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Non-Pollen Palynomorphs from the Tiber delta area as indicators of past human activity

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Acadamic year 2019- 2020

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Masters Thesis

DIPARTIMENTO DI BIOLOGIA AMBIENTALE
Botanica

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Abstract

This research paper on Non-Pollen Palynomorph was conducted in Tiber delta (Rome, central Italy). Specifically, sediments from Lingua d'Oca-Interporto (LOI) near Fiumicino airport. By using palynological approach of Non-Pollen Palynomorph the relationship between humans' cultural behavior with the surrounding environment were studied. Thus, the main objectives of the research were to reconstruct the livestock activity of the area by focusing coprophilous fungi and also to evaluate the importance of NPP in the understanding of paleoenvironmental reconstruction.

In order to have adequate coverage of the lithological phases and to have good coverage of the environments identified by the pollen record, 25 samples were selected for NPPs analysis ca. 10 cm interval along the whole sedimentary sequence.

As a result, the sequence from Stagno di Maccarese reveals a total of 1563 Non-Pollen Palynomorphs. A total of 58 taxonomical groups were recognized with the highest number represented by fungal spores, plant fragments, spores of algae and animal remains. Cercophora sp., cf. Endophragmiella, Coniochaeta cf. ligniaria and cf. round charcoal is the most frequent palynomorph in the samples.

According to the research, the findings of the study provide new insights to reveal the subsistence selection of prehistoric humans and the history of livestock habitation in the surrounding archaeological sites. In conclusion, the identified NPPs shows the continual presence of animals in the past landscapes, supporting the evidence of pastures and livestock activities in the region. It also recorded the presence and the effect of humans on the landscape of the region with other natural factors

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First of all, I would like to thank the European Union Archaeological material science consortium for giving me this opportunity. I would also like to acknowledge, My Supervisors Professor Federico Di Rita and Professor Donatella Magri for providing guidance and feedback throughout this project.

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Abbreviations

AD-Anno Domini

AMS- Accelerator Mass Spectrometer

BRN-BuRreN

BP-Before Present

BC-Before Christ

EMA-Ernst-Moritz-Arndt-University of Greifswald

IBB- Innsbruck Institute of Botany – Austria

HdV-Hugo de Vries Laboratory, University of Amsterdam — The Netherlands

IBB- Botanical Institute of Barcelona

HCL-Hydrochloric Acid

HF-Hydrogen Fluoride

NaOH-Sodium Hydroxide

NPP- Non-pollen Palynomorphs

TM-University of Toulouse

LCE- Laboratoire Chrono-Environnement, University of Franche-Comté – France

LOI-Lingua d'Oca-Interporto

UAB- Universitat Autònoma de Barcelona – Spain

UG- Universiteit Gent – Belgium

PLN- Paleoecology Laboratory, Nevada – USA

µm-Micro meter

List of Figures

Plate 3

Figure 1 *Sordariaceae/ Apiosordaria verruculosa ?*

Figure 2 Glomus cf. *fasciculatum*

Figure 3 *Thielaviopsis/ Thielaviopsis basicola*

Figure 4 *Podospora sp./Zopfiella sp.*

Figure 5 *Pseudoschizaea*

Plate 4

Figure 1 Cf. round charcoal

Figure 2 Type UG-1277

Figure 3 Type UAB-15

Figure 4 *Thecaphora* Sp.

Figure 5 *Cymatiosphaera*

Figure 6 Scalariform perforation plate of *Alnus / Corylus / Betula*

Plate 5

Figure 1 cf. *Endophragmiella*

Figure 2 *Zopfiella lundqvistii*

Figure 3 conidium of cf. *Excipularia fusispora*

Figure 4 *Sporormiella*-type

Figure 5 *Diplocladiella scalaroides*

Figure 6 Type UAB-20

Plate 6

Figure 1 *Podospora* type

Figure 2 *Valsaria* Type

Figure 3 *Chaetomium* sp.

Figure 4 Type HdV-83

Figure 5 *Diporotheca sp. / D. rhizophila*

Figure 6 Type HdV-361

Plate 7

Plate 8

Plate 9

List of Tables

Chapter One

Introduction

Non-pollen palynomorphs (NPPs) are various organic microscopic objects with high resistance to the taphonomic process and chemical reactions that can be recovered after laboratory sample preparation for pollen extraction (Cugny et al 2010, Van Geel 2001, Van Geel 2006). Their identification can provide information about the depositional environment; thus, they have the potential to improve palaeoecological reconstructions. The term NPPs is used to describe organic remains from different organisms (e.g. spores of fungi, cysts of algae, eggs of animals, etc.).

The study of other organic remains from a pollen slide has begun in 1917 by Karl Rudolph. He published the first study about the fungal remain and other organic remains beside the study of pollens from south Bohemian peat bogs (Rudolph, 1917). Afterwards, Hebert, (1929) and David G. Frey (1964) published similar studies about the microfossil content found with pollen and micro charcoal remains in pollen slides. The first systematic research on NPP however was conducted in the 1980’s by Bas Van Geel from the Amsterdam University of Hugo de Vries. Since then the research of NPPs is increased both in terms of interpretation and studied sites all over the world.

Most NPPs are composed of sporopollenin-like material (a resistant material for both pollen and spores) which provide its resistance to various chemical treatments (Limaye et al, 2007). This special characteristic of the organic material enables them to record various information about the surrounding environment. The major groups of NPPs include algae (Nielsen &

Sørensen, 1992; Komárek and Jankovská, 2001., animalia (Brinkkemper and van Haaster, 2012) and fossil spores of fungi (Van Geel 2001; Kalgutkar and Jansonius, 2000; Shumilovskikh et al. 2015a; Webster and Weber 2007). Other NPPs which are rarely researched includes plant remains, (Van Geel, 1978 and Van Geel et al 1989), protozoa (Van Geel, 1978; Van Geel et al 1981,1989; Kuhry 1997; Prager et al, 2006), and ferns (Bakker et al, 1981).

Each non-pollen palynomorph can narrate specific environmental and human processes of the ecological context that they are recovered from. Accordingly, by analyzing NPPs, one can obtain information on the presence of livestock and different fauna species (Van Geel et al. 1983, 2003; Ralska-Jasiewisczowa and Van Geel 1992; Mazier et al. 2009), pH and salinity of water (Van Geel et al 1996; Van Geel et al 1994), farming and the utilization of fertilizers (Ralska-Jasiewiczowa and Van Geel, 1992; Van Geel et al., 1994), human impact surrounding the water bodies (McCarthy et al. 2018), development of parasites (Charman et al 2000; Mitchell et al. 2008), eutrophication, pollution, and various hydrological changes (Payne et al. 2012) environmental and cultural changes (Bosi et al., 2019; Carrion et al., 1999), lake responses and land use (Riera, López-Sáez, & Julià, 2006; Di Rita et al 2010; Feeser et al 2010; Jones et al 2018; Van Geel 2001). Despite their potential for the understanding of cultural, biological and environmental history of a given archaeological site, NPPs are still understudied.

1.1.Statement of the problem

The present study investigates the presence, type, and interpretation of NPPs from the Tiber delta (Rome, central Italy). Specifically, sediments from Lingua d'Oca-Interporto (LOI) near Fiumicino airport. Nearby, archaeological settlements from the Copper Age site Le Cerquete-

Fianello to the Roman saltworks have yielded much information about the cultural background of the area (Di Rita et al. 2010). Therefore, analyzing the non-pollen palynomorphs data will help us understand the relationship between humans' cultural behavior with the surrounding environment.

This area has been a focus for various researches for a long time in archaeological, geological, and palaeobotanical disciplines. Palynological research about the vegetation and environmental changes between 8300-0 cal BP of the Satagno di Maccaresa has been published. This research was based on the analysis of pollen, seeds, wood, and micro-charcoal (Di Rita et al., 2010). The result shows that the area was unstable due to changes in lake level, introgression of marine water, eutrophic phase, flood events, desiccations, and opening of the forest vegetation. Despite their potential in the narration of a given archaeological site's cultural, biological, and environmental history, NPPs of this site were not fully investigated. Therefore, this research will help to understand the use of NPPs in the reconstruction of paleo-environment processes and human presence, and it is the first comprehensive examination of NPP from this site.

1.2.Objectives

The research's general objective is to reconstruct and understand the presence of stock breeding activities in the area with human activity and vegetation change.

The specific objectives of the research include

1. To reconstruct the livestock activity of the area by focusing coprophilous fungi
2. To evaluate the importance of NPP in the understanding of paleoenvironmental reconstruction

1.3.Study area

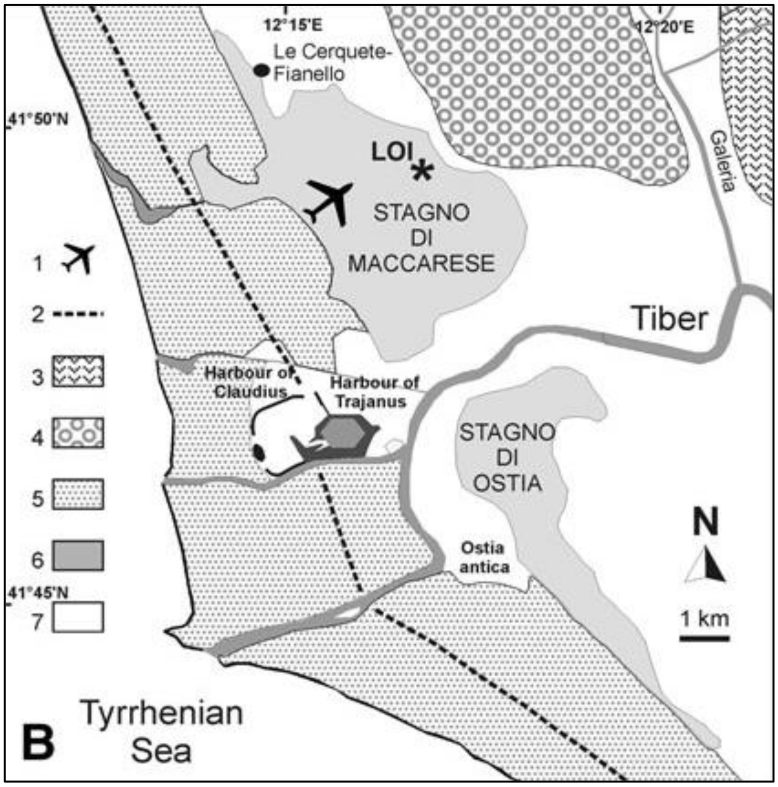
The research was conducted from Lingua d'Oca Interporto near the Fiumicino international airport, which was occupied by the Stagno di Maccarese of Tiber river, 20 km southwest of Rome.

The Tiber river is a microtidal wave-dominated delta supplied by mud-sandy sediments (Galloway, 1975). Tiber river delta is stretching over 38 km along the Tyrrhenian coasts of the Latium region (Milli et al., 2013; Di Rita et al., 2015). Inner alluvial-marshy sediments and outer strand-plain characterize the plain of the Tiber river formed during the Holocene period, which was transformed from the former estuary, a submerged Tiber paleo-valley, to the present wave dominated delta (Bellotti et al., 1995).

The hills adjacent to the Tiber delta, which are characterized by coastal and fluvial deposition, result from sea-level fluctuation occurred around 100 ka BP (Amorosi & Milli, 2001). During the Holocene, the Tiber delta topography was split into two main phases caused by different types of depressions, Stagno di Ostia southern side of the Tiber mouth and Stagno Maccaresse at the northern side (Bellotii et al., 2007). The main vegetation type covers of the area including sclerophyllous, riparian, aquatic vegetation, and mixed deciduous. (Lucchese 1996; Manes et al. 1997).

According to pollen sequence, abrupt climatic fluctuation of the late-glacial and Holocene period, regional environmental shift, and hydrological changes play a significant role in vegetation changes in the area (Di Rita et al, 2010).

A palaeobotanical analysis was carried out on the lacustrine sediments excavated during an archaeological survey in the area of Fiumicino international airport (Di Rita et al., 2010, 2012). Around 8300 and 5400 cal. BP, the area was dominated by evergreen and deciduous vegetation. At about 5400, cal BP the pollen record shows an abrupt change in plant distribution and landscape, dominated by Poaceae and Cyperaceae's open landscape, accompanied by other herbaceous taxa. This period also presents the first glimpse of human activity, evidenced by local fire and cereal type pollen, which is in the same chronological context of the nearby Eneolithic settlement of Le Cerquete Fianello, dated between 5200-4950 cal BP. Human activity from the Eneolithic to Etruscan period lacks appropriate evidence in the pollen research. Subsequently, the first evidence of saltworks dates back to 2500 cal. BP during the Etruscan period. Followed by the extensive exploitation of the Romans in about 1800 cal BP. A new vegetation cover of arboreal type is recorded. Human markers were abundantly available since the Romans (Di Rita et al., 2010).



1. Fiumicino airport
2. Coast line of the 1st c AD (from Tuccimei et al. 2007)
3. Plio-Pleistocene sands
4. middle and upper Pleistocene sands and gravels.
5. Holocene beach ridges
6. Ancient ponds near the Tiber delta,
7. Holocene alluvial deposits (modified from Giraudi 2004; Tuccimei et al. 2007)

Fig. 1. Location of the Lingua d'Oca (LOI) site (Modified from Di Rita et al, 2010)

1.4. Literature Review

In order to put the main research in an appropriate frame, the review of a related literature will briefly describe research conducted around Tiber River delta. The selected researches are mainly focused on palynological and archaeological investigations.

Di Rita et al. (2010) published an article by conducting a palynological and palaeobotanical, study on the Tiber delta and the same samples that this thesis also analyzes. The research's primary objectives were to understand how local vegetation history was influence by local geomorphic and regional climatic changes. Moreover, to understand the role of human impact in the evolution of coastal landscape surrounding the study area. Accordingly, for the last 8300 years, the area witnessed vegetation cover and environmental fluctuations. Local geomorphic process and environmental factor plays a significant role in the process of the vegetation history. Despite the presence of archaeological settlements in the area, human activity in the area's landscape evolution seems to have had a minor role than geomorphic processes in shaping the vegetation landscape. Except for salt works, whose vegetation signal is well represented in the pollen diagram, most human activity lacks direct evidence in the pollen record of Lingua d’Oca-Interporto.

Based on geomorphic, stratigraphic, faunistic, palynological, and carbon isotope (Bellotti et al., 2011) conducted systematic research in Tiber estuary, specifically of the Roman town Ostia. The project was aimed to tackle four research questions. 1) to reconstruct the progression of the deltaic cusp of the river, 2) to reconstruct the vegetation landscape of Tiber delta from the early bronze age to the end of the roman empire, 3) to understand and reconstruct the development of Ostia by comparing the scientific approach with historical documents, 4) to evaluate the role of human vs. natural impact on the river mouth of Tiber delta. As a result, the research shows that Tiber delta's vegetation experienced a significant change involving both terrestrial and aquatic environments in response to the deltaic cusp's progression and the Tiber shifts distributary. Furthermore, between 1900 and 600 BC, the pollen sequences show that the landscape was dominated by a mixed oak-dominated woodland with a patchy evergreen plant. Around 600 BC, the marshland was changed into a chenopod dominated saltmarsh by unexpected intrusion of seawater into the basin. Human activities were existing from 450 BC onward, which is evidenced by cultivated and anthropochore plants.

Belloti et al. (2018) conducted a multiple approach to reconstruct the how the landscapes of Tiber delta plain changed over the past 3000 years. The research team trying to relate and identify the human and natural factors that alter the landscape. Accordingly, the natural factors include climatic changes, sea level rise, tectonic and local subsidence. Whereas, anthropic factor consists of population density, farming, breeding practices, reclamation, construction of ports, canals and saltworks.

Another paleobotanical research aimed at understanding human-environment interaction in the Tiber mouth region was carried out by Sadori et al. (2010). The research focused on the Tiber delta of Rome's ancient harbor, which was constructed by Claudius in 42 AD. Two core samples were examined for the analysis. A dock core dated between 2nd to 4th century AD yielded a plant landscape that is a typical coastal environment character and scarce evidence of human presence (Lack of cultivated weed and ruderal taxa). On the other hand, the sediment of channel core presents a strong human impact in the 1st century AD, evidenced by the high percentage of synanthropic taxa and fire use evidence. Accordingly, human impact shows a significant increase over the centuries. The presence of Portus town evidences this increase in population. The authors concluded that this is an excellent example of how the human population can shape the plant landscape.

In order to understand the transition of the Tiber delta from the wave-dominated estuary to a wave-dominated delta Milli et al. (2013) carried out a petrographical and paleontological study. The stratigraphic architectural structure of the Tiber delta between the late Pleistocene and Holocene period was examined from the sediments of Pesca Luna site. Accordingly, the preserved succession, the completeness and thickness of sediments showing the transitional passage from a wave-dominated estuary to a wave-dominated delta. The research confirms that wave-dominated estuary's transgression and infilling affected the lithofacies distribution of the area (Mille et al., 2013).

Giraudi (2011) conducted a geo-archaeological investigation around the Campo Salino area, a drained part of Stagno di Maccarese. The sediment analysis, erosion surface, and artifacts were done for the last 3 millennials based on the stratigraphy. Accordingly, during the Holocene, the Maccarese marsh experienced extensive and frequent natural and artificial environmental alterations. Extreme variations in salinity and water level changes are the most important modifications. The researcher agreed with increasing human influence during the 1st century AD as already reported in Di Rita et al. (2010) and (Sadori et al., 2010).

Pepe et al. (2016) carried out a research around the ancient Holocene Tiber meander to reconstruct the paleoenvironmental evolution from the 4th century BC to present day. They utilize analysis of pollen and Non-pollen palynomorphs in a sediment core drilled from southern part of palaeo meander Fiumo Morto (Dead River), in the Tiber delta area near ancient Ostia central Italy. Accordingly, the research revealed that forest canopy dominated the area with some riparian, Mediterranean and mesophilous arboreal floras. Those vegetation covers are evidencing a transition environment. In addition, the presence of cultivated and ruderal taxa shows strong human presence.

Palynological sediments, and archaeological sites from the Eneolithic, Middle/Late Bronze Age, to Roman and medieval age were identified various human settlement sites surrounding the delta (Manfredini, 2002; Morelli et al., 2004).

One of the most important archaeological sites is the lake dwelling of Le Cerquete, Fianello, dated back to 5370-4920 cal BP and located at the margin of the ancient lake. This site presents the existence of pastoral and agricultural activities. Excavation reveals a large settlement area with the shreds of evidence of huts and activity areas with high pottery concentration (Manfredini, 2002; Forte, 2012). A mid-late Bronze Age settlement site was also discovered near the Le Cerquete Fianello (Arnoldus-Huyzendveld et al. 1998; Carboni 2002). In 6th century BC, the Etruscans built a large artificial saltworks in the Maccarese area, whose economic resource attracts Romans (Morelli et al., 2004). The Romans developed the system of saltworks called *Campus Salinarum Romanarum*, which was active until the 15th century AD (Lanciani, 1888). Because of its important location, different emperors preferred the area as a harbor and saltworks area. The western part of Stagno di Maccarese was selected to reconstruct the harbor by emperor Claudius 54-60 AD, then by Emperor Trajanus 98-117 AD. Up until the 12th century, the harbors and saltworks occupied both sides of the delta (the harbor of Ostia and Portus were giving service for the community (Verduchi, 2004). The presence of these archaeological sites surrounding the Tiber river delta helps us understand the impact of humans on the area's environment and *vice versa*.

Chapter Two

Materials and Methods

This research on the NPPs of the Tiber delta was carried out based on both primary and secondary data. Processed soil samples for pollen analysis (Di Rita et al. 2010) and Non-pollen palynomorph information were included in the primary data. Works of literature about NPPs, about the Tiber delta vegetation history, palynological, sedimentological studies, and the area's archaeological records are amongst the secondary data. Furthermore, the research applies both qualitative and quantitative data analysis methods. Qualitative data were gathered from the description of the NPPs from various books, articles, and databases quoted below. Quantitative data was gathered by counting the NPPs from each sample.

This research will only focus on the NPPs data of Stagno di Maccaresa, precisely the Lingua d'Oca Interporto sedimentary sequence (LOI). Furthermore, to meet the research objectives, the researcher will focus on palynomorphs, which are indicators of human and livestock presence.

2.1. Sampling and analytical procedures

The samples for pollen NPPs analysis were taken from the sediments that emerged after an archaeological excavation and survey carried out in 2001 in the modern-day Fiumicino international airport near the Lingua d'Oca canal. For pollen analysis, samples were taken at 5 cm interval along 320 cm thick sedimentary sequences (Di Rita et al., 2010). In order to have adequate coverage of the lithological phases and to have good coverage of the environments identified by the pollen record, 25 samples out of 64 samples were selected for NPPs analysis ca. 10 cm interval along the whole sedimentary sequence. NPPs were extracted in the same procedure of pollen extraction. Each sample was chemically treated with HCl (37%), HF (40%), and NaOH (10%). In order to estimate the pollen concentration, exotic *Lycopodium* spores were added in the first phase of the treatment (Di Rita et al., 2010).

The examination of the obtained palynomorphs includes the taxonomic identification of fungal, algae, plant, and animalia micro-remains and the determination of the relationship between different taxa. The identification of taxa is by the examination of the grains, shape, size, color, wall structure, and other morphological characteristics. Usage of the term 'Type' follows van Geel (1978). For each sample, an average of 80 NPPs was counted. To count and scan the NPPs, light microscope of 400x magnification was used, and the S-Eye microscope camera was used to take images. Furthermore, Microsoft excel and Psimpoll computer software were employed to plot the NPPs in the diagram. The taxonomic identification of the NPPs was based on referring the descriptions and illustrations from the NPP database (i.e., http://nonpollenpalynomorphs.tsu.ru/index.html and https://www.wikis.unikiel.de/non_pollen_palynomorphs/doku.php/home). Additionally, some publications on NPP's morphology were used for taxonomic identification. (e.g. Feeser and O'Connell 2010; Haas 1996; Mudie et al. 2011; Cugny, et al. 2010; Hillbrand et al. 2014; Van Geel, 2001, Gelorini et al. 2011).

The acronym followed by a hyphen and a progressive number indicates that the NPP type could not be referred to a specific taxon at the moment of publication, e.g., HdV-303. Furthermore, the acronym identifies the lab in which the NPP type was described and where specimens preserved in palynological slides should be stored. In some cases, a capital letter follows the type number without space, e.g., HdV-128A, HdV-128B, and HdV-55A. It indicates a different NPP type whose morphology may indicate a taxonomical relationship with the type having the same number (Miola 2012).

2.2. Lithology and Chronology

Identifying the lithology of a particular sample is vital in understanding the nature, survivability, and existence of any biological organism. The relationship between the lithology with the NPPs type is used to understand the nature of the non-pollen palynomorphs and how those soil types affect palynomorphs. The sediments of (LOI) shows the following sedimentary sequence and stratigraphic composition.

Depth	Sediment lithology
0-80	Greyish silt, rich in Cerastoderma sp. and other brackish molluscs
80-134 cm	Grey silt with dark levels of organic mud
134-185 cm	Black organic silt
185-275 cm	Peat with abundant wood remains

Table 1 Stratigraphic composition and sedimentary sequence (Modified from Di Rita et al., 2010)

AMS radiocarbon dating was carried out on the organic content of the sediments sample at the CEDAD Laboratory, University of Lecce. Radiocarbon dates were calibrated using the program Bcal with IntCal04 calibration datasets. Furthermore, the age-depth models were calculated by the Psmipoll computer program.

Lab. code	Depth (cm)	^{14}C a BP	Calendric age (cal. a BP	Material	Rejected
LTL2073A	30-32	2318 ± 45	2311 ± 153	Molluscs/sediment	x
LTL1494A	70-72	1955 ± 50	1898 ± 144	Bulk sediment	
LTL1495A	138-140	2967 ± 60	3118 ± 165	Bulk sediment	
LTL2074A	198-200	706 ± 65	643 ± 97	Wood/sediment	x
LTL1496A	256-260	4529 ± 55	5089 ± 174	Bulk sediment	
LTL2075A	278-280	4618 ± 60	5427 ± 189	Bulk sediment	
LTL2076A	313-315	7191 ± 60	7984 ± 139	Bulk sediment	

Table 2 AMS Radiocarbon dating of Lingua d'Oca sediments (After Di Rita et al., 2010)

The age-depth model produces five reliable dates (see fig 2). Two dating errors were observed and excluded from the data. First, at 198-200 cm, a sample taken from the root shows a recent date, and at 30-32 cm dated to 2311 ± 153 cal a. BP show inconsistent with the other dates, it might be the presence of marine mollusks (Di Rita et al., 2010).

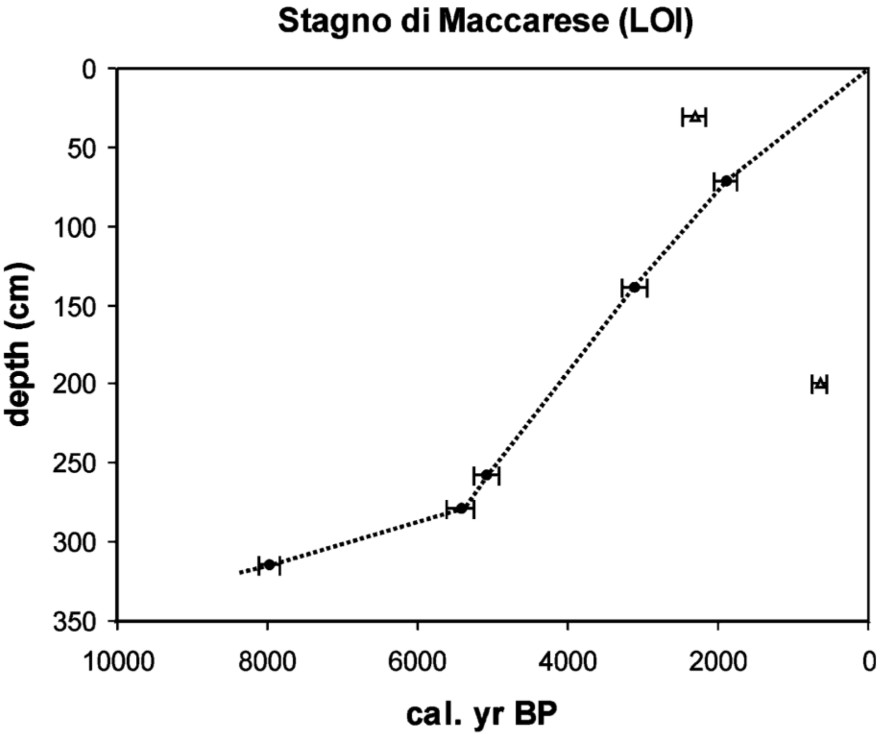


Fig. 2. Age-depth model for Lingua d'Oca-Interporto sequence

Chapter Three

Result

The sequence from Stagno di Maccarese reveals high number of NPPs. From the analyzed 25 samples a total of 1563 NPPs were identified. A total of 58 taxonomical groups were recognized with the highest number represented by fungal spores, followed by plant fragments, spores of algae and animal remains (fig. 3). The maximum number of NPPs counted in one sample is 353.

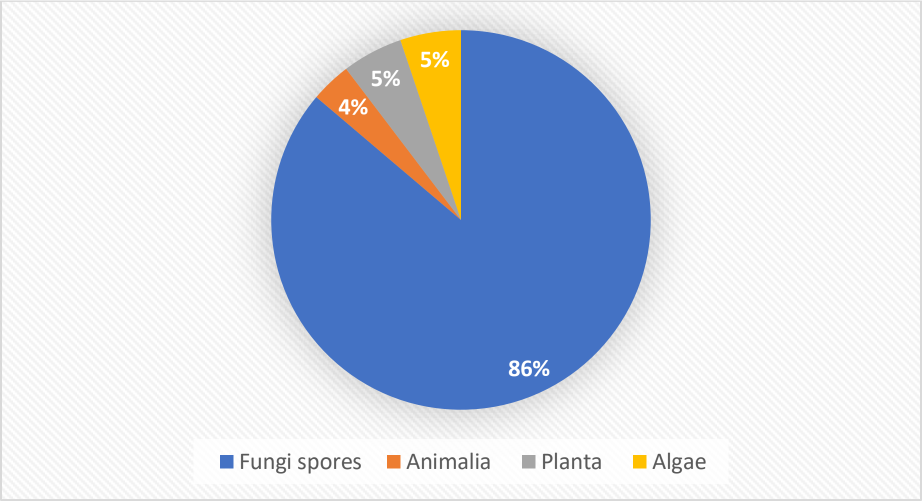


Fig. 3. Taxonomical composition of the NPPs

Cercophora sp. is the most frequent palynomorph in the samples, with a remarkable presence especially in LOI 240 cm. This fungus shows high concentration between LOI 270-200 cm. Another fungi cf. *Endophragmiella* is also present in a high concentration in LOI 200 cm and LOI 180 cm with a sparse distribution in the other samples. A type of NPP derived from plants, cf. round charcoal (Barthelemes et al, 2006), is present in a high concentration between 90 and 30 cm. A fungi ascospore which is present from 270 to 160 cm is *Coniochaeta cf. ligniaria* and it is observed also in other two samples LOI 60 cm and LOI

40 cm. *Chaetomium sp.* is also one of the most frequent fungi ascospores. It is highly concentrated in the interval 250-200 cm, while it is present only once in the upper part of the sequence in LOI 180 cm.

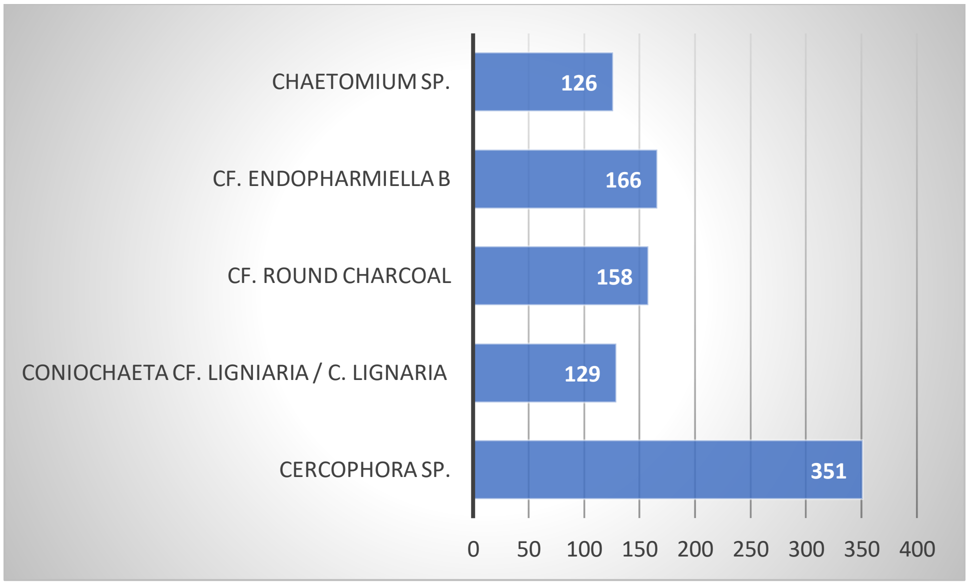


Fig. 4. Graph showing most frequent NPP taxa identified

According to the dated samples, the number of NPPs are high in LOI 256-260 cm (5089 ± 174 cal a BP) followed by LOI 138- 140 (3118 ± 165 cal a BP) and LOI 70-72 (1898 ± 144).

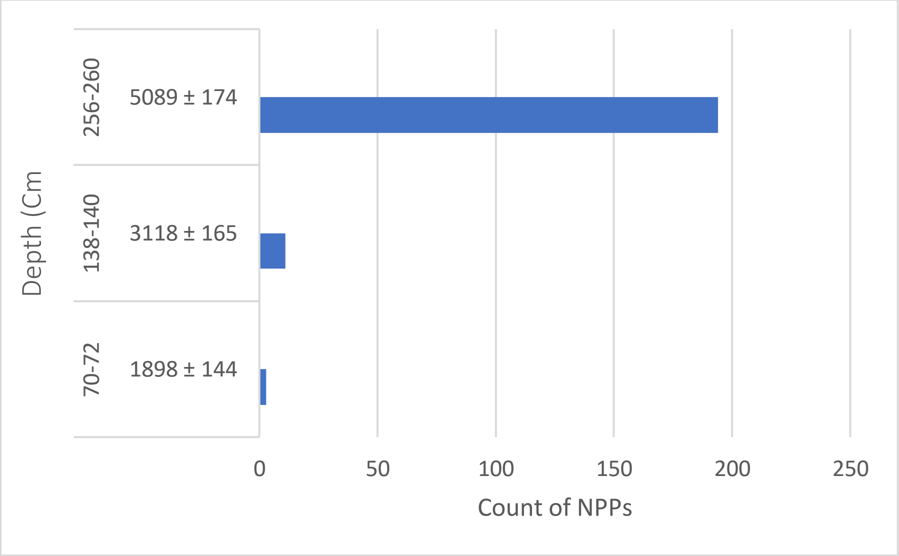


Fig. 5. Dated samples and the abundance of NPPs

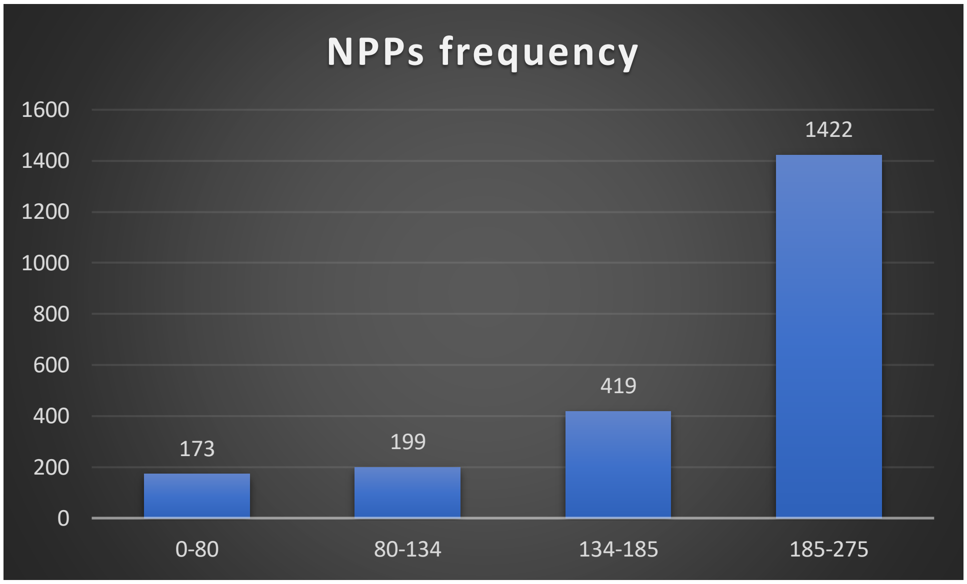


Fig. 6. Soil Type and frequency of NPPs

The different lithological facies show a different frequency in the presence of Non pollen palynomorphs (see fig 6). Peat sediments are characterized by abundant wood remain (185-275 cm) with a high number of NPPs. Black organic silt (134-185 cm) it also has high amount of NPPs, comparing with the Grey silt with dark levels of organic mud (80-134 cm) and Greyish silt with brackish molluscs (0-80 cm). Generally, the lowest frequency of NPPs was observed in the samples at 10 and 20 cm represented by Greyish silt with brackish molluscs. Conversely, the highest concentration of palynomorphs was in the sample 270, 240 and 200 cm, which are represented by peat sediments with abundant wood remains.

In order to facilitate the comparison of the the NPPs record with the results of pollen analysis published in Di Rita et al. (2010), I have included the NPPs of the present study in the Local Pollen Zones identified by Di Rita and co-authors (2010).

NB. The percentage presented here is only for the dominant NPP types and the one which is important indication of livestock and human presence..

Zone LOI-2 (270 -260 cm ; 5400–5100 cal. a BP)

This zone presents a total of 408 NPPs. It is dominated by *Coniochaeta cf. ligniaria* 22 % (see fig 7) *Podospora* sp. / *Zopfiella* sp. (HdV-466) 18%, Type HdV-121 which takes 17% of the distribution followed by cf. *Podospora polysporus* and 15 %, *Delitschia* spp. 12% and mentioning 8% of Glomus and 8% of *Cercophora* sp. is important.

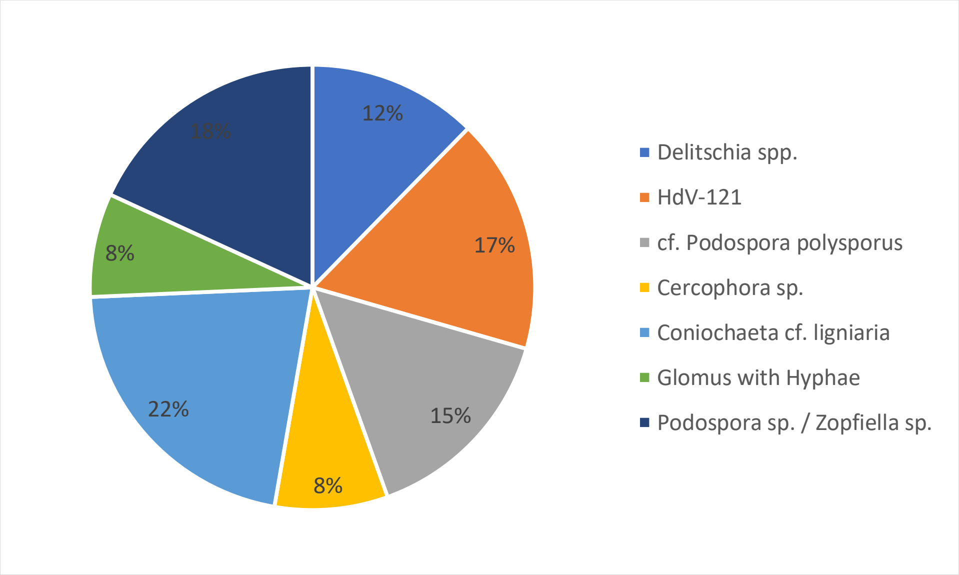


Fig.7. Dominant NPPs in Zone LOI-2 (270 -260 cm ; 5400–5100 cal. a BP)

Zone LOI-3 (260- 140 cm ca 5100–2900 cal. a BP)

1223 NPPs were counted. It is mostly represented by *Cercophora* sp (see fig. 8). shows high frequency with 42% followed by *cf. Endophragmiella* 21% and *Chaetomium* sp. 16 %. *Podospora* and *Coniochaeta cf. ligniaria* has both 8 %. *Delitschia* spp. reach 5%.

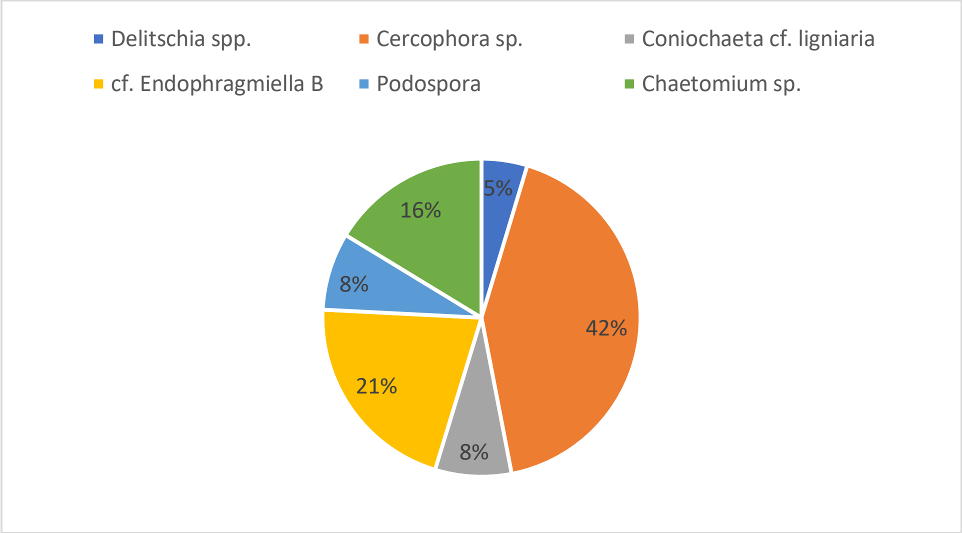


Fig.8. Dominant NPPs in Zone LOI-3 (260- 140 cm ca 5100–2900 cal. a BP)

Zone LOI-4 (140-80 cm ca 2900–2000 cal. a BP)

A total of 230 NPPs were counted. This zone is dominated by Type HdV-200 (49%), Type HdV-361 (15%), cf round charcoal 15% (see fig.8.). With a sparse frequency and distribution of Type-30B (7%), Type UG-1277 (8%) and Type UAB-20 (6%).

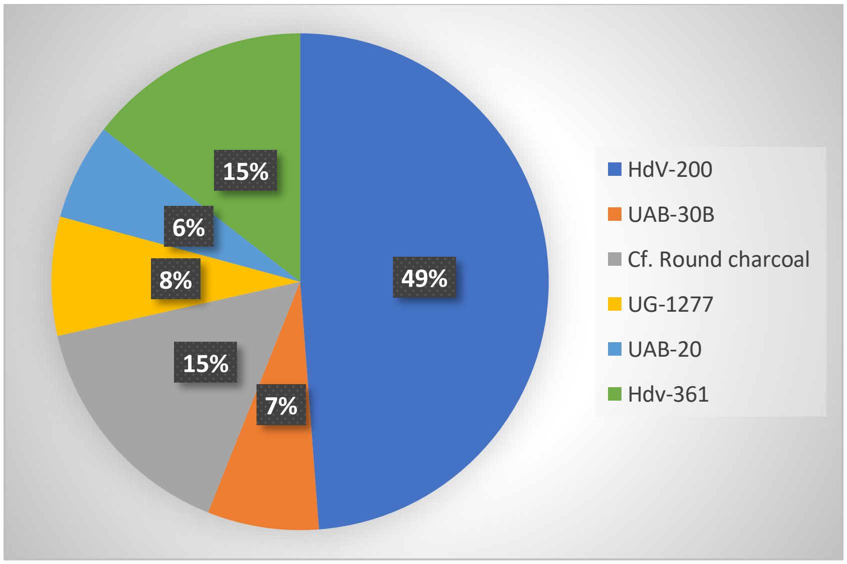


Fig.9. Dominant NPPs in Zone LOI-4 (140-80 cm ca 2900–2000 cal. a BP)

Zone LOI-5 (80-10 cm ca. 2000–0 cal. a BP)

The diversity and abundance of non-pollen palynomorphs decrease in this biozone. a total of 153 NPPs were counted. This zone is dominated by cf round charcoal 93% (see fig.11). Another noteworthy mentioning NPPs includes *Coniochaeta cf. ligniaria* has 5%, and *Sporormiella*-type (2%).

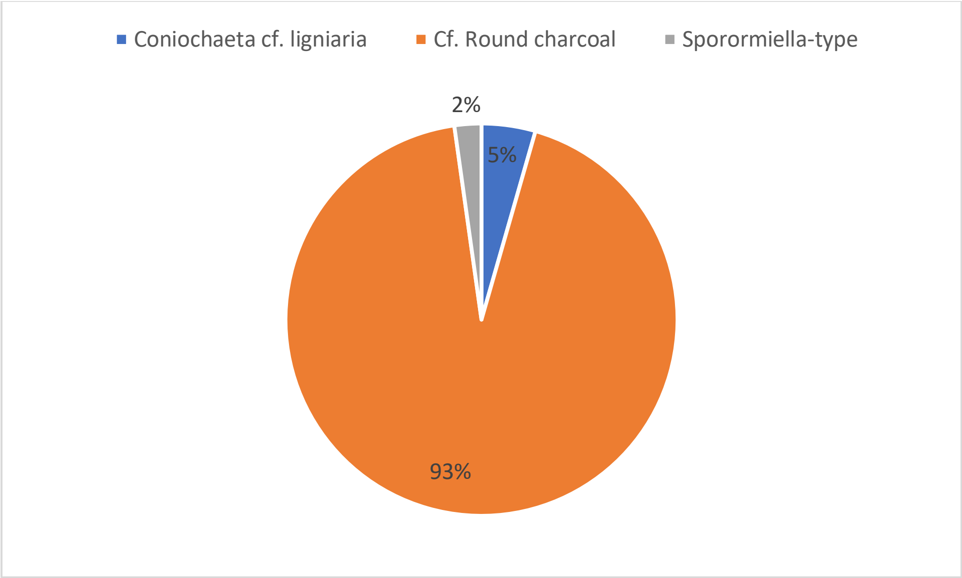


Fig.10. Dominant NPPs in Zone LOI-5 (80-10 cm ca. 2000–0 cal. a BP)

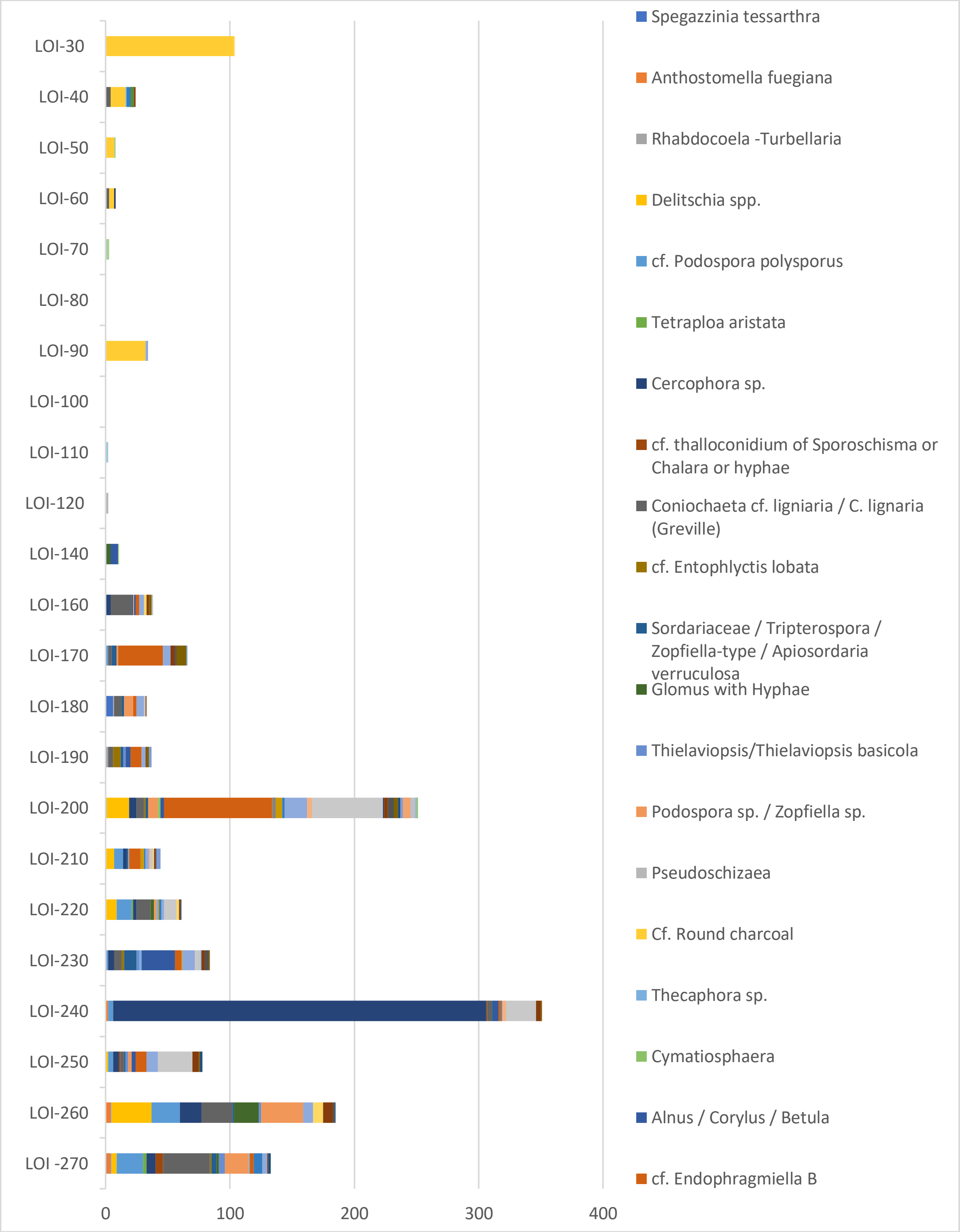


Fig. 11. Graph showing the Presence of NPPs in different samples

3.1. Description, illustrations and indicators of the NPPs

Type HdV-1018B; cf *Spegazzinia tessarthra* (Plate 1-1)

Van Geel et al. (2011): septate conidia, 14–18 µm in diameter, excluding spines up to 3 µm long (Type HdV-1018A). Smooth conidia also occur (Type HdV-1018B). Gelorini et al. (2011): this fungal morphotype occurs in two more or less distinct types. Conidia of Type B are septate, equally and symmetrically 4-celled, brown, 14–18 µm in diameter, smooth, thick-walled. The species is known to occur especially on various tropical plant species (Ellis, 1971). Gelorini et al. 2011: *Spegazzinia tessarthra* is widespread in tropical and subtropical regions. It is particularly common on dead leaves and stems from various monocotyledonous plants, such as maize, grasses, and *Andropogon* (Ellis, 1971; Subramanian, 1971). In our data 8 specimens were counted.

Type HdV-4; *Anthostomella fuegiana* (Plate 1-2)

Van Geel (1978): Ascospores 18-25 x 5-7 µm, inequilateral (one side almost straight), tapering apically into a sharp point, basal end truncate. Germ slit extending over about half of the length of the spore along the straight side. The palaeoecological context shows that the fossil fruit-bodies' presence indicates that it grew in the peat vegetation. The identifiable fossil remains of *Eriophorum vaginatum* are subterranean parts of the plants, occurring in the peat at a lower level than the unrecognizable leaves in which fruit-bodies of *A. fuegiana* could develop. Ascospores and especially fruit-bodies, may be good indicators of the presence of *E. vaginatum* (and possibly of other *Eriophorum* spp.) in the local vegetation. However, the absence of the fungus does not necessarily imply the absence of the host plant. A total of 9 specimens were identified in LOI samples.

Type HdV-353A/B; *Rhabdocoela Turbellaria* (Plate 1-3)

Van Geel et al. (1981): bag-shaped microfossil type. 90-150 µm in length; wall ca. 0.5 µm thick, smooth. HdV-353A - D have in common that they have a large opening at one side initially covered by a valve. The characteristic of HdV-353A is the apical appendage. Specific identification of these microfossils is highly desirable because Frey (1964) reports a restricted temperature range of some of the species (Van Geel et al. 1981). According to illustrations of Frey (1964), HdV-353A and 353B are cocoons of representatives of the non-parasitic flatworm order *Rhabdocoela* (Class Turbellaria). A total of 7 specimens were counted.

Type HdV-200 (Plate 1-4)

Van Geel et al. (1989) described it as a coiled cluster of 5-10 globose fungal cells; the diameter of the clusters is (18)30-38(-45) µm. Individual cells vary in size; the smaller cells are often concentrated at one side of the cluster. One of the smaller cells is often tapering into a short ca. 2.5 µm wide hypha, broken off at the end (the former connection with a mycelium). High amounts of HdV-200 and -201 in a sequence coincide with a phase with *in situ* occurrences of *Equisetum fluviatile*, *Phragmites*, and *Carex rostrata*. Several unspecific plant tissue pieces were observed with many HdV-200 fungi in organic connection with it. Repeatedly, HdV-201 spores were present on the same tissue. After an aquatic phase, the fungal Types 200 and 201 were the first fungi to show manifest increases. This fungal microfossil indicates relatively dry microhabitats on the standing culms of helophytes or plant remains in temporary desiccating bottoms of pools. In our sample a total of 126 specimens were identified.

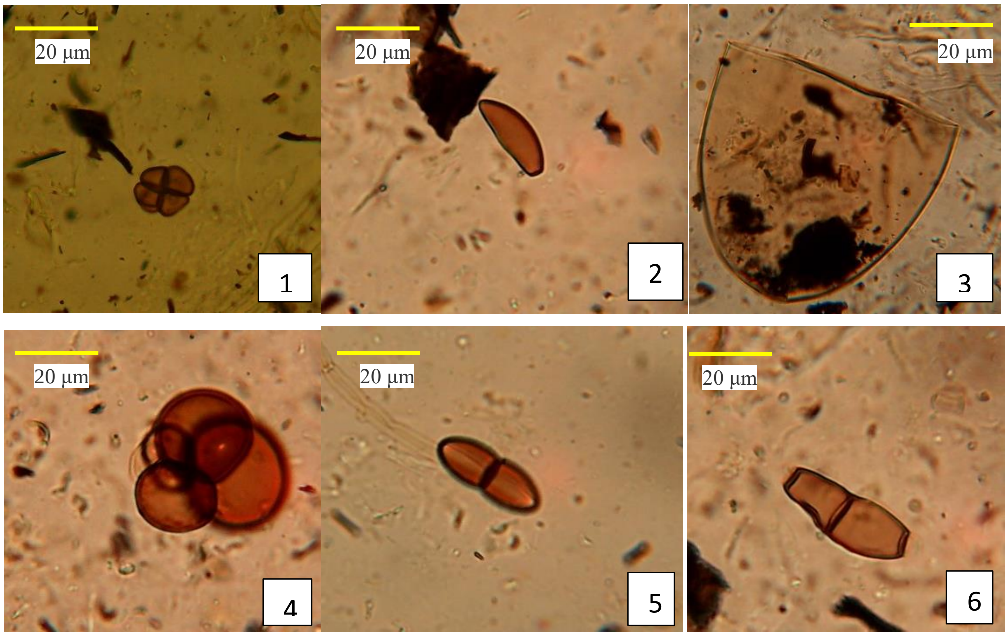
Type UG-1066*; Delitschia* spp. (Plate 1-5)

Gelorini et al. (2011): ascospores ellipsoid to broadly fusiform, unequally and unsymmetrically 2-celled, brown to dark brown, 20–30(37)x9–10(15) µm, smooth, thick-walled, often constricted at the septum; each cell with a straight germ slit parallel to the long axis of the ascospores. Based on differences in the size of the ascospores and position of the germ slits, this morphotype may include some *Delitschia* species. *Delitschia* species are mostly coprophilous, occurring worldwide on various dung (Bell, 1983; Hanlin, 1990). 72 specimens were counted from LOI.

Type HdV-121;(Plate 1-6)

Pals et al. (1980): spores three-septate, somewhat constricted at the septa. The two end cells very pale, often totally damaged. The two central cells measure 29-36 x 9-13 micron. Type HdV-121 seems to have its optimum in the lake deposits. Kuhry 1997: This Type was found in low frequencies in *Typha*-dominated phases. Its occurrence is probably related to temporary drying up of the wetland surfaces, especially in the late summer and early fall. A total of 83 specimens were counted.

Plate 1



Type HdV-89; *Tetraploa aristate* (Plate 2-1)

Van Geel (1978): Conidia verrucose, consisting of (3-)4 columns of 2 or 4 cells, each column terminating in a septate appendage, verrucose pigmented. Gelorini et al. (2011): conidia ellipsoid to rectangular, 3–4 columns with four cells to each column, yellowish-brown, 35–40×20–25 µm, verruculose, thick-walled; terminating in septate appendages, 12–80 µm long (frequently broken), 5–8 µm wide. *Tetraploa aristata* is a widespread fungus, usually found on leaf bases and stems just above the soil. It is relatively rare only 4 specimens were present.

Type PLN-03 (Plate 2-2)

Tunno and Mensing (2017) described a fungi mono-septate, yellow hyaline fungal spore with one aperture on each opposite side. So far, no ecological importance is identified for this fungus. Only 1 specimen were counted.

Type HdV-112; *Cercophora* sp. (Plate 2-3)

Van Geel et al. (1981): ascospores of (13)15-18(21) x 6-8(14) micron. Truncate at basal tile side and tapering at the apical end. One subapical pore ca. 0.7 micron in diameter. Sometimes with one septum, and if so, there is no constriction at the septum. Some specimens still with the hyaline appendage at the basal side. Van Geel et al. (2003): these ascospores are 13–27 (-36) x 6–14 (-18) µm in size, truncated at the basal side and tapering at the apical end. The subapical pore is ca. 0.7 µm in diameter. Ascospores with one septum also occur, and if so, there is no constriction at the septum. The fossil spores may still have the hyaline appendage at the basal end. The *Cercophora* spores mainly occur in the peat formed by a birch cart and, therefore, decaying wood was available. However, at that time, the locality may have been accessible to greater mammals. In that case, also dung was available as a matrix for *Cercophora* species. Van Geel et al. (2003) recorded many spores of this type, combined with other dung indicators, at a Bronze Age site in The Netherlands. According to Lundqvist

(1972), representatives of the sordariaceous genus *Cercophora* are coprophilous or occur on decaying wood, culms, and other herbaceous stems and leaves. According to Barthelmes et al. (2012) it occurs in open fen vegetation, alder and birch carrs with different water levels. A total of 351 specimens were present in LOI sample.

Type EMA-12: cf. thalloconidium of *Sporoschisma* (Plate 2-4)

Prager et al. 2006: Fungal spore, cf. *thalloconidium* (e.g. of *Sporoschisma* or *Chalara*, cf. Ellis and Ellis, 1997) or hyphae (pers. comm. Rogers 2005), rectangular elongated, mono septate, 21–28×5–8 µm, wall (1 µm) thicker than septum, ends detached, dark brown, psilate. Common in surface samples. Mostly scarce, absent in deadwood samples. In fossil samples, present in the peat of wet alder carr and sedge fen (Barthelmes et al., 2006). Indicative of wet alder carr. not restricted to a single carr substrate type. 8 specimens were present in the sample.

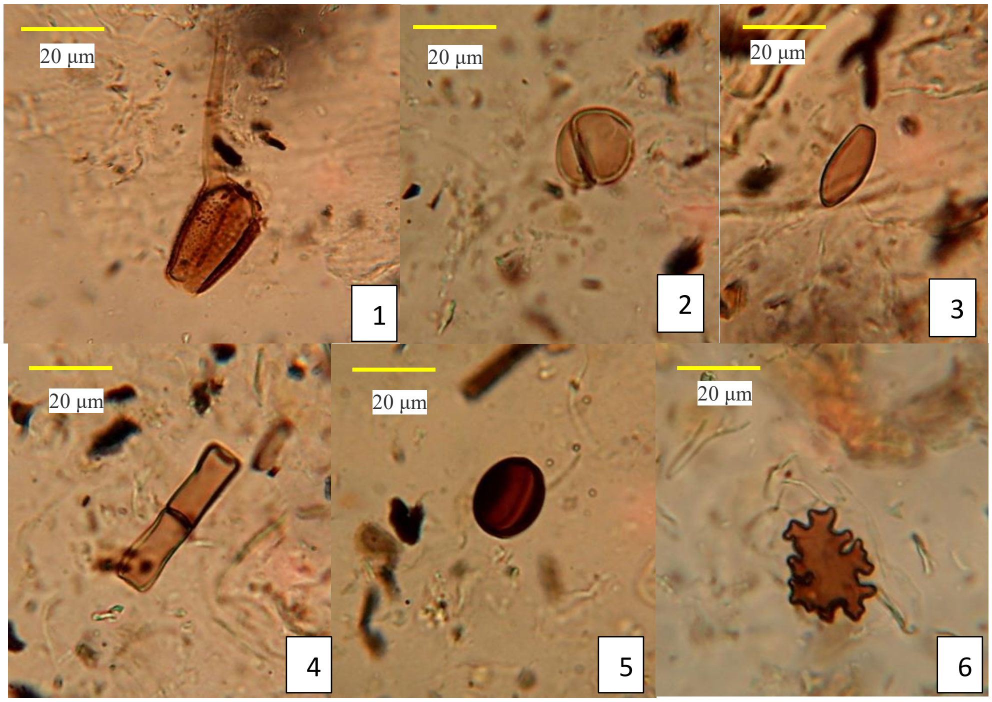
Type HdV-172: *Coniochaeta cf. ligniaria* (Plate 2-5)

Van Geel et al. (1983b): Ascospores ellipsoid, 14.5-19.9 X 11.6-14.6 (-15.7) µm, one-celled, bilaterally flattened, the two flattened sides dark brown and a light brown zone encircling the spore. Germ slit along the narrow side in the light brown zone. Type 172 is common in the local zone, dominated by *Thelypteris palustris*. Van Geel et al. (2011) argue that Jarzen and Elsik (1986) described and illustrated similar spores from river deposits in Zambia as *Exesisporites*. Ascospores of this species were found in soil surface samples from a Roman Period settlement site with strong evidence for a high density of domesticated animals (van Geel et al., 2003; van Geel and Aptroot, 2006). This type is common throughout the Challa record, particularly during the Holocene. Prager et al. (2012) suggest its presence in open fen abundant in reed and sedge vegetation. It is possibly indicative of the specific open fen environment rather than of single plant taxa. It occurs in *Phragmites* litter and surface peat layers. *C. ligniaria* is also common on dung and wood. In LOI sample a total of 129 specimens were present.

Type HdV-13: cf. *Entophlyctis lobata* (Plate 2-6)

Van Geel (1978) crenulate sporangia of 18-40 x 18-32 micron, with walls about 0.5 microns thick. Kuhry (1997) describe it as crenulate sporangia, with a central porefound attached to the leaves of *Polytrichum alpestre, Aulacomnium palustre, Scheuchzeria palustris, Calluna vulgaris*, and *Andromeda polifolia*. They were also found inside the water-cells of *Sphagnum imbricatum* leaves. Godwin and Andrew (1951) associated similar microfossils in postglacial deposits with microthyriaceous fruit-bodies. A total of 10 specimens were present in the sample.

Plate 2



Type HdV-169: *Sordariaceae/ Apiosordaria verruculosa ?* (Plate 3-1)

Van Geel et al. (1983b): ascospores, 19.4-23.0 x 13.4-16.5 µm, ellipsoid with a somewhat eccentrically placed, 1.0-1.4 µm wide, germ pore at the apex, the truncated base 5.0-10.8 µm in diameter. Some spores still showing the remains of a ca. 8 µm long hyaline basal pedicel. Van Geel et al. (2003): these ascospores are 19–24 x 13–17 μm in size, ellipsoidal with somewhat eccentrically placed 11.5 μm wide germ pore at the apex, with the truncated base ca. 10 μm in diameter. Some spores still show the remains of ca. 9 μm long hyaline basal pedicel. Van Geel et al. (1983b): ascospores, 19.4-23.0 x 13.4-16.5 µm, ellipsoid with a somewhat eccentrically placed, 1.0-1.4 µm wide, germ pore at the apex, the truncated base 5.0-10.8 µm in diameter. Some spores still showing the remains of a ca. 8 µm long hyaline basal pedicel. Type 169 ascospores probably were produced by a representative of the Sordariaceae (cf. Lundqvist, 1972) related to the genus *Tripterospora* (*Zopfiella*, Lundqvist, 1969). A sum of 29 specimens were present.

Type HdV-207: Glomus cf. *fasciculatum* (Plate 3-2)

Van Geel et al. (1989): globose spores, (17.5-) 25-68 (-138) μm in diameter exclusive of the hyphal attachment. Wall of small chlamydospores ca. 0.5 μm thick, that of larger ones up to 5 μm thick. Van Geel et al. (2003a) chlamydospores of *Glomus* occurred isolated in microfossil samples, but they were also observed–in clusters still attached by mycelium–in macrofossil samples. The spores of *Glomus* are extremely variable in size (17–138 μm). HdV-207 spores closely resemble to chlamydospores of *Glomus fasciculatum*; a relationship of this vesicular arbuscular endomycorrhizal fungus (it occurs on a variety of host plants) with the roots of local stands of *Betula.* Fossil chlamydospores of *Glomus* were also described and illustrated by Wagner and Taylor (1982) and Berch and Warner (1985). Anderson et al. (1984) identified *G. fasciculatum* in postglacial lake sediments in Maine

(USA). The fungus became established with tundra vegetation on newly developing soils soon after the melting of Wisconsin ice. It was postulated that erosion accounts for the abundance of *Glomus* in Lateglacial sediments. The reduced abundance in Holocene sediments was attributed to a decrease in the rate of soil erosion after the establishment of trees. A total of 28 specimens were counted in our sample.

Type EMA-31: *Thielaviopsis/ Thielaviopsis basicola* (Plate 3-3)

Fungal spore, very likely the end fragments of arthroconidia of *Thielaviopsis* (*Thielaviopsis* state of *Ceratocystis moniliformes* (Hedgc.) C. Moreau and of conidia of *Thielaviopsis basicola* (Berk. and Br.) It is grown on rotten wood (Ellis and Ellis, 1997). Hemispherical to bowl-shaped, Ø 10– 17 µm, convex wall ("outside") continuously ~1.3 µm thick, planar and concave wall ("inside") thinner, no pores observed, bright brown, psilate. Common in surface samples. Usually scarce, but scarce to abundant in deadwood samples. Probably indicative of wood (Prager et al., 2006). 13 specimens were counted.

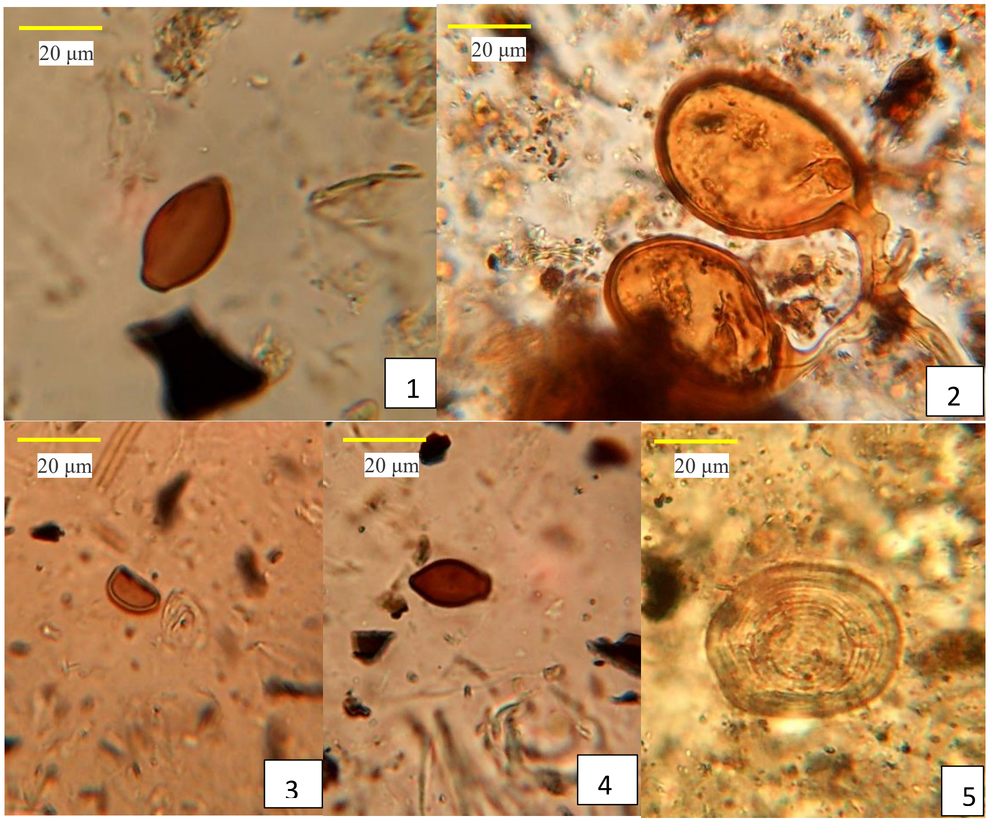
Type HdV-466: *Podospora sp./Zopfiella sp.* (Plate 3-4)

Kuhry (1985): fungal spores, elongated, asymmetrical, convex, 29 x 13-15 with a truncated tip about 5-6 µm wide. The other end acute with an apical pore of ca. 1.5 µm in diameter. Representatives of the sordariaceous genera *Podospora* and Zopfiella are coprophilous. A palaeoecological indication is unknown. A total of 75 specimens were counted.

Type NN-61: *Pseudoschizaea* (Plate 3-5)

These disc-shaped algae, 35 µm with a spiral kind of decoration. The presence of Pseudoschizaea supports this hypothesis, suggesting seasonal desiccations (Scott 1992) and freshwater flows with erosional processes in the basin (Pantaleón-Cano et al. 2003). Very rare in LOI sample with only 2 speciemns.

Plate 3



Type EMA-57: cf. round charcoal (Plate 4-1)

Barthelmes et al. (2006): cf. round charcoal, round to slightly elliptic (4–15 µm), sometimes slightly ruptured margins, black. Frequent in fossil samples. Recurrent to abundant, especially concentrated in alder carr peat. A total of 158 specimens were counted.

Type UG-1277: (Plate 4-2)

Gelorini et al. (2011) spores from globose to subglobose, 40–57×40–43 µm, smooth, with more than 20 brown, thick-walled septated cells (slightly variable in size and shape), and some subhyaline, thin-walled basal cell(s), slightly constricted at the septa. The palaeoecological context is unknown. 27 specimens were counted.

Type UAB-15 (Plate 4-3)

Fungal spores, globose-sub globose, brown to dark brown, 10–12.5 μm in diameter, with some small pores. Similar to type TM-334 (Cugny, 2011). A total of 16 specimens were present.

Type HdV-364: *Thecaphora* Sp. (Plate 4-4)

Van Geel et al. (1981): basidiospores, mostly occurring as groups of 3-6 cells, each cell 10-13 μm in diameter, with 2.5-3 μm wide pore. Small protuberances on the surface, densely distributed. *Thecaphora* species are usually plant pathogens of Fabaceae and Convolvulaceae. Only 7 specimens were counted.

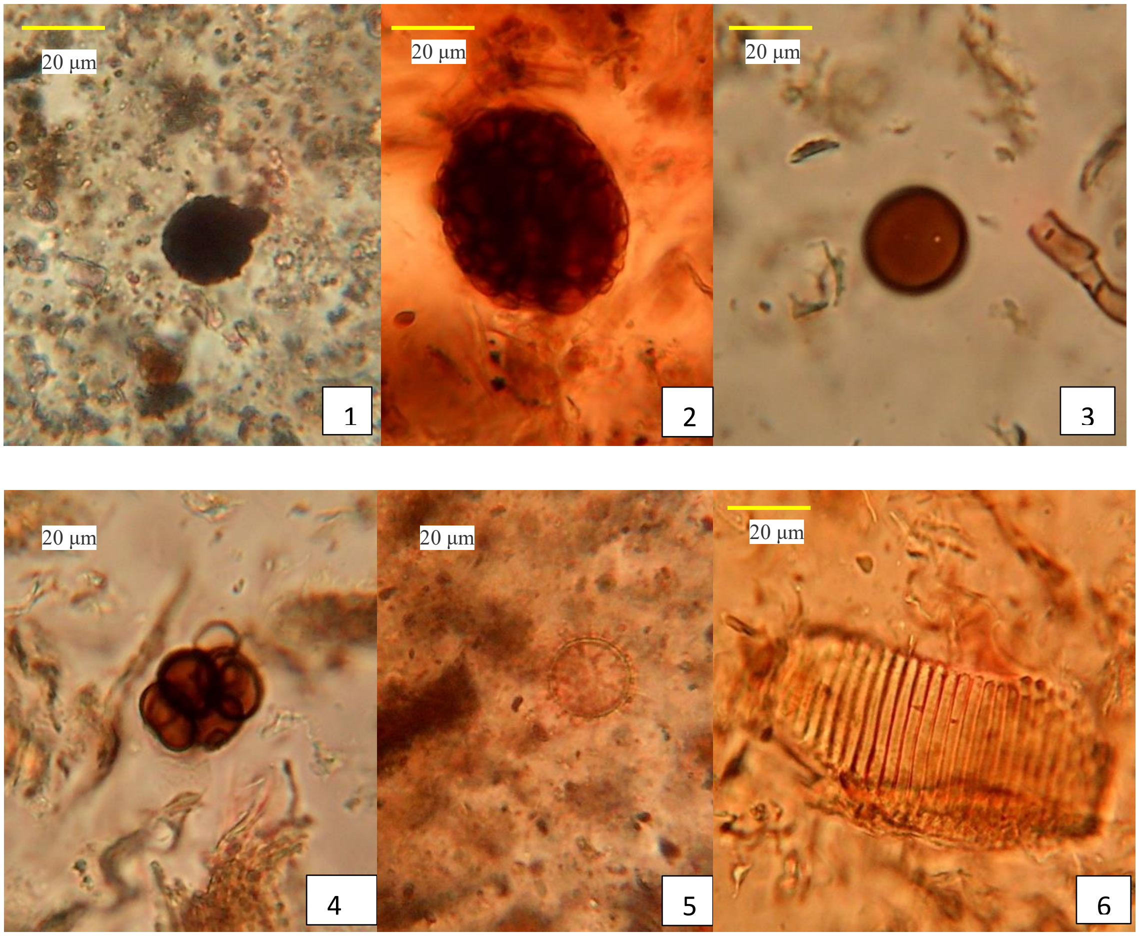
Type HdV-116*: Cymatiosphaera* (Plate 4-5)

Pals et al. (1980) spores globose, reticulate, 16-20.5 micron in diameter, inclusive of the 2.0-2.5 μm thick reticulum with meshes 3.5-6 micron wide. Type HdV-116 belongs to the indicators of saline water conditions. Bakker et al. (1982) note that in the Ilperveld section, Type HdV-116 is found in small numbers chiefly in peat, but only in two cases under clear marine conditions. In the peat of the Ilperveld section, Type HdV-116 shows a preference for relatively wet conditions. In lOI sample a total of 3 specimens were counted.

Type HdV-114: Scalariform perforation plate of *Alnus / Corylus / Betula* (Plate 4-6)

Pals et al. (1980): scalariform perforation plate, occurring in vessels of *Betula*, *Alnus*, *Corylus*, and *Myrica*. The number of rungs can be used to identify the genus level, but small fragments were often observed, often making identification impossible. Type HdV-114 is restricted in its occurrence to the clay, which was sedimented under marine conditions. This means that these wood fragments were always water-eroded, and the wood had decomposed into tiny fragments before sedimentation. Barthelmes et al., 2012: related this Type to EMA-15. Points at heavy decomposition – even of wood – which implies low or strongly fluctuating water levels. Prager et al., 2012: Indicator group: carr undefined. Associated both with alder and with birch carr and are thus regarded as general indicators of forested fens (carrs). A total of 48 specimens were identified.

Plate-4



Type TM-224: cf. *Endophragmiella* B (Plate 5-1)

Fungi, in which its palaeoecological purpose is not yet known (Cugny et al 2010). A total of 126 specimens were identified.

Type HdV-501: *Zopfiella lundqvistii* (Plate 5-2)

Van Geel et al. (1986): three-lobate, flattened fungal spore, diameter c. 37 μm. A pore, c. 2 μm wide, with the annulus, at one lobe. A second lobe with a ~1 μm wide pore and the third lobe without a pore. In LOI sediment only 3 specimens were identified.

Type EMA-38: conidium of cf. *Excipularia fusispora (*Plate 5-3)

Slightly bent six septate fungi, light brown color. Common in surface samples. Abundant in deadwood (*Alnus*) sample. This is good indicator of alder carrs resulted from above ground decay of dead wood. These fungi may also be produced during the decay of alder roots. As such, they are potentially present primary as well as in secondary (displacement) alder wood peat concentrate in deadwood samples (Prager et al. 2012). Barthelmes et al. (2012) point that *E. fusispora* is common on decaying wood and bark of deciduous trees (e.g., *Alnus*, *Populus*, *Salix)*. A total of 10 specimens are identified as EMA-38.

Type HdV-113: *Sporormiella*-type (Plate 5-4)

Ascospores of extant *Sporormiella* species are three to many-septate, smooth and dark brown. The septa are transverse to oblique, every cell showing an elongated germ slit, extending the cell's entire length. Ascospores easily split up in separate cells, and as a consequence, in the fossil state, usually no observations of complete ascospores can be made. Nevertheless, the separate ascospore cells show sufficient features for identification. Species identification of *Sporormiella*-type is not possible because fruit-bodies, asci, and complete ascospores are not available. Fossil cells of ascospores are of 14–21 x 11–17 μm in size. There are two types of *Sporormiella*-type cells: (i) half-ovoid cells (10–13), which are the terminal cells of ascospores, the flattened part initially forming the connection with the adjacent ascospore cell, and (ii) the central ascospore cells (14–16) which are more or less cylindrical. All cells show an oblique to diagonal germ slit: Van Geel et al. 2003a.

Van Geel et al. (2003a) indicate that fossil spores of *Sporormiella* were distinguished by Davis et al. (1977), Davis and Turner (1986), and Davis (1987), who concluded that ascospores of these coprophilous fungi could be used as indicators for increased population densities of herbivores. Most *Sporormiella* species are coprophilous (Ahmed and Cain, 1972). The representatives of the related, coprophilous genus *Sporormia* are without germ slits, but as Ahmed and Cain (1972) are based on non-germinated spores, a slit may appear after germination of *Sporormia*-spores, and therefore we cannot exclude that also *Sporormia* is among our fossil spores. A total of 16 specimens were identified in LOI.

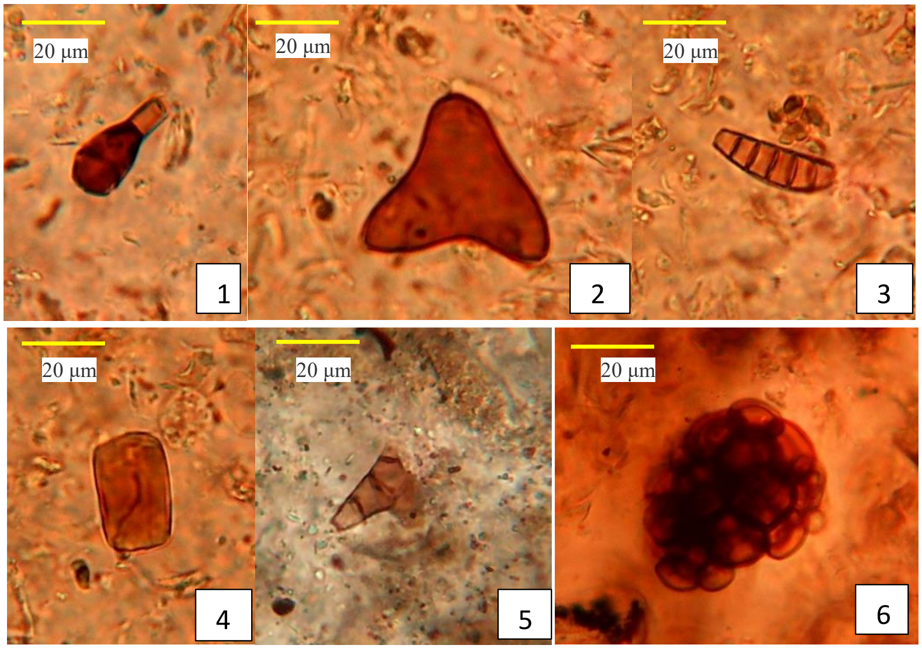
Type EMA-19: *Diplocladiella scalaroides* (Plate 5-5)

Barthelmes et al. (2006): conidia of *Diplocladiella scalaroides* have a triangular shape, with two-horned (Ø 20 µm), psilate, each arm 11 µm long and with 1–2 septa, hyaline to pale brown, tips hyaline. Rare in fossil samples and restricted to Willow shrub peat, but probably not related to wetland vegetation (Ellis, 1997). Only 3 specimens were counted.

Type UAB-20: (Plate 5-6)

Globose clusters of fungal cells (each one ca. 7.5 µm). Pale brown to dark brown, 25–42.5 µm in diameter. The presence of this type is probably related to lacustrine environments deficient in nutrients and rich in calcium (Revelles et al. 2016b). A total of 56 specimen were identified.

Plate 5



Type HdV-368: *Podospora* type (Plate 6-1)

Van Geel et al. (1981): ascospores, one-celled, ellipsoidal, smooth, brown, 39-44 x 16-23 µm. With one protruding pore 2 µm in diameter, directly below the apex. A relation with the presence of man or cattle (providing dung as a substrate) and a sordariaceous origin seems probable. Van Geel et al. 2003: Spores of the Podospora-type (coprophilous Sordariales) are of a regular occurrence in samples from archaeological sites. In LOI sample, 75 individual speciemens were identified as *Podospora* type.

Type HdV-263: *Valsaria* Type (Plate 6-2)

Van Geel et al. 2003a: These ascospores are uniseptate, ca. 42 x 22 µm in size, inclusive of the characteristic velum, which forms longitudinal ribs and an equatorial ring around the septum. Like HdV-140, these seem to be ascospores of a (different) member of the genus *Valsaria*. A total of 8 specimens were counted.

Type HdV-1009: *Chaetomium* sp. (Plate 6-3)

Van Geel et al. (2011): ascospores lemon-shaped, ca. 8–10 X 7–8 µm, bilaterally flattened with apical pores. Apart from the occurrence of their fossil ascospores in deposits from natural habitats (Van Geel, 1978; Van Geel et al., 1989), fossil *Chaetomium* spores also appeared to be linked to archaeological sites (Buurman et al., 1995; Van Geel et al., 2003). *Chaetomium* species are common, saprophytic cellulose-decomposers occurring on cloth, leather, bones, feathers, decaying herbaceous stems, etc. They are seldom found on dung, except when it is mixed with straw (Buurman et al., 1995). A total of 126 specimens were identified as *Chaetomium* sp.

Type HdV-83: (Plate 6-4)

Van Geel (1978): spores unequally one-septate, slightly constricted, brown, 24-29 x 11-12 um, wall becoming thinner at both ends and forming a pore about 1 µm wide (or broader due to corrosion). Kuhry (1997) points that this fungal spore is most prominent under ombrotrophic conditions at the Beauval (zones E and F) and Gypsumville (zone D) sites. In these sequences some, but not all, of this type's maxima correlate with higher charcoal concentrations in the peat. A total of 6 specimens were identified.

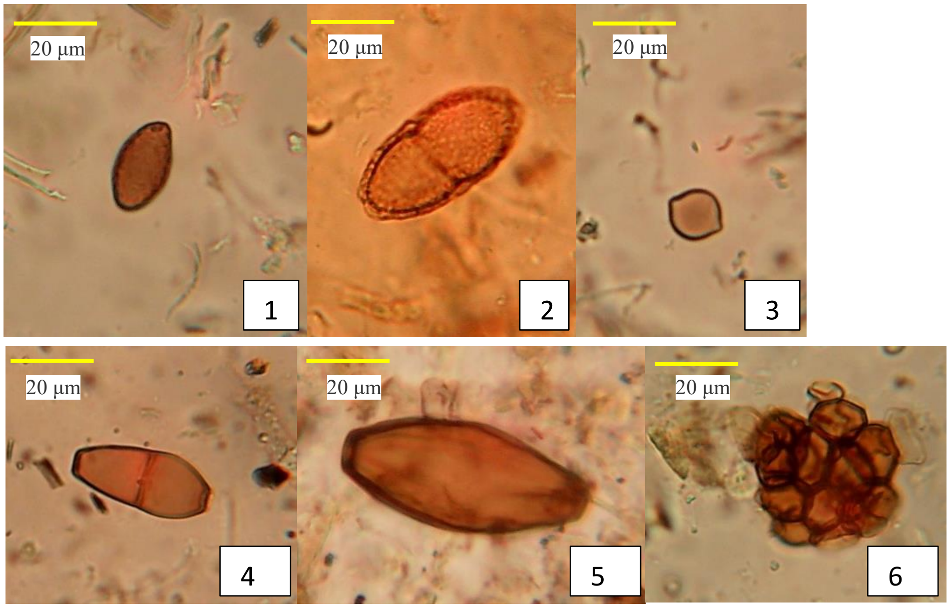
Type HdV-143: *Diporotheca sp. / D. rhizophila* (Plate 6-5)

Van der Wiel (1982): fungal spores, light brown, biseptate, 47-57 (52) x 17-25 (22) µm, fusiform, truncate at both ends. Both end cells of the spore are provided with a pore of ca 3 µm in diameter. Van Geel et al. (1986) describe this fungus as having an ovoid fruit-body of c. 180-300 µm, blackish-brown, with a coarsely verrucose wall, verrucae 15-20 µm high. It occurs regularly in Holocene deposits formed in eutrophic to mesotrophic conditions (Van der Woude, 1983). A total of 14 individual specimens were counted.

Type HdV-361 (Plate 6-6)

Van Geel et al. (1981): group of fungal cells (361.c), often fallen apart (361.a and 361.b) into polyhedral cells, 10-15 µm in diameter with a ca. 0.5 µm wide pore in each plane. In LOI sample a total of 61 specimens were counted.

Plate 6



Type Hdv-132: *Spirogyra sp*. (Plate 7-1)

Reticulate *Spirogyra* spores, measuring 100-144 x 60-70 micron. The curve includes Type 131 and is too low for any interpretation. It is even possible that the spores were not locally produced but that such low frequencies may result from transport (e.g., by animals). Consultation of the literature (Czurda, 1932) shows that this type can include several species. Only 2 specimens were identified.

Type LCE-33: cf. *Alternaria (Plate 7-2)*

It is a multi-cells microfossil, about 100 µm long. Modern-day occurrence is found only in a dung sample from one grazed forest (Dietre et al 2012). A total of 4 specimens were counted.

Type BRN-9: *Podospora /Cercopora* (Plate 7-3)

Feeser et al. (2009): fungi with brown, ellipsoidal spores, c. 12 x 20 micron, truncated at one end, and a slightly subapical pore opposite. A total of 35 individual specimens were counted.

Type BRN-2: (Plate 7-4)

Feeser et al. (2009): spindle-shaped fungal spore, c. 18-37 micron, with pores at both ends; one end tapered. The palaeoecological context is unknown. A total of 9 specimens were counted.

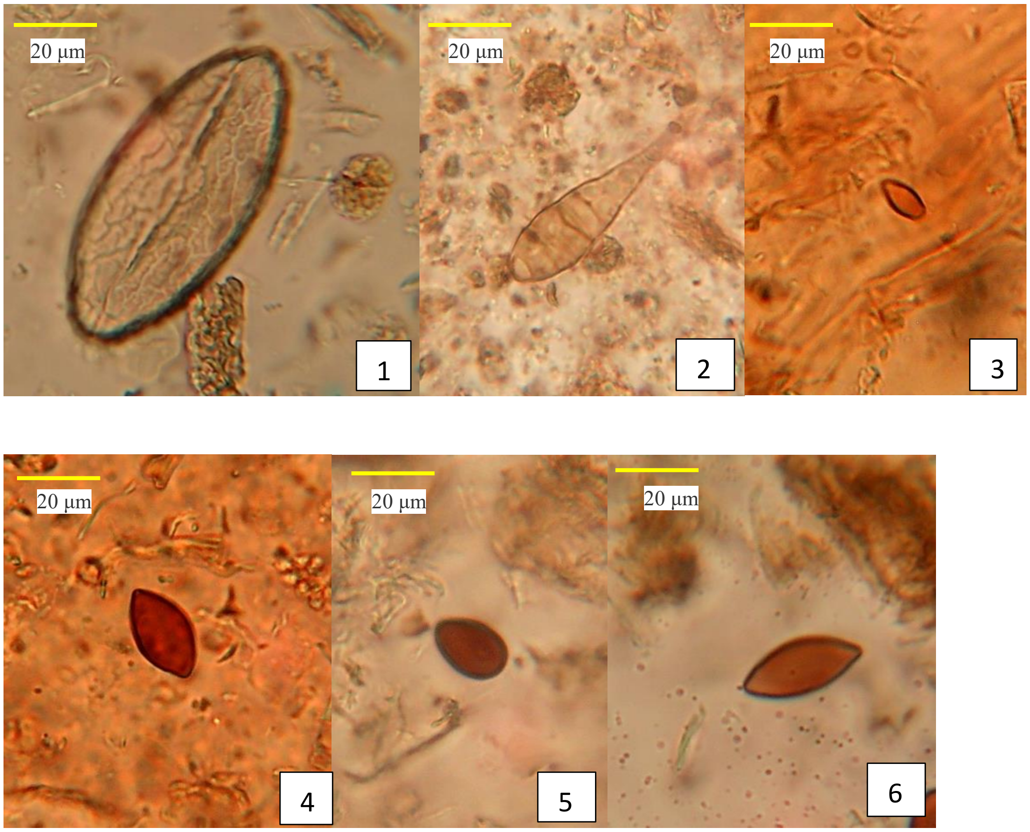
Type BRN-7: Cf. *Schizothecium conicum* (Plate 7-5)

Feeser et al. (2009): Elliptical, non-septate, fungal spore, ca. 16 x 25 micron with the protruding apical pore. It resembles spores of *Schizothecium conicum*, a common coprophilous species. A total of 7 specimens were counted.

Type HdV-261: *Arnium* type (Plate 7-6)

Van Geel et al. (2003a): one-celled ascospores, ellipsoidal, 52–86 x 30–36 µm in size, with ca. 1.5 µm wide pore at both ends. The large spores are characteristic, but not with certainty identifiable to any genus. They seem to belong to a member of the Sordariales, most probably to the genus *Arnium*, one of the few genera with ascospores with two germ pores (Lundqvist, 1972). A total of 18 specimens were identified as *Arnium* type.

Plate 7



Type BRN-5: *Dematacious hyphomycete* (Plate 8-1)

Feeser et al. (2009): brown, 2–3 septate fungal spore, ca. 23 x 16 micron. The palaeoecological context is unknown. This is a very rare type with only 1 specimen counted.

Type HdV-715: (Plate 8-2)

Bakker et al. (1982): triseptate fungal microfossils, somewhat curved, slightly constricted at the septa, c. 26-30 × 6-12 micron (central cells only). Terminal cells small, hyaline, sometimes absent. Wall smooth or with inconspicuous spinules not over 0.3 microns in height. It indicates a preference for eutrophic to mesotrophic helophyte marsh conditions. Prager et al. (2012) indicate that it is abundant in open reed and open sedge vegetation, it possibly reflects the specific conditions (high light availability) of open vegetation rather than single plant taxa. A total of 17 specimens were identified.

Type MM-478: Large pittet xylem (Plate 8-3)

Vascular cell with large, elongated perforations. A total of 7 specimens were identified.

Type UG-1155: (Plate 8-4)

Gelorini et al. (2011): ellipsoid spores, equally and symmetrically 2-celled, brown, 23–32×12–15 µm, smooth, thick-walled, constricted at the septum; with slightly tapering ends; small opening/pore at one end (only visible in strictly polar or equatorial orientation). The palaeoecological context is unknown. Only 1 specimen were identified.

Type HdV-47: (Plate 8-5)

Van Geel 1978: Ascospores one-septate, 18-23 X 6-7µm, slightly constricted at the septum. Two or three longitudinal hyaline zones, each about 2 µm wide. The palaeoecological context is unknown. A total of 12 specimens were identified as Hdv-47.

Type UG-1002: *Sporoschisma spp. / Sporoschisma cf saccardoi* (Plate 8-6)

Conidia cylindrical with flattened ends, unequally and sub symmetrically 4- or 6-celled, 50–61x 12–14 µm, smooth, thick-walled, slightly constricted at the septa; central cells pale to dark brown, almost of equal size; end cells paler, short, discoid or somewhat truncate, flattened or slightly rounded at free ends (Gelorini et al. 2011).

Gelorini et al. (2011), described that, these conidia probably belong to *Sporoschisma saccardoi*. Specimens, whose end cells are missing, are possibly fragmented conidia of the same species (due to decay) or 3-septate conidia of other *Sporoschisma* species (e.g., *S. juvenile* Boud., *S. mirabile* Berk. & Broome). Shumilovskikh et al. (2017). The same authors associated also the type UG 1002 with *Sporoschisma* spp., possibly with *Sporoschisma saccardoi*. *Sporoschisma saccardoi* is distributed in tropical (e.g., Indonesia, Taiwan, Ecuador, and South Africa) and more temperate regions (e.g., Europe) (Gelorini et al. 2011). It is mainly found on submerged wood in freshwater habitats (Goh et al., 1997). A total of 6 specimens were identified.

Plate 8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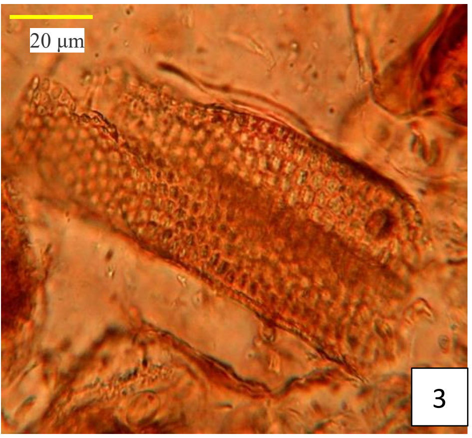
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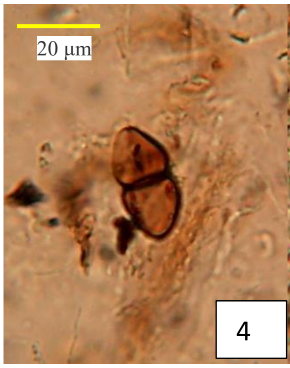
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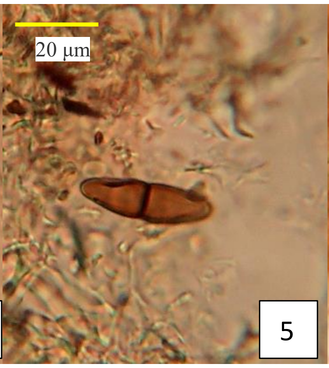
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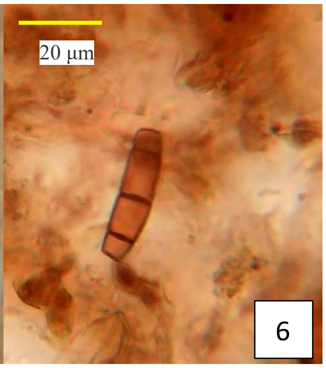
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Type HdV-18 (Plate 9-1)

van Geel (1978): one-septate ascospores (35)37-43(48) x (10)11-14(17) µm. At both ends with 0.8 µm wide pores. The septum is often no longer attached to the spore wall. Rather pale, non-septate spores were sometimes found (Type HdV-18B: Van Geel, 1972) together with Type HdV-18A spores in the same fruit-bodies, probably fossilized before maturation. Fruit-bodies ostiolate, 125-450 µm in diameter; wail 7-10 µm thick, an outer layer composed of interwoven, 2-4 µm wide, septate hyphae. Fruit-bodies immersed in long, cylindrical, hyaline pieces of a host plant usually measure 70-125 µm in diameter. However, where the tissue is infected by the fungus and fruit-bodies are present, this tissue has a diameter of 220-500 µm. The cylindrical cells of the host tissue measure 10-20 x 35-70 µm. Through the host tissue and the fruit-body walls, the ascospores can be observed, and most of the fossil fruit-bodies still contain ascospores. Van Geel (1978) affirms that in the section Wietmarscher Moor III, the occurrence of Type 18 spores could be correlated with *Eriophorum vaginatum*, which formed thick peat layers in that section. The analysis of Engbertsdijksveen section revealed fruit-bodies immersed in host tissue (as yet unidentified), possibly leaf fragments or rootlets of *E. vaginatum*. A total of 8 specimens were counted.

Type EMA-56: *Alysidium resinae* (Plate 9-2)

Barthelmes et al. (2006): conidia of *Alysidium resinae*, conidia often in chains of 3–4, globose (Ø 7–12 µm) to elliptic-truncate (10–13×7–10 µm), brown, psilate. This type is common in fossil samples. Scarce, concentrated in Alder carr peat., *Alysidium resinae* occurs on rotten wood of different species (Ellis and Ellis, 1997). A total of 6 specimens were identified.

Type EMA-33: *Chaetosphaerella fusispora* (Plate 9-3)

Prager et al. (2006): fungal spore, ellipsoid, tri-septate, 15–18×7–8 µm, both middle cells dark brown, 5–7 µm long, terminal cells hyaline, 1.5 µm long, truncated conical, often detached, spore psilate. *Chaetosphaerella fusispora* is a species described from dead branches of *Acer pseudoplatanus* (Ellis and Ellis, 1997). A total of 5 specimens were identified.

Type HdV-713: (Plate 9-4)

Bakker et al. (1982): uniseptate, ellipsoid microfossils, slightly constricted at the septum, ca. 20-30 x 6-10 micron. Surface smooth with three or four longitudinal grooves with a ca. 0.4 μm thick wall. Type 713 is found in phases associated with eutrophic to mesotrophic conditions. A very rare specimen with only 2 counts.

Type IBB-34: Tardigrada IV (Plate 9-5)

Unidentified microfossil remains which could proceed from Tardigrada egg remains. A very rare specimen with a total count of 1.

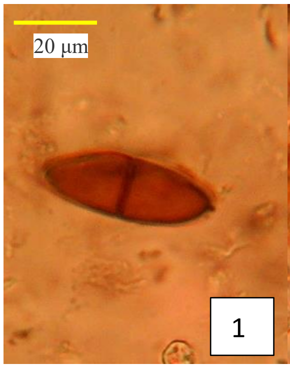
Type UG-1113: *Meliola* sp (Plate 9-6)

Gelorini et al. 2011: Ascospores oblong to rarely sub ellipsoid, unequally and asymmetrically 5-celled, brown, 38–45×12–18 μm, smooth, thick-walled, constricted at the septa. In LOI it observed only once.

Type 1-A: oocyte; *Gyratrix hermaphroditus* Ehrenberg (Plate 9-7)

It is a roughly "Bordeaux-wine-glass" shaped palynomorph. It has a typical stalk of a minimum of 125 μm long, with articulation just beneath the main body, and an adhesion plate at the opposite end. No macrosculpture on the outer oocyte wall with fine operculum suture. In LOI sample it was observed only once.

Plate 9



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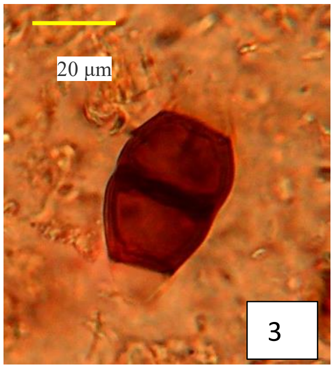

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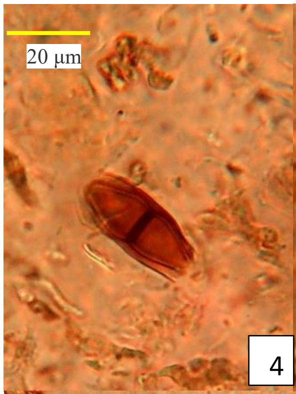

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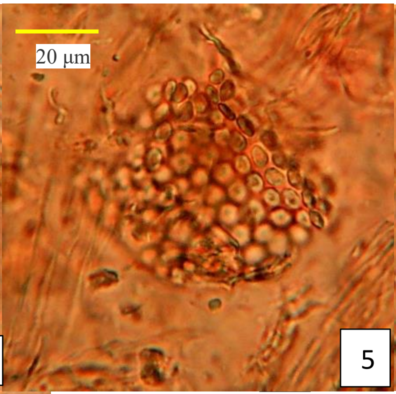

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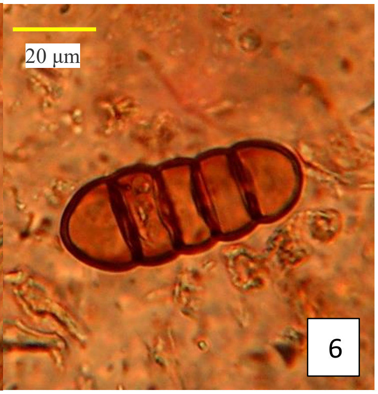

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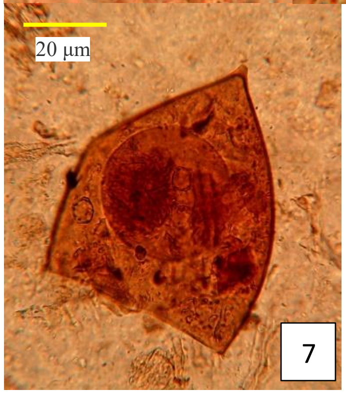

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Chapter Four

Discussion

Fungal spores as indicator of Livestock herding

The history of human adaptation in this part of the region goes back to the Neolithic period. Various sites are yielded ample information about permanent human settlements (Manfredini, 2002; Morelli et al., 2004). In this case, understanding the history of the past livestock herding is crucial for considering the process and mechanisms of the prehistoric settlement of the nearby archaeological sites. The use of non-pollen palynomorphs in Quaternary paleoecology and archaeology has increased during the last decades, and nowadays, it is recognized as a powerful tool for past inference (van Geel, 2001). The study of fungal remains preserved in lacustrine sediments can complement palyno-data in interpreting the palaeo-vegetation and past climate in the region (Van Geel, 1978, 1986, 2001; Cugny et al. 2010). Among the vast diversity of NPPs, coprophilous fungal spores are especially relevant for understanding livestock presence (Basumatary and McDonald 2017). Coprophilous fungi provide a useful tool for assessing past grazing activities (Baker et al., 2016; Blackford and Innes, 2006; Van Geel et al., 2003). Spores of these fungi have become one of the most important means for the palaeoecological studies on natural and anthropo-zoogenic landscape changes (Felauer et al., 2012; Galorini et al., 2011). Generally, the identification of Coprophilous fungi in prehistoric cultural deposits may help refine the history of the study area's livestock presence. A detailed documentation and interpretation of NPPs from the Tiber delta is a vital study to correlate the less researched Non-Pollen Palynomorphs with the extremely studied pollen data of the region. The findings of this study provide new insights to reveal the subsistence selection of prehistoric humans and the history of livestock habitation in the surrounding archaeological sites.

In the mid-Holocene (5400–5100 cal. a BP) period, the NPP data shows a high number of *Coprophilous* fungi, including *Sporormiella* spp, *Sordaria* spp, and *Delitschia* spp. The presence of these fungal spores is a valuable indicator of past grazing presence and intensity. Moreover, *Glomus* spores' occurrence backs these grazing activities, which probably leads to erosion and exposing the plant roots. The vegetation history of Stagno di Maccarese records an open landscape related to the development of a harsh environment (Di Rita et al., 2010). Furthermore, the presence of *Tertraploa* aristate and *Glomus* in the sample's assemblages suggests waterlogged conditions with a rich plant, water, and soil, as these fungi are commonly found on the leaf bases, roots, and stems of Poaceae and Cyperaceae (Ellis, 1971). Also, In Stagno di Maccarese records an open landscape related to the development of a marshy environment, as indicated by Poaceae's successive dominance, followed by Cyperaceae (Di Rita et al., 2010). The presence of *Glomus* in our sample makes a good correlation with the pollen record of the area. Previous studies of lake sediments linked an increase in *Glomus* percentages to intensified soil erosion in the catchment area of lakes (Anderson et al., 1984; van Geel et al., 1989), to a more reduced vegetation cover (Leipe et al., 2014) and anthropogenic activities (Ghosh et al., 2017; Miehe et al., 2014).

The presence of local fires evidenced by peak of micro-charcoal concentration is illustrated by pollen data and charcoal analysis (Di Rita, et al. 2010) and it is also attested by NPPs, which is the presence of the fungus *Coniochaeta* cf. *ligniaria* suggests wildfires (López Sáez et al., 1998).

The presence of *Sporormiella* has proved to be a reliable indicator of dung (Davis and Shafer, 2006; Feranec et al., 2011; Raper and Bush, 2009). Baker et al. (2013) suggest that *Sporormiella*-, *Sordaria*- and *Podospora*-types are the most reliable feces indicators. This NPPs are supported by the evidence of human impact on the landscape, documented by micro charcoal concentration indicating local fires and cereal type pollen (Di Rita et al., 2010). One of the nearby archaeological sites of the Middle Eneolithic settlement of Le Cerquete Fianello (5370 and 4920 Cal. BP) yielded archaeological, archeozoological, and archaeobotanical evidence. For instance, animal remains *of Ovis* vel *capra, Bos taurus, Sus scrofa, Equus caballus, Canis familiaris, Felis sylvestris, Capreolus capreolus,* and *Testudo* species (Tagliacozzo et al., 2002) may account for the presence of abundant *coprophilous* fungi in this interval. Accordingly, the presence of a variety of *coprophilous* fungi can be a robust indicator of the nearby settlement of livestock herding community. In addition. coprophilous fungi *Sporormiella* and *Podospora* suggests that the medium forest cover zone has been subjected to a degree of disturbance by herbivores and basin erosion (Loughlin et al. 2018).

Around 5100–2900 cal. a BP middle Holocene to Late Holocene period, the *coprophilous* fungi frequencies are decreasing. This is probably caused by the decreasing amount of grazed land in the vicinity of the environment's taphonomy condition. For this, the development of marshland around 4000 cal. a BP matches the geological evidence of a temporary lowering of the water level documented by an evaporitic level dated around 4000 cal. a BP at Le Cerquete-Fianello (Giraudi, 2004). It is possible that the arid event did not determine a significant decrease of the forest cover in such a wet site like Stagno di Maccarese, where only a temporary lowering of the water level occurred. Ingold and

Marshall, 1962 stated that a high amount of coprophilous fungi and other associated fungal spores indicate warm and humid condition in a particular region since water availability is crucial for germination sporulation of *Sporormiella*. Therefore, the probable lowering of the water level might have disturbed the germination of the fungi species. However, it is reasonable to suppose that some of the coprophilous fungi spores are also related to the contamination of lake waters with organic fertilizer runoff from cropland. Such a scenario may be further supported by the presence of *Chaetomium*, which frequently populates manure composed of a mixture of dung and straw (Ellis and Ellis 1988). Furthermore, *Diporotheca* fungi presence on this date can also be linked to unstable soil conditions (López-Merino et al. 2012; Reveles and van Geel 2016a); however, this should be researched further.

In the Late Holocene, ca. 2900–2000 cal. a BP, livestock and pastureland indicators of fungi species exhibit serious decline. The preservation of the soil type might be the reason. The increase in salinity is observed around 2600 Cal a BP. Furthermore, this lack NPPs indicators of livestock and human settlement is consistent with the lack of archaeological sites and the scarcity of anthropogenic markers indicating cultivation (e.g., cereals, *Olea*, *Juglans*) or pastures (e.g., *Rumex* and *Plantago*). An intensification of the usage of the area as saltworks since the first century BC is documented by an increase in chenopods pollen and macrofossils of halophytes (*Atriplex* and *Salsola*) around 2200 cal. a BP. (Di Rita et al., 2010).

Late Holocene period after 2000 Cal a. BP not only the coprophilous fungi spores but also the frequency of the NPPs shows a considerable decline. This might be due to site-specific conditions (e.g. soil chemistry, moisture, surface disturbance) which probably influenced their preservation. These microfossils are not necessarily always present in the same deposit, so that they should be regarded as complementary rather than alternative proxies (Morandi, 2020). Overall NPP taxon richness is certainly also affected by in situ decay processes, since after burial in bottom sediments the microfossils are differentially sensitive to chemical (oxidation), mechanical (abrasion) and biological (feeding) degradation (Gelorini et al., 2011). This issue is supported by the presence of Type UAB-20 probably related to lacustrine environments deficient in nutrients and rich in calcium. Highest values in an alkaline environment low in nutrients (Revelles et al. 2016b). Despite the scarcity of the NPPs, the *Coniochaeta cf. ligniaria* and *Sporormiella*-type presence evidenced human impact around the sites. It is well documented that Romans were exploited the area since 2000 cal. a BP. Etruscan saltworks were also located near the southern corner of Stagno di Maccresse (Manfredini, 2002; Morelli et al., 2004). The palynological research indicated that an anthropogenic marker is continuously found since the Roman exploitation of the area (Di Rita et al., 2010).

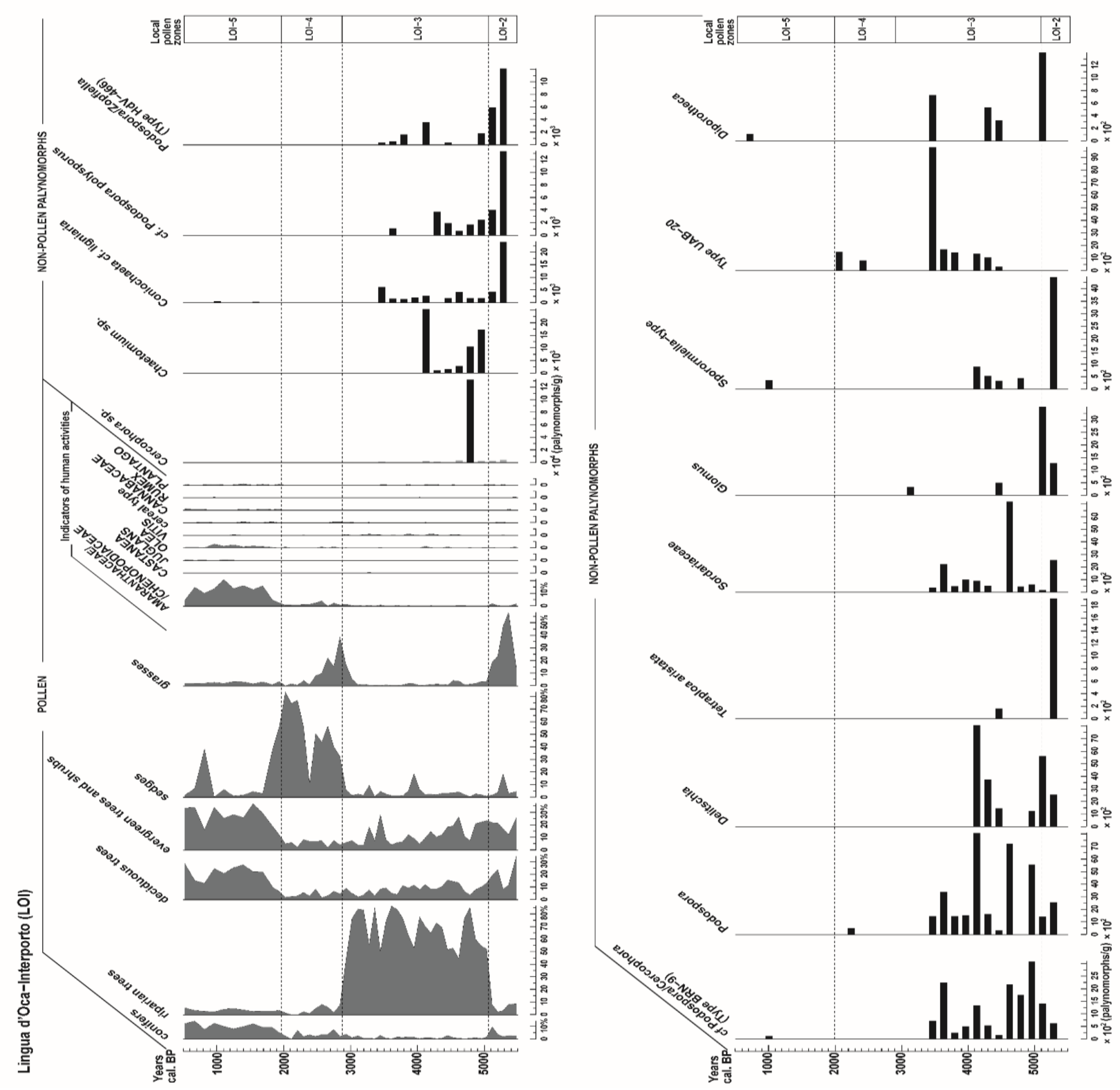

Fig. 12. Figure shows pollen data as main ecological groups, main anthropogenic pollen taxa and concentrations expressed in numbers of palynomorphs/gram of sediment.

Chapter Five

Conclusion

The analysis of Non-Pollen Palynomorphs from Stagno di Maccarese (LOI sequence) confirm that these markers are important tools to understanding the human settlement with the presence of livestock. The findings of this study, with special attention to the detailed record of well-preserved assemblages of coprophilous fungi, provide new insights to reveal the subsistence selection of prehistoric humans and the history of human habitation in the surrounding archaeological sites. In particular, my research highlights the following main resuts:

- The NPP record evidences the continual presence of animals in the past landscapes, supporting the evidence of pastures and livestock activities in the region. It also recorded the presence and the effect of humans on the landscape of the region with other natural factors.
- In addition, the implication of all above mentioned palynomorphs can lead to general and detailed palynological conclusions about past environment of Tiber river. Further research of NPPs may significantly contribute for interpretations based on pollen and spores and assists in improving detailed palynological conclusion.
- Furthermore, the comparison of fungal NPP assemblage data with independent environmental proxy like pollen data is an important way of understanding the environment in a holistic way. Archaeological studies should also incorporate the NPP data to get a better understanding of their cultural context. Thus, the NPP data

so far derived demonstrated good potential for palaeoecological consideration and to interpret the paleoclimate changes during the Quaternary period.

- Finally, the absence of some NPP identifications and environmental association of the study area illustrates that much research is still required. To progress understanding of NPPs in the Tiber delta, I recommend that all NPP remains should be recorded as part of any palynological investigation, even when the identities of the NPP morphotypes are unknown.

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