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## Article

# Pollen and Molecular Biomarkers from Sedimentary Archives in the Central Po Plain (N Italy): Assessing Their Potential to Deepen Changes in Natural and Agricultural Systems

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**Abstract:** This paper proposes to improve the information provided by biological indicators from sedimentary archives by integrating biomolecular techniques and botanical skills. This study represents a first proposal for combining pollen and biomolecular markers to detect land use and improve knowledge of past environmental change drivers. The specific aim of the research is to verify the relationship between miliacin (a pentacyclic triterpene methyl ether, usually interpreted as a broom-corn millet biomarker) and *Panicum* pollen in three near-site stratigraphic sequences of the Terramara S. Rosa di Poviglio (Po Plain, N Italy). The three cores span the last ~15,000 years and potentially record the beginning of *Panicum miliaceum* cultivation attested in the area since at least the Bronze Age within the Terramare culture. Despite the fact that *Panicum* pollen grains were rare in the spectra and miliacin was barely detectable in most of the 31 samples selected for biomolecular analyses, their combined evidence testifies to the local presence of the plant. *Panicum* pollen and sedimentary miliacin suggest the adoption of millet crops during the Recent Bronze Age by the Terramare culture, when climatic instability led to the diversification of cereal crops and the shift to drought-tolerant varieties.

**Keywords:** *Panicum*; miliacin; pollen; millet crops; palaeoenvironment; archaeological site; pentacyclic triterpenes methyl ethers



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## 1. Introduction

Molecular biomarkers detected in sedimentary archives are being increasingly utilised as proxies for examining past environmental conditions and anthropic impacts (e.g., [1–5]). These organic compounds possess, in specific cases, high taxonomic specificity and are reputedly resistant to biodegradation [6], providing information on both the overall ecological conditions and the local presence of species. Tricyclic diterpenes are specific molecular biomarkers of vascular plants, mostly produced by gymnosperms, and pentacyclic triterpenes are mainly derived from angiosperms [7,8]. Among them, pentacyclic triterpene methyl ethers (PTMEs) have been shown to be specific mostly to Poaceae [9], whereas triterpenyl acetates are mostly produced by Asteraceae [10]. These plant-derived biomolecules could be particularly informative of the past local presence of a plant, even in the absence of any vegetal macroremains in the sedimentary archives.

Recent research is focused on the detection of molecular biomarkers specific to *Panicum* in sedimentary archives as traces of the past presence of millets in paleosols and sediments. Increased attention has been paid to the studies of miliacin, PTME specific to *Panicum*, and some other Poaceae. Miliacin is mainly concentrated in seeds, and it is also normally present in leaves and stems but absent from hulls and roots [11]. In sedimentary deposits, the detection of miliacin is commonly regarded as trace evidence of the past presence of the broomcorn millet [11–14]. *Panicum miliaceum* L. (Poaceae) is a C4 warm-season annual grass

that is highly tolerant of poor soils, drought, and high temperatures. This small-grained cereal is used in foods and as a forage plant as it requires very little water and has a short growing cycle. Today, it is extensively cultivated in arid regions, which have poor soils and hot, dry weather [15]. The earliest finds of *P. miliaceum* were recorded in China in the form of charred grains from Early Neolithic sites dated to the early–mid 6th millennium BC [16]. Archaeobotanical research indicated that millet was widely spread in Europe by the 2nd millennium BC [17–19]. Uncertainties about the timing and diffusion path of its adoption by European societies were mostly resolved thanks to an extensive program of AMS dating of charred broomcorn millet grains from prehistoric sites in Europe [20].

The detection of *P. miliaceum* caryopses in archaeobotanical records, which is crucial for the reconstruction of the time and spread of its cultivation, could also be used to support the interpretation of miliacin as a plant-specific biomarker. In fact, some controversy remains over its interpretation as evidence of broomcorn millet since miliacin is produced by a variety of plants (53 species), mainly Poaceae (45) [21]. Recent biomolecular studies [9,11,13] have restricted the production of miliacin, in combination with other PTMEs, to a few plants belonging to *Panicum* and *Pennisetum* species. Nevertheless, if miliacin is found as the sole PTME and in high concentration, it is very likely that *P. miliaceum* is the source [11]. Nevertheless, the need for an unequivocal association with *P. miliaceum* alone remains. Actually, *P. miliaceum* caryopses are hardly preserved in archaeological deposits due to biodegradative or taphonomic processes. Additionally, the identification of broomcorn millet in phytolith records [22,23] can support the attribution of miliacin as a biomarker specific to *P. miliaceum*, but this is still an underexplored research field in European countries (e.g., [24]). Furthermore, when present, both of these *P. miliaceum* macro- and microremains show evidence of the use of this plant at a site but not necessarily its local cultivation.

When coupled with pollen, could miliacin be considered reliable evidence of broomcorn millet growing locally? Indeed, palynology is the most powerful tool to reconstruct vegetation cover and environmental changes, providing detailed information on vegetation composition and distribution and allowing for the identification of anthropogenic environments and cultural landscape evolution (e.g., [25–29]). Especially when related to archaeological contexts, pollen evidence could elucidate aspects of agricultural systems, land-use types, and crop cultivation (e.g., [30–32]). *Panicum* is mainly a self-pollinating plant and a low-pollen producer [33]. When found in pollen samples from sedimentary contexts, millet pollen grains confirm the local past presence of the plant.

Biomolecular investigations integrated with pollen studies are innovative and yet rarely applied methods for certifying the presence of broomcorn millet and its use in the agricultural activities of an archaeological site. In stratigraphic sequence samples, the comparison of miliacin concentrations and pollen data for *Panicum* could be particularly informative regarding this topic. This is conditioned by several factors that affect both the production, transport, and archiving of these two tracers from the same plant. On the one hand, in *P. miliaceum*, miliacin is very abundant only in seeds (0.5% weight; [11]), and, in general, its detection is dependent on the site. For example, detecting miliacin in lake sediments supposes that a sufficient (depending on the lake's catchment area and surface area) number of seeds were produced in the catchment and that the mechanism of transportation of miliacin from the field to the lake sediment (more plausibly after ingestion and excretion by humans) was efficient [2]. On the other hand, millet pollen is transported by the wind a short distance from the source plant and attests to its presence in situ [34].

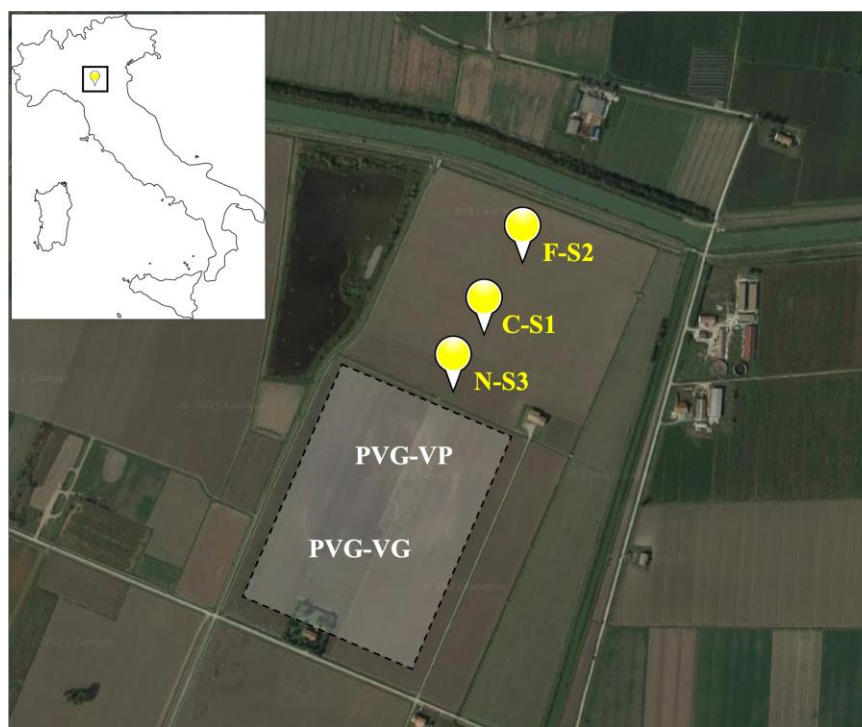
This paper aims to verify the relationship between miliacin and *Panicum* pollen content in the near-site cores of the Terramara S. Rosa di Poviglio (3500–3120 cal yr BP, Po Plain, N Italy), where sediment records continuously span at least the last ~15,000 years and provide detailed and high-resolution information on vegetation history and land-use changes in the area [35]. Pollen analyses at the Terramara Bronze Age sites (3500–1950 cal yr BP) in the Po Plain have indicated the presence of *Panicum* during increasing climate aridity [36–38], and the first biomolecular studies attest to traces of miliacin in contexts inside Terramara

settlements [39]. The integration of biomolecular techniques and pollen analyses may improve the information on broomcorn millet adoption by the Terramare culture.

## 2. Materials and Methods

### 2.1. Study Area

The Terramara Santa Rosa di Poviglio (lat. 44°52'21" N; long. 10°34'3" E) is located in the alluvial plain of the Po River, about 3 km southward from the present-day course of the river (Figure 1). The climate is semi-continental, with mean annual temperatures of 12–14 °C and mean annual rainfall between 600 and 800 mm. This area was occupied by a swamp until the 15th century [40], and even today, it is poorly drained. The territory is characterized by intensive industrial agriculture with the production of crops (mainly cereals for food, tomatoes for industrial use, and *Medicago* for fodder), orchards (Rosaceae–Prunoideae, including cherries, plums, apricots, and grapes for wine production), and animal breeding (especially pigs for meat and cattle for milk). Hygrophilous forests are spread near rivers, canals, and springs, while sparse woods consisting of mixed oakwood are the most common forest cover, as attested from past pollen diagrams in the region [41–44]. Almost 40 years of archaeological excavations of the site and a geophysical survey [45] have revealed that the archaeological structures of the Bronze Age settlement consist of an earthen rampart, wood fences, and two dwelling areas named “Villaggio Piccolo”—“Small Village” (PVG-VP) and “Villaggio Grande”—“Large Village” (PVG-VG; Figure 1) [37].

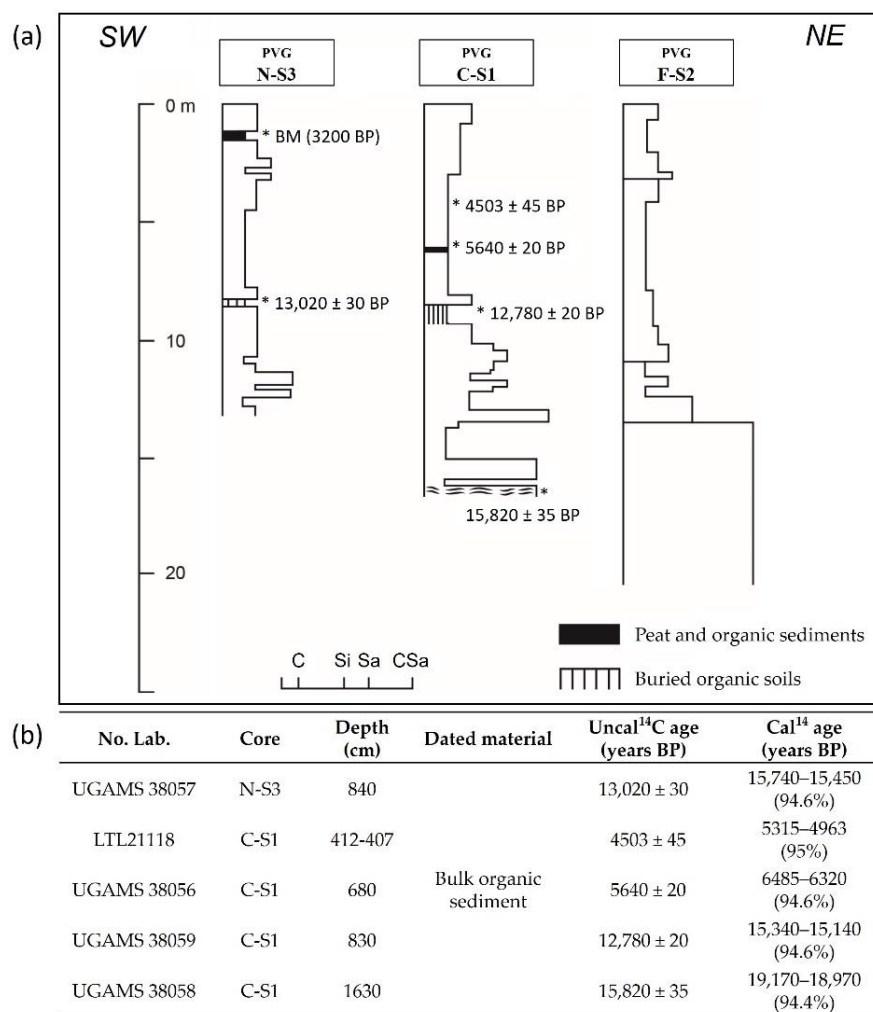


**Figure 1.** Location map of the Terramara Santa Rosa di Poviglio (PVG) site (VP = “Villaggio Piccolo”—“Small Village”; VG = “Villaggio Grande”—“Large Village”) in the central Po Plain, N Italy, and position of the three cores (N-S3, C-S1, and F-S2) drilled in a SW-NE direction at ~150 m, ~320 m, and ~525 m, respectively, from the Terramara (GoogleEarth™).

### 2.2. Sediment Samples

During the excavation campaign carried out in 2018, three continuous near-site cores (Near-S3, Central-S1, and Far-S2) were collected in a SW-NE transect at different distances (~150 m, ~320 m, and ~525 m) from the Terramara S. Rosa di Poviglio [35] (Figure 1). A total of 292 pollen samples were collected (N-S3: 77 samples; C-S1: 150 samples; F-S2: 65 samples) at about 10–30 cm intervals, according to the stratigraphy and archaeological phases. These

cores span about ~15,000 years; the chronology relies on radiocarbon dates obtained from organic sediments collected along the stratigraphic sequence of the N-S3 and C-S1 cores and ceramic fragments (Figure 2). The organic layer of the N-S3 core is archaeologically dated to the Middle Bronze Age (BM) and stratigraphically correlates to the occupation layers of the “Villaggio Piccolo” stratigraphy that has been archaeologically and radiometrically dated to a well-defined window of the Italian Middle Bronze Age [46]. The F-S2 core has been stratigraphically correlated to the other two cores.



**Figure 2.** Near-site cores (N-S3, C-S1, and F-S2) of the Terramara S. Rosa di Poviglio: (a) stratigraphy and uncalibrated age BP marked by \*; C = clay, Si = silt, Sa = sand, CSa = coarse sand; (b) AMS-<sup>14</sup>C ages for N-S3 and C-S1; the calibration into calendar years (2σ range) was calculated with R Clam using IntCal20 Northern Hemisphere <sup>14</sup>C calibration curves [47].

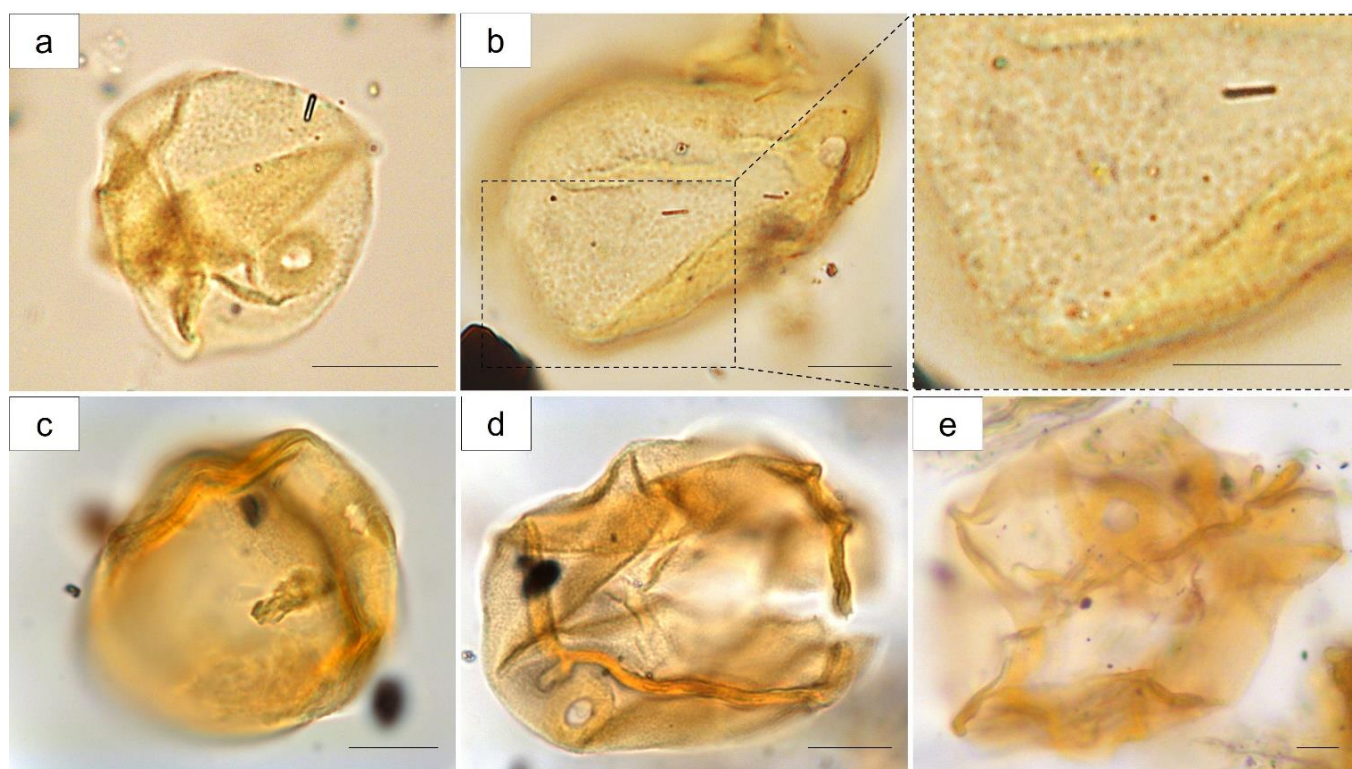
In 2020, a subsampling step was performed for biomarker analysis with a selection of 31 samples mainly related to Holocene chronology. Thick sediment discs ranging 1–2 cm in size were taken in correspondence with the 2018 pollen sampling, and then a subsampling of 1 cm<sup>3</sup> for each disc was taken and wrapped into aluminum sheets to avoid any contamination with plastic materials.

### 2.3. Pollen Treatment and Analysis

Pollen was extracted from 195 selected samples (N-S3: 77 samples; C-S1: 90 samples; F-S2: 28 samples), according to the routine method in use in the Laboratory of Palynology and Palaeobotany of Modena-LPP [48,49]. About 2 g of sediment from each sample were treated through sieving and heavy-liquid flotation with sodium metatungstate hydrate.

*Lycopodium* spore tablets were added to calculate the concentration (pollen per gram = p/g). Pollen was identified at 1000× magnification with the help of atlases/keys (e.g., [50,51]) and the LPP reference pollen collection.

Particular attention was paid to the identification of different types of Poaceae pollen grains (Figure 3), which are divided into morphological groups according to the literature [52,53], with the correction factor for glycerol jelly [25]. Pollen grains smaller than 37 µm (only a few wild grasses are larger) with annulus diameters smaller than 8 µm were included in the wild grass group [53,54]. According to Beug [52], cereal pollen is larger than 37 µm. The *Hordeum* group includes pollen between 37 and 50 µm in size with an annulus diameter between 8 and 12 µm, while the *Avena/Triticum* group includes pollen larger than 50 µm, with a mean annulus diameter larger than 12 µm [25,52]. The presence of *Zea mays* (mean pollen size larger than 60 µm) was identified according to Beug [55]. Morphological characterization of *Panicum* pollen can be difficult, as it includes pollen with medium to large sizes (32–62 µm in diameter) and *porus* plus annulus between 8 and 13 µm; as such, it overlaps, especially with the *Hordeum* group. However, it could be identified by the presence of insulate exine, which is absent in the *Hordeum* group [52,53,55,56]. Different species of *Panicum* and other genera (e.g., *Setaria* and *Echinochloa*) could be included in the same pollen type. Therefore, the term *Panicum* s.l. was used to indicate this pollen type, which includes both cultivated millet (*P. miliaceum*) and wild grasses. In this paper, “wild cereals” refer to large Poaceae pollen (including the *Avena/Triticum* group, *Hordeum* group, and *Panicum* s.l.) observed in pre-Holocene samples.



**Figure 3.** Poaceae pollen grains: (a) wild grass group (23 µm); (b) *Panicum* s.l. (43 µm) and details of its verrucate exine; (c) *Hordeum* group (40 µm); (d) *Avena/Triticum* group (51 µm); (e) *Zea mays* (101 µm). The scale is 10 µm.

On average, about 350 pollen grains per sample were counted [35]. Percentages were calculated based on a pollen sum, including both identified and unidentified pollen grains. Only data on Poaceae (wild grass group, cereals—*Avena/Triticum* and *Hordeum* groups, and *Panicum* s.l.) were reported in this paper.

Pollen diagrams, drawn with Tilia 3.0.1 [57], also show selected pollen curves as follows: Arboreal Pollen/Non-Arboreal Pollen (AP/NAP), hygrophilous herbs (Cyperaceae, *Juncus* type, *Phragmites*, *Sparganium emersum* type, and *Typha latifolia* type), and Anthropogenic Pollen Indicators—API group (*Artemisia*, *Centaurea*, Cichorieae, *Plantago*, *Trifolium* type, *Urtica*, and cereals; [58]).

#### 2.4. Biomolecular Analysis

The lipid extraction and separation were based on Jacob et al. [9]. The samples were crushed to powder after being dried in an oven at 50 °C for 48 h. About 2–3 g of sample powder were ultrasonically extracted with a mixture of dichloromethane and isopropanol (2:1 v:v), which was repeated three times. After being combined, the extracts were separated into neutral and acidic fractions by solid-phase extraction using silica bonded with aminopropyl groups. The neutral compounds, including PTMEs and triterpenyl acetates, were eluted with dichloromethane and isopropanol (2:1 v:v). Additionally, 5 $\alpha$ -cholestane was added as a quantitation standard prior to the analysis. The identification and quantification analyses were performed using a gas chromatography instrument (GC; Trace 1300, ThermoScientific, Waltham, MA, USA) coupled to a mass spectrometer (MS; ISQ QD, ThermoScientific). The gas chromatograph was fitted with a BPX5 capillary column (60 m; 0.32 mm i.d.; 0.25  $\mu$ m film thickness). The GC operating conditions were as follows: the temperature was held at 80 °C for 1 min, then increased from 40 to 120 °C at 30 °C min<sup>-1</sup> and 120 to 310 °C at 3 °C min<sup>-1</sup>, with a final isothermal hold at 310 °C over 40 min.

The sample was injected splitless, with the injector temperature set at 280 °C. Helium was the carrier gas at 1 mL/min. The mass spectrometer was operated in the electron ionization (EI) mode at 70 eV ionization energy and scanned from 50 to 650 Da. PTMEs, such as miliacin and triterpenyl acetates, were identified using authentic standards [10,11]. The concentrations were determined by comparing the peak areas of specific biomarkers on the GC trace with those of 5 $\alpha$ -cholestane and reported to the mass of the sample to provide a concentration value. Replicate injections of some samples lead to a reproducibility better than 3 ng/g.

### 3. Results

#### 3.1. Pollen of *P. Miliaceum* and Other Poaceae

Poaceae pollen grains are ubiquitous in the three cores. The Poaceae wild grass group is dominant among herbs (N-S3: 15.3%, C-S1: 12.6%, and F-S2: 12%, on average; NAP values: N-S3: 54.7%, C-S1: 56%, and F-S2: 50.2%). Among the cereals, the *Hordeum* group is the most common in the pollen spectra, and it is present in at least half of the samples (N-S3: 0.8%, C-S1: 0.9%, and F-S2: 0.6%).

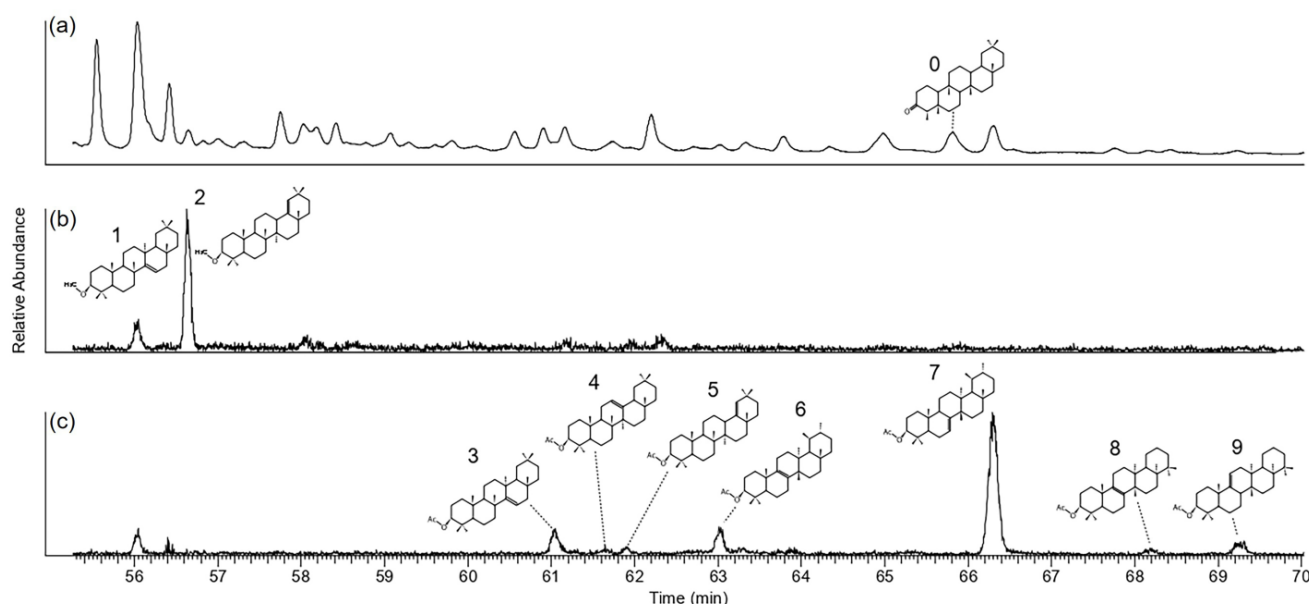
In the C-S1 core, the almost continuous occurrence of high values of *Panicum* s.l. in the pre-Holocene samples (up to 3.3% at a depth of 846 cm) is probably not indicative of the presence of *P. miliaceum* but rather wild grasses producing large pollen (e.g., *Echinochloa*, *Glyceria*, and *Setaria*), as no pre-Holocene broomcorn millet (or any other) cultivation is attested. However, in lower percentages, *Panicum* s.l. pollen is also present in the pre-Holocene levels of the N-S3 (0.3% at 1013 cm) and F-S2 cores (0.9% at 830 cm). Similarly, the *Hordeum* group (C-S1: up to 4.6% at 825 cm) and, to a lesser extent, the *Avena/Triticum* group (N-S3: up to 0.6% at 1165 cm) also show significant percentages in pre-Holocene levels, both in N-S3 and C-S1.

Even for the Holocene levels, the C-S1 core is the one with the highest presence of *Panicum* s.l. pollen, although always in lower percentages compared to the samples described above. The *Hordeum* group and, to a lesser extent, the *Avena/Triticum* group have been observed continuously during the Middle–Late Neolithic period (ca. 7050/6450–5450 cal yr BP) and the pre-Bronze Age in all cores. The higher percentages of cereals characterize the Middle Bronze Age levels (*Hordeum* group: 0.8% in N-S3, 1.2% in C-S1, and 1.4% in F-S2; *Avena/Triticum* group: 0.4% in N-S3, 0.1% in C-S1, and 0.7% in F-S2; mean values). The peak of the *Hordeum* group is reached in the N-S3 core (2.2% at 128 cm), while

the highest value of the *Avena/Triticum* group is attested in the F-S2 core (1.4% at 281 cm). The traces of *Panicum* s.l. pollen in the C-S1 core (0.3% at 177 cm—combined evidence of miliacin up to 17.2 ng/g) indicate an increased crop diversity (mainly wheat and barley until then). The increase in cereals is evident in the most recent levels of the cores, with especially high percentages for the *Hordeum* group (e.g., N-S3: 5.6% at 99 cm and 4.8% at 45 cm; C-S1: 4.9% at 81 cm and 6.2% at 61 cm; F-S2: 1% at 73 cm). *Zea mays* pollen is present in the C-S1 core (0.8% at 61 cm and 0.3% at 81 cm).

### 3.2. Molecular Biomarkers

Apart from classical molecular biomarkers, such as *n*-alkanes and *n*-alcohols, the neutral fraction of the lipid extracts of Poviglio samples contained a wide variety of pentacyclic triterpenes, of which several original ones could be detected and identified (e.g., Figure 4). Miliacin (olean-18-en-3 $\beta$ -ol methyl ether) and crusgallin (taraxer-14-en-3 $\beta$ -ol methyl ether) were the only PTMEs detected. Pentacyclic triterpenyl acetates specific for Asteraceae were also found in some samples. These comprised taraxeryl (taraxeryl), olean-12-en-3 $\beta$ -yl ( $\beta$ -amyrenyl), olean-13(18)-en-3 $\beta$ -yl (germanicyl), bauer-8-en-3 $\beta$ -yl (isobauerenyl), bauer-7-en-3 $\beta$ -yl (bauerenyl), pichier-8-en-3 $\beta$ -yl (isopichierenyl), and pichier-9(11)-en-3 $\beta$ -yl (pichierenyl) acetates.



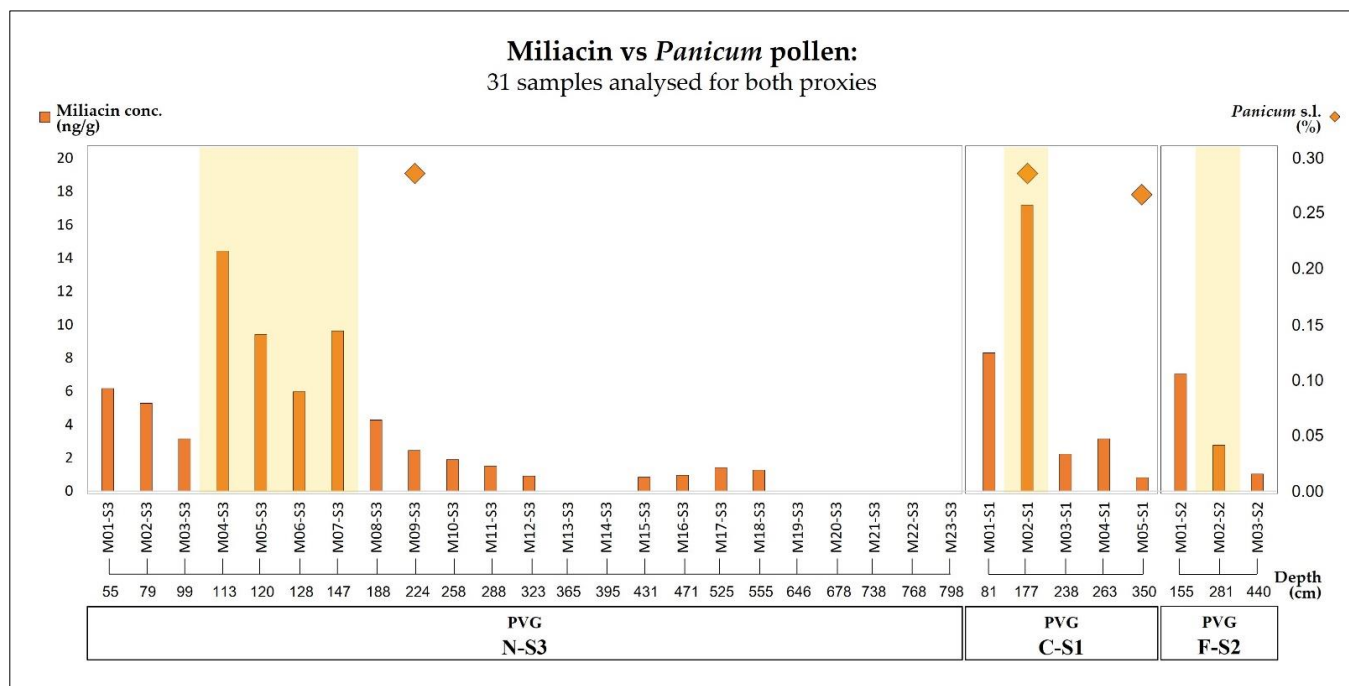
**Figure 4.** Partial chromatograms of the neutral fraction of the lipid extract of sample M04-S3 (113 cm). (a) Total Ion Current chromatogram showing the peak of friedelin (0); (b)  $m/z$  425 + 440 ion-specific chromatogram revealing the peaks of pentacyclic triterpene methyl ethers (1—crusgallin; 2—miliacin); (c)  $m/z$  453 + 468 ion-specific chromatogram illustrating the peaks of pentacyclic triterpenyl acetates (3—taraxeryl acetate; 4— $\beta$ -amyrenyl acetate; 5—germanicyl acetate; 6—*isobauerenyl* acetate; 7—*bauerenyl* acetate; 8—*pichierenyl* acetate; 9—*pichierenyl* acetate).

Up until now, *bauerenyl*, *pichierenyl*, and *isopichierenyl* acetates were only described in *Picris hieracioides* (Asteraceae) [59–62]; thus, they are specific at a species level. Apart from Lavrieux [63], this study constitutes their first detection in sediments. The samples also contained friedelin (*friedelin-3-one*), a pentacyclic triterpene that is found in many angiosperms but is not specific at the genus or even species level.

Miliacin concentrations are very low (<4 ng/g), below 250 cm, in the N-S3 core (pre-Bronze Age), and then increase upward to reach 6 to 15 ng/g in Bronze Age sediments. The same pattern is observed in the C-S1 core, with concentrations maximizing at 18 ng/g at 177 cm, i.e., for samples deposited during the Bronze Age (Figure 5). Crusgallin was only detected in one sample from N-S3 and two samples from C-S1, all of which date back to the Bronze Age. Only *bauerenyl*, *pichierenyl*, and *isopichierenyl* acetates were quantified.



Significant concentrations (>5 ng/g) in bauerenyl, pichierenyl, and isopichierenyl acetates were only found in samples corresponding to the Bronze Age.



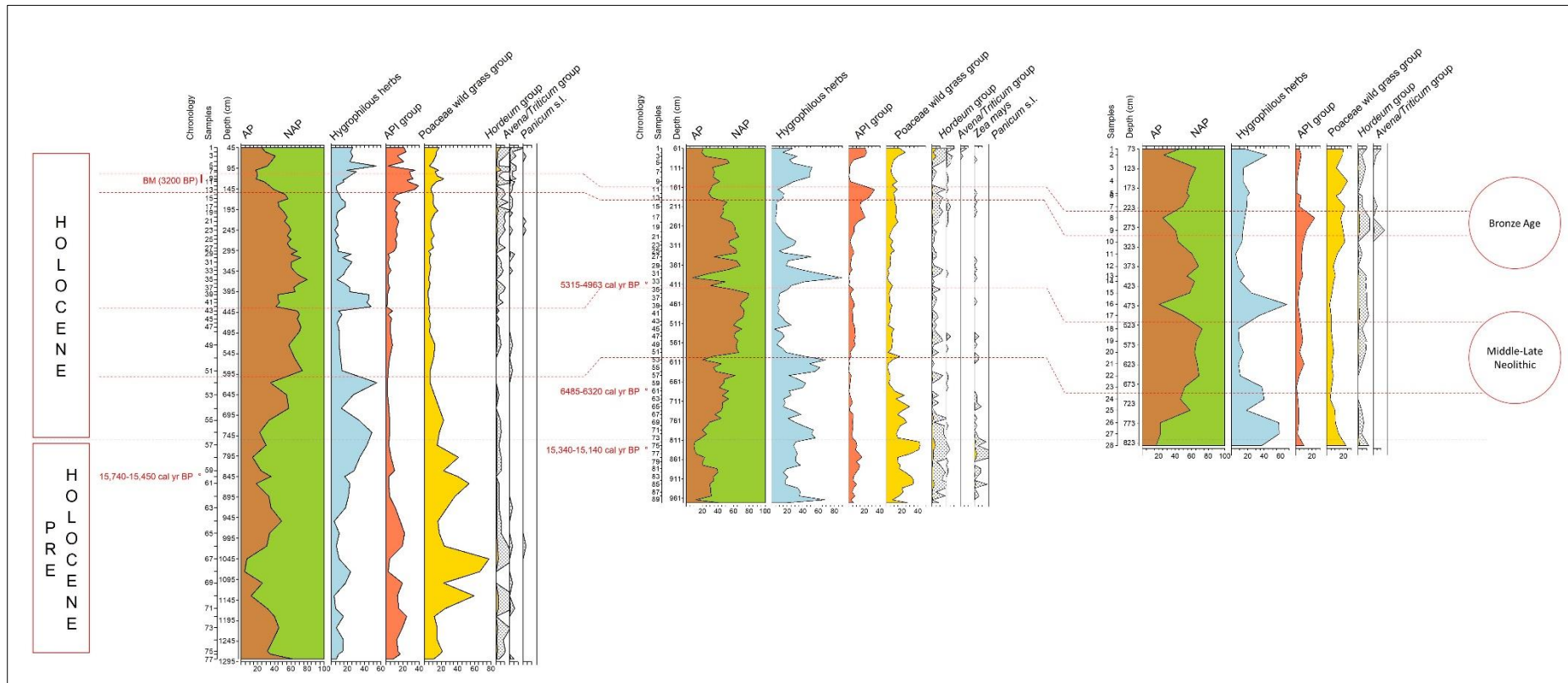
**Figure 5.** Near-site cores (N-S3, C-S1, and F-S2) of the Terramara S. Rosa di Poviglio: concentration of miliacin (ng/g) and *Panicum* s.l. pollen percentages from the 31 samples studied for lipid biomarkers. The selected samples come from the Holocene levels; the orange bands mark the Bronze Age phase in each core.

#### 4. Discussion

The integrated analysis of pollen and molecular biomarkers from the near-site cores of the Terramara S. Rosa di Poviglio provided new data for improving knowledge on agriculture and human impact in the central Po Plain. Despite the low concentrations of miliacin along the cores, its specific presence in key phases of the local environmental transformation described by pollen records may contribute to detailing the reconstruction of the spread of *Panicum miliaceum* in N Italy. For the sake of clarity, in the following section, the pollen and biomarker samples are indicated with the depth of sampling.

##### 4.1. The Dualistic Interpretation of the Open Environment Inferred from Poaceae Pollen Curves

Pollen analyses from the N-S3, C-S1, and F-S2 cores provide information on vegetation dynamics in the central Po Plain from a long-term perspective (from at least the last ~15,000 years; [35]). According to the stratigraphy, radiocarbon dates, and possible comparison with the main reference pollen records for the study area (e.g., [64–66]), the bottom of the cores intercepted the pre-Holocene phases. Pollen spectra from the pre-Holocene levels of the cores indicate the presence of an open vegetation dominated by Poaceae (Figure 6). According to the presence of plants in wet environments, the occurrence of increasing values of *Panicum* s.l. at the bottom of the cores is probably not indicative of the presence of *Panicum* itself but rather wild grasses producing large pollen (e.g., *Echinochloa*, *Glyceria*, and *Setaria*), which are typical of wet and ruderal environments, such as banks, riverbeds, and swamps, and warm temperatures [67]. Marsh and alluvial plain deposits from almost the whole Late Pleniglacial/Lateglacial have also been identified at other sites in the lower central Po Plain [68].



**Figure 6.** Near-site cores (N-S3, C-S1, and F-S2) of the Terramara S. Rosa di Poviglio: percentage pollen diagram of selected sums for palaeoenvironmental reconstruction (AP/NAP, hygrophilous herbs, API group [58], wild and cultivated Poaceae). Enhanced curves  $\times 10$ .

Pollen records indicate that vegetation changes in the early Holocene were mainly related to climate oscillations, and fairly open riparian communities (dominated by hygrophilous herbs: Cyperaceae, *Sparganium emersum* type, and *Typha latifolia* type) characterized the study area [69]. The open landscape is also indicated in the upper part of the cores and is associated with the increasing pressure of human activities in the area, as evidenced by the rapid and significant increase in Anthropogenic Pollen Indicators (API group [58] in Figure 6). Palynological data show vegetation changes linked to the interplay between climatic and human forces acting on the environment. The openness of the landscape strongly increased toward the Bronze Age, as indicated by an expansion of the space dedicated to cereal crops [37]. Cereals were mainly barley (represented by the *Hordeum* group), but wheat (*Avena/Triticum* group) was also cultivated in the surrounding area (Figure 6). Pollen curves suggest that the distribution of barley fields was almost ubiquitous, while wheat is documented mainly in the N-S3 and F-S2 cores. In the C-S1 core, the presence of *Panicum* s.l. could be an indication that crops were probably more diversified.

According to Cremaschi et al. [37], broomcorn millet spread during the Bronze Age in the Po Plain, and it was probably introduced by the population of the Terramara S. Rosa di Poviglio during drier phases (Bronze Age Warm Period, or BAWP; [70]) or sown at the end of the season in the same fields where other cereal crops had already been harvested. Widespread arid conditions and poor nutrient supply are also indicated by the decrease in cyanobacteria colonies and riparian vegetation [37,69] and by the increase in Cichorieae and other Asteraceae [37], suggesting an expansion of dry meadows and pasturelands in the area. The biomolecular analyses also indicated the presence of triterpenyl acetates specific for Asteraceae-*Picris hieracioides* (such as bauerenyl and pichierenyl acetates) in the Bronze Age samples.

During the Roman Period, the territory was densely occupied, and the plain was organized into a rural system known as “centuriation” (from about the 2nd century BC until the 5th century AD; [71]). The high percentages of cereals (up to 6.4% at 99 cm in N-S3) agree with this new human occupation. It is worth noting that a Roman *villa rustica* with its own agricultural productive area was located in the same territory occupied by the Bronze Age settlement [72,73].

In the Middle Ages (6th–10th centuries AD), a palustrine environment spread in the territory following the “Roman empire crisis” [40,73], with a large diffusion of hygrophilous herbs, including plants belonging to Paniceae [35].

During the Renaissance, a rapid loss of wet environments occurred after land reclamation (15th–16th centuries AD). The agrarian landscape began to characterize the Po Plain, dominated by cereal crops in the lowland. The recovery of *Zea mays* pollen in the upper part of the core (Figure 5) matches historical sources documenting the introduction of this cultivation in the Emilia-Romagna Region between the end of the 16th century and the first half of the 17th century [74].

#### 4.2. The Combined Evidence of *Panicum* Pollen and Sedimentary Miliacin during the Holocene

*Panicum* pollen was identified in the three Poviglio cores, but only in 3 of the 31 samples analyzed for lipid biomarkers (N-S3: 224 cm—pre-Bronze Age; C-S1: 350 cm—pre-Bronze Age, 117 cm—Bronze Age; Figure 5). *Panicum* s.l. pollen grains were identified in the Middle–Late Neolithic samples (C-S1 and F-S2; Figure 6). Miliacin was detected in low abundance in at least three Neolithic samples in the N-S3 core (Figure 6). In a recent synthesis of miliacin sedimentary records from six lakes in the European Western Alps by Jacob et al. [14], a low concentration of miliacin before the supposed introduction of millet in the Alps was considered an indication of the presence of another miliacin producer, such as wild *Panicum* sp. or *Pennisetum* sp. At that time, broomcorn millet was already a “Neolithic package of founder crops” in Europe (e.g., [75–78]). Based on *Panicum* macroremains and biomolecular markers, the challenging topic of reconstructing the timing and spread of millet cultivation in the study area requires further investigation.

Miliacin concentrations increased slightly in the pre-Bronze Age samples, indicating the presence of miliacin producers. According to our hypothesis, the presence of broomcorn millet is suggested when the combined evidence of pollen and miliacin occurs (N-S3: 224 cm; C-S1: 350 cm; Figure 5).

*Panicum* pollen grains are almost absent in the Bronze Age levels. *Panicum* s.l. has been found only in the C-S1 core (177 cm), whereas miliacin was documented in six samples of the three cores (Figure 5). As the occurrence of *Panicum* pollen grains was quite rare in the sediment samples (the plant is a low-pollen producer, as are the other cereal species), when it is recorded, it testifies to the occurrence of the plant. Archaeobotanical evidence reveals that millet was widespread in Europe in the 2nd millennium BC, but there is uncertainty about the timing and diffusion path of its adoption by European societies [20]. Current research indicates that millet cultivation was spread for alimentary purposes by the Terramare civilization [20,79,80]). Archaeobotanical remains from Terramare sites (e.g., Montale [36]; Fondo Paviani [39]) indicate the diffusion of millets from the Early Bronze Age with an increasing trend of spread up to the Recent Bronze Age. During this last phase, a climatic crisis, including the depletion of water, affected the agro-sylvo-pastoral system of Terramare, mainly based on irrigated agriculture [37]. The increasing arid conditions toward the Recent Bronze Age are reflected by the diversification of cereal crops, shifting to drought-tolerant varieties as millets adopted by the Terramare people [37,81].

The combined evidence of *Panicum* pollen and miliacin in the Bronze Age level of C-S1 (177 cm) suggests that broomcorn millet could have been cultivated at some distance (~300 m, i.e., C-S1 position; Figure 1) from the settlement. Interestingly, in the same sample, the *Avena/Triticum* and *Hordeum* pollen groups decreased. Nevertheless, here as in other Terramare sites, intercropping practices are attested (e.g., [34,36,37]). In the N-S3 core, the continuous presence of miliacin in the last phases of the Bronze Age can be considered an indicator of the local presence of *Panicum* sp.

Miliacin concentrations in the three Poviglio cores are always lower than those found in sediment samples from the Terramara of Fondo Paviani (Verona, N-E Italy; [39]). Many factors can control miliacin concentrations, such as dilution by the mineral matrix and transport of millet from cultivation or consumption sites [14]. Even at Fondo Paviani, the lowest miliacin yields came from a minerogenic unit constituting the base of the peripheral earthen rampart. The highest miliacin yields in the study by Dal Corso et al. [39] came from ash lenses of anthropogenic origin in the ditch fill. The presence of phytoliths derived from the chaff of pooid and panicoid cereals, grass leaves and stems, and panicoid inflorescences have suggested that the ashes were derived from the burning of dung from domestic animals [24,82].

As for the more recent chronological phases, the ubiquitous presence of miliacin seems to suggest a growth of *Panicum* in the area. However, the presence of this plant is not reflected in the pollen record. Broomcorn millet became a common culture during the Iron Age in northern and central Italy (ca. 3100-2400 cal yr BP; [83]), while in Roman times, the consumption of millet was reserved for animal feed and it was deemed suitable only for the poor as it was considered a low-quality cereal [84]. In general, archaeobotanical evidence suggests a minor presence of millet compared to other cereal crops at Roman sites [85]. A recent synthesis of Roman food from about one hundred Roman-period archaeological sites (from the 3<sup>rd</sup> century BC to the 6th century AD) located in northern Italy [86] highlights the presence of broomcorn millet grains in inhabited places and infrastructures and, in lesser amounts, in cemeteries and cult places. Carpological remains indicate the importance of “minor” cereals, especially broomcorn millet, during the Early Middle Ages in northern Italy, motivated by environmental and cultural factors that modified habits concerning food production and diet [84,87].

#### 4.3. Wild-Cereal Hints from Large-Size Cereal Pollen (Pre-Holocene Phases)

Pollen spectra from the three cores, together with those from previous palynological research carried out in stratigraphic records from the Terramara S. Rosa di Poviglio, revealed

the presence of wild cereal pollen grains even in phases prior to the settlement. This feature is particularly evident in the pre-Holocene levels (Figure 6), when common meadow grasses covered most of the lawns. Poaceae exploit a wide variety of environments as pioneer taxa; as such, Poaceae spread in woodland clearings and swamps in the alluvial plain. The same environmental conditions allowed for the spread of cereals, mainly represented by the *Hordeum* group and *Panicum* s.l., produced by wild and not domesticated grass species. However, the morphology of wild cereal pollen cannot be distinguished from that of domesticated species [53]. In particular, the pollen size of wild Paniceae (e.g., pollen of *Setaria* and *Echinochloa*) is mainly medium to large (e.g., [56]), partially overlapping with cereal pollen's diameter measurements.

Wild cereals (e.g., most of the wild progenitors of cultivated cereals) are characterized by key adaptive features that allow them to thrive in disturbed soils and easily adapt to past environmental changes and conditions of environmental instability [56]. These plants are often described as pioneering, ruderal, weedy, and generalist and, thus, are able to colonize a wide range of habitats, from swamps to open grasslands to semi-desert habitats. The same behavior that allowed these plants to survive in a human-disturbed environment, described by Mercuri et al. [88], could also be suggested for Paniceae and other wild cereals in the central Po Plain during the pre-Holocene phases. At that time, unstable environments subject to alluvial events were present in the lowlands [35]. Pollen records from the plain identify a large open area dominated mostly by shrub-herbaceous communities related to alluvial processes that led to less-arid soil conditions and the temporary formation of wetlands and wet meadows (e.g., [68]). Arid grasslands and ephemeral pioneer meadows were probably present, defining the xerophilous herbaceous component. Open spaces and large meadows may have favored the frequentation of the area by grazing wild animals, as also suggested by the presence of coprophilous fungi in the palynological record [35]. However, the low quantities of coprophilous fungal spores [35] cannot support the hypothesis on the genetic modifications of the Poaceae taxa and the appearance of "proto-cereals" as a consequence of continuous overgrazing, as described in a different context by Andrieu-Ponel et al. [89].

In our record, the presence of large Poaceae pollen should probably be linked to climate change in the central Po Plain during the pre-Holocene [42], when the atmospheric drought may have favored the appearance of polyploidy in some species of Poaceae [89–92]. A recent study from the Early–Middle Holocene site of Takarkori in central Sahara [93] suggests that the occurrence of pollen anomalies, mainly in Paniceae, has derived from multiple genomes of some wild species as well as from plants' ability to respond to environmental stresses. The Poviglio pollen record suggests that climate could have played a major role in the spread of Poaceae with large pollen in pre-domestication phases. The presence of such proto-cereals and evidence of polyploid grasses seem to indicate that a genetic modification of Poaceae might be related to adaptive responses to environmental changes that appeared long before the emergence of agriculture.

## 5. Conclusions

The results obtained from this research provide a new contribution to the reconstruction of the history of the broomcorn millet crop in northern Italy by combining the molecular tracer of *Panicum miliaceum* and its pollen evidence. Archaeocarpological records often lack grains of broomcorn millet due to preservation issues; hence, further approaches to investigate its introduction and adoption are necessary. Whereas on their own, *Panicum* pollen and miliacin as proxies are ambiguous, and the co-occurrence of this pollen type and miliacin in the same deposits can be taken as evidence of the presence and local cultivation of *Panicum miliaceum*. This is what we see in the sediment samples from the near-site cores (N-S3, C-S1, and F-S2) of the Terramara S. Rosa di Poviglio. Our study demonstrates the potential of considering both types of materials preserved in the sediments and the importance of such an approach at sites where other (micro- or macro-) botanical evidence is poor or lacking altogether. In the cores of the Terramara S. Rosa di Poviglio, miliacin

shows the highest concentration in the samples attributed to the Bronze Age, confirming that the Terramare population cultivated millet fields. When recorded, the joint occurrence of *Panicum* pollen and sedimentary miliacin provides evidence of the local presence of the plant and suggests the adoption of millet crops, especially during the Recent Bronze Age, when the diversification of cereal crops shifting to drought-tolerant varieties has been documented [37,81].

In addition to the information obtained by the combination of pollen and biomolecular proxies, the detailed study of Poaceae curves provides new information on the presence of open areas related to different environmental conditions in the central Po Plain from a long-term perspective. Pollen spectra from the three cores indicate the presence of wild cereal pollen grains in the pre-Holocene levels. The adaptation of wild cereals to past environmental changes and environmental instability is attributed to their key adaptive features [88]. In this context, wild Poaceae with large pollen could result from adaptive responses (e.g., polyploidy) to climate changes, and this might be linked to phases of natural environmental changes occurring during the pre-Holocene phases in the central Po Plain.

The novel approach based on the combined evidence of *Panicum* pollen and miliacin indicated unequivocally the presence of broomcorn millet in sedimentary archives. In the current state of research, the precise chronology of the introduction and diffusion of millet crops in several large regions of Europe has been addressed [20], but some uncertainties remain about their local cultivation. Tracing millet through the two proxies could provide new, essential information on the timing of the earliest introduction of broomcorn millet in northern Italy.

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