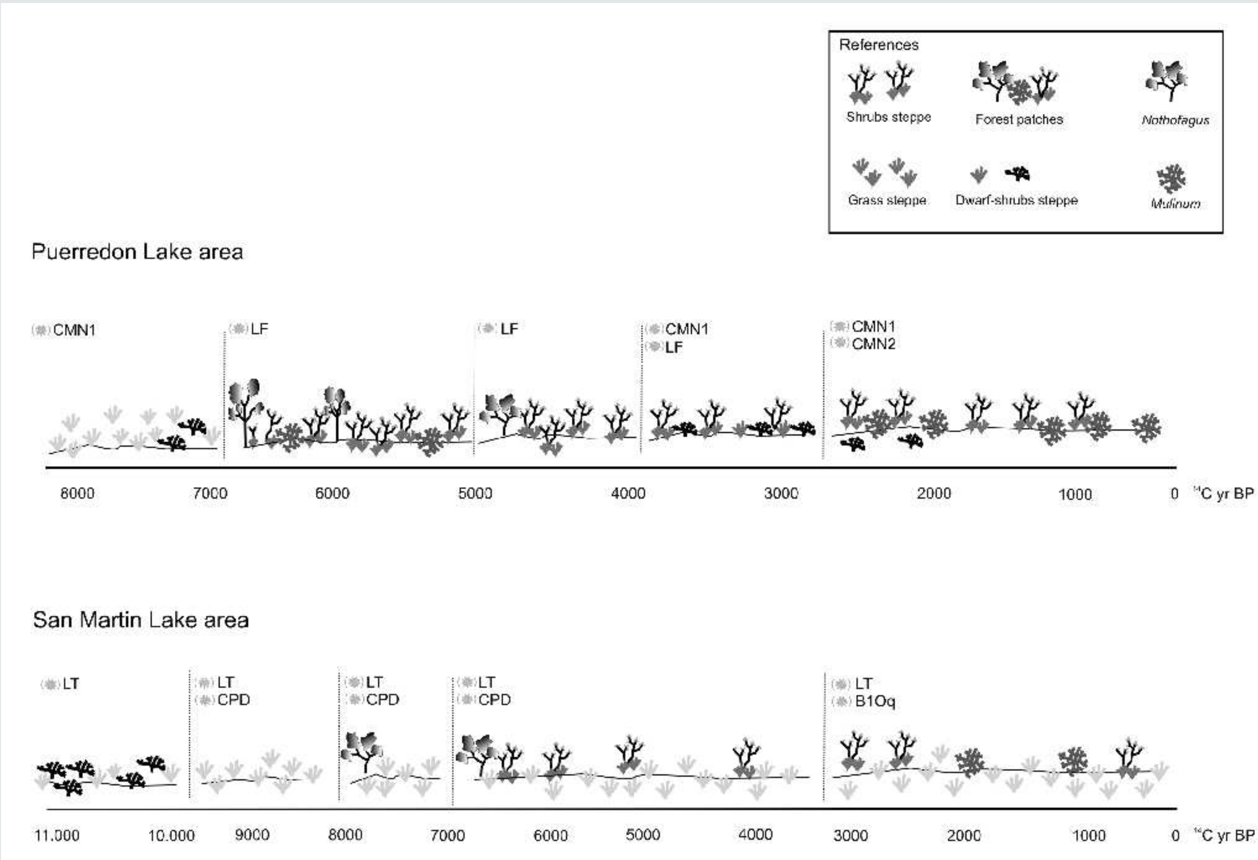

Figure 4: Schematic model of the vegetation dynamic of (1) Pueyrredón Lake area and (2) SanMartín Lake area. The starts indicate the site where the palaeoenvironmental information was obtained for each period. Details of the pollen information of each site has been published in Bamonte et al. [34], Marcos et al. [71].

Conclusion

According to the biochemical and mechanical indices, the samples of archaeological sites analyzed in this paper present a good and/or moderate preservation. In this sense, we concluded that this archaeological caves and rock-shelter (CMN1, B1Oq and CPD) studied in this paper present relevant information to perform palaeoenvironmental reconstructions. However, the preservation problem and low pollen sum present in CMN2 indicated that it is not rigorous enough to carry out apalaeoenvironmental reconstruction. The low pollen sums present in CMN2 are mainly due to the high temperatures (bonfire) at which the pollen grains were exposed and that completely destroyed them.

In the archaeological sites CMN1, B1Oq and CPD, there was evidence of preservation problems (biochemical and mechanical deterioration), but these were not significant. The main factors that affected pollen preservation were exposure to high temperatures, abrasion of the sediments, oxidation, pH effect that caused mechanical damage and the presence of fungi associated with biochemical damage.

In addition, we consider in this paper other complementary items in pollen analysis (contribution of *anemophilous* and exotic pollen types, antropogenic factors and post-deposition processes). The influence of the westerly winds and their consequent contribution of long-distance pollen overrepresented the *Nothofagus* signal in CMN1. In CMN2, the high values found of *Mutisieae* and *Euphorbiaceae* may be due to the fact that these ornamental flowers could have been brought to caves by humans. Antropogenic and post deposition factors had no significant influence on pollen deterioration. The analysis of these factors complements the pollen information obtained from each archaeological layer, as well as it allows to comprehension of other causes that have affected the preservation of the pollen.

The most samples of archaeological site analyzed in this paper present a good preservation condition. The general results show that the problems of pollen preservation of CMN1, CPD and B1Oq were low and / or moderate. In this sense, these sequences provide important information to perform palaeoenvironmental reconstructions.

At last, taking into account that archaeological sites in general represent temporary windows of palaeoenvironmental information, the interpretation of the information as a whole of the archaeological and non-archaeological sites allows to complement the palaeoenvironmental history available for the study areas.

Acknowledgements

This research was financially supported by CONICET (PIP 1265) , UNMdP (EXA 602/12, EXA 738/15) and PICT 1722/15. We thank Carlos A. Aschero, Teresa Civalero, Luis Horta, Juan Bautista Belardi, Silvana Espinosa for providing archaeological samples and Alejandro Súnico for his personal comments.

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