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Ancient Starch Remains Reveal the Vegetal Diet of the Neolithic Late Dawenkou Culture in Jiangsu, East China

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The Liangwangcheng site, located in Pizhou County, Xuzhou City, northern Jiangsu Province, is one of the most important Neolithic Dawenkou Culture archeological sites in the Haidai area of China's eastern seaboard. In recent years, archaeobotanical studies in the Haidai area, mainly focusing on Shandong Province, have yielded fruitful results, while relatively few such studies have been undertaken in northern Jiangsu Province. Here, we report the results of dental residue analysis conducted on 31 individual human skulls unearthed from the Late Dawenkou Culture Liangwangcheng site. The starch granules extracted from these residue samples indicate that foxtail and broomcorn millet, rice, roots and tubers, and legumes comprised the vegetal diet of Liangwangcheng's occupants. Evidence suggests that mixed rice–millet agriculture played a definite role, with the coexistence of gathering as an economic element. According to archaeobotanical evidence from surrounding cotemporaneous sites, the Late Neolithic human groups that lived in the lower Huang-Huai River drainage shared similar subsistence patterns. Our results provide new evidence for a more comprehensive understanding of plant resource utilization and agricultural development in northern Jiangsu during the Dawenkou period.

Keywords: Liangwangcheng site, Neolithic, ancient starch, prehistoric subsistence, Dawenkou Culture

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INTRODUCTION

The Haidai Cultural Region (abbreviated Haidai) refers to prehistoric cultural groups that include a continuous and rich sequence of Neolithic and Bronze Age archeological cultures, covering Shandong, northern Jiangsu and Anhui, eastern Henan, and the southern Liaodong Peninsula in eastern China (Gao and Shao, 1984; Luan, 1997; **Figure 1**). This region plays a significant role in the Neolithic archeology of China by providing a long, continuous cultural sequence, exhibiting regional characteristics that distinguish it from other parts of China, and by preserving extensive deposits of ancient human occupations. This area's prehistoric human activity peaked during the Dawenkou (ca. 4100–2600 BCE) and Longshan (ca. 2600–1900 BCE) Neolithic periods, then

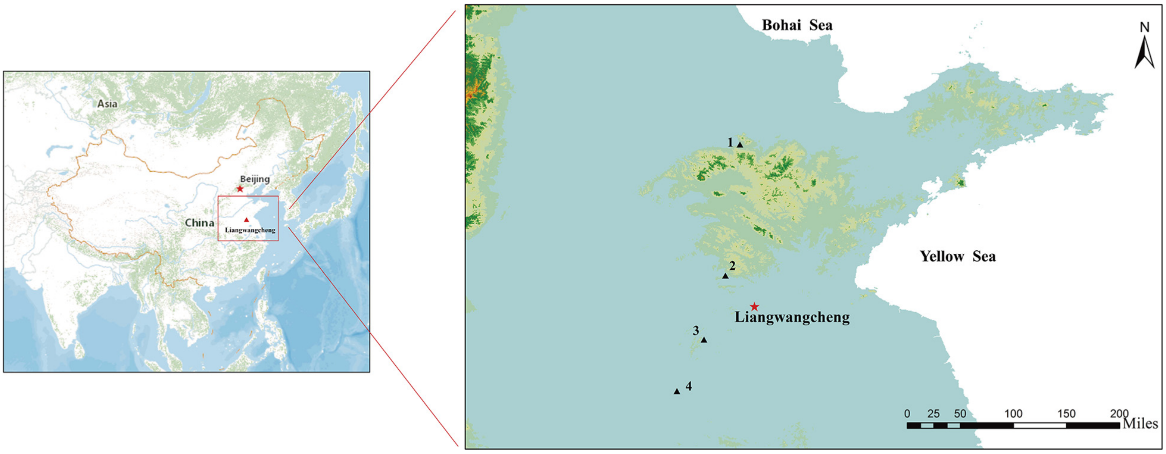

FIGURE 1 | Geographic location of Liangwangcheng and other Dawenkou Culture sites mentioned in this paper. (**1**, Jiaojia site; **2**, Jianxin site; **3**, Yangpu site; and **4**, Yuchisi site).

declined, gradually vanishing during the Yueshi period (ca. 1900–1500 BCE). Thus, Dawenkou and Longshan cultural remains in the Haidai area provide key evidence for understanding the evolution of social behavior and subsistence transformations during the Middle and Late Neolithic. Based on Neolithic archeological discoveries made in China thus far, the Haidai Culture is considered a distinct local cultural entity, differing from cotemporaneous prehistoric complexes on the Chinese Central Plain in Shanxi, Henan, and Shaanxi provinces. Therefore, it is necessary to pursue a comprehensive study of Haidai Culture, especially during the Dawenkou stage, when prehistoric populations expanded over a much more extensive geographical range in comparison with the preceding Houli (ca. 6500–5500 BCE) and Beixin (ca. 5300–4100 BCE) periods.

Ancient human subsistence patterns have been considered one of the most important issues in the scientific study of Neolithic adaptations, including the analysis of ancient diets, productivity, human social structures, and human–environment interaction, which are collectively extremely useful in the reconstruction of prehistoric societies. Among these subjects, the utilization of plant resources and the origin of agriculture in the Haidai region have been widely addressed, especially with respect to the appearance of rice–millet mixed agriculture (Jin et al., 2014; Wu et al., 2014; Guedes et al., 2015; Wu, 2019). It is thought that the development of Neolithic cultures in this region was related to diachronic climatic and paleoenvironmental changes (Jin and Wang, 2010a,b). The region's warm and humid climate present ca. 8000–6000 years ago (i.e., the postglacial hypsithermal) provided suitable natural conditions for the development and florescence of the Houli and Beixin cultures and the development of early agriculture. From 5500 to 5000 years ago, continuous fluctuations between relatively warm and relatively cool climatic conditions forced the Dawenkou people to respond to consequent environmental changes, triggering significant alterations in their subsistence systems and social structures (Dong et al., 2021). In addition, during this stage, ancient populations in the Haidai region were distributed across multiple varied landforms (e.g., coastal, in proximity to mountains, and near rivers), which offered different natural and ecological conditions, resulting in a plethora of economic adaptations. For example, during the late Neolithic Longshan period (ca. 2600–1900 BCE), populations inhabiting coastal areas of southeast Shandong took advantage of local hydrothermal conditions to intensively cultivate rice.

Contemporaneously, people occupying inland areas of northwest Shandong principally developed dry farming agricultural techniques (Jin et al., 2010; Guedes et al., 2015). Marine transgressions occurred several times during the early Dawenkou period (ca. 4100–2600 BCE) on the northern Jiangsu Plain and in Jiaozhou Bay (Huang, 1998), frequently altering the coastline, which had a dramatic impact on human settlement and subsistence activities. The overall number of archeological sites in this area decreased during the early Dawenkou period, and more shell mounds are found in coastal areas dating to this stage. Artifacts associated with fishing and hunting and remains of marine animals have been unearthed in such shell middens, and aggregate evidence from such contexts suggests a poorly developed agricultural economy which was quite different from the subsistence systems apparent at contemporaneous inland sites (Shandong, 1973; Yan and Zhang, 1987; Wang and Wu, 1992). It is clear that marine transgressions had a considerable impact on ancient people's subsistence practices in this area.

Recent research indicates that in the Haidai area, mainly in Shandong Province, incipient agricultural practices were initiated by the Houli Culture about 8000 cal BP, based on the discovery of morphologically cultivated millet plants from that context (Jin, 2012). Carbonized rice remains were discovered at the late Houli period Yuezhuang site and ^{14}C dated to 7050 $\pm$ 80 BP (Gary et al., 2006, 2013). However, during the Houli period, foxtail millet-based farming was not dominant, and a hunting–gathering–foraging economy still prevailed (Wu, 2019). Rice, foxtail millet, and broomcorn millet have been discovered at several sites dating to the following Beixin Culture period (5300–4100 BCE) (Chen, 2007; Wang H. et al., 2011; Wang and Jin, 2013; Jin et al., 2016, 2020), indicating the initiation of a rice–millet mixed agricultural economy. In addition, during this period, the proportion of agriculture as a component of the overall economy increased in comparison with the Houli. During the Dawenkou period (4100–2600 BCE), which followed the Beixin, agricultural economies continued to develop and accounted for a greater proportion of the subsistence system than previously. Based on the analysis of carbonized plant remains, however, broomcorn millet replaced foxtail millet in the agricultural repertoire at the beginning of the Dawenkou period (Jin et al., 2016).

Located on the margin of the Haidai Cultural region, northern Jiangsu Province shares a similar climate with southern Shandong but seems to have been less intensively occupied during the Neolithic than other parts of the Haidai. There, archeological sites are thinly distributed and have been the subject of very few chronological studies, leading to a lack of systematic understanding of Neolithic cultural development and subsistence patterns of the later Stone Age occupants of Jiangsu. Zooarchaeological and archaeobotanical studies were not systematically conducted at most early-excavated sites, thus the reconstruction of prehistoric economic forms has been limited by a lack of substantive evidence. The Liangwangcheng site is one of the most potentially valuable prehistoric sites uncovered thus far in northern Jiangsu Province and is widely considered an important archeological discovery (Nanjing et al., 2013), thus providing the opportunity and materials to explore prehistoric social constructs in this area. However, since flotation has not been conducted at the site, there are no macrobotanical remains available to support the discussion of scientific issues relating to plants or incipient agriculture. Plant micro-remains open the window for us to reconstruct plant utilization at Liangwangcheng and assist the interpretation of subsistence patterns in the Haidai area.

SITE AND ENVIRONMENT

The Liangwangcheng site (34°30′713″N, 117°47′629″E, 23–28 m above mean sea level) is located in Pizhou County, Xuzhou City, northern Jiangsu Province, on the east bank of the Beijing–Hangzhou Canal (Nanjing et al., 2013). From 2004 to 2009, the Nanjing Museum carried out fieldwork at this site. The cultural components of the Liangwangcheng site are multiple and diverse, including prehistoric remains from the Dawenkou Neolithic period to the historic era. Numerous Dawenkou period settlement remains were unearthed. Houses, pits, and 139 human interments representing more than 140 individuals were discovered, including adults and juveniles. According to Dong Yu's study (Dong et al., 2019), Carbon-14 dating suggests that these humans were interred between 4055 ± 20 BP (4425–4780 cal BP, 95.4% probability) and 4175 ± 25 BP (4586–4833 cal BP, 95.4% probability) [modeled in OxCal v.4.4, using IntCal20 calibration curve (Bronk Ramsey, 2009; Heaton et al., 2020; Reimer et al., 2020)]. In addition, based upon the burials and mortuary evidence and the results of stable isotope analysis of human bones, Dong and her colleagues suggested that social complexity was great and competition and differentiation between human groups and social classes were becoming more and more intense during the Dawenkou period. People began to choose various expressive forms to define and signal their identities, such as the number of funerary objects included in burials and the like. Therefore, during the Dawenkou period, the occupants of Liangwangcheng were likely undergoing fundamental transformations of their social complexity.

Northern Jiangsu, located in eastern China on the lower reaches of the Yellow River and the northern Huai River region, currently has a warm, temperate climate characterized by moderate rainfall and abundant sunshine. At present, a humid and semi-humid monsoon climate prevails (Köppen climate classification Cfa). Natural climatic conditions in this area are similar to those in southern Shandong. Northern Jiangsu is part of the transition zone between the Huanghuai Plain and Jianghuai Plain, bordering the mountainous area in southern Shandong Province to the north, with high terrain in the northwest and low topography in the southeast. The region is part of the Yi River, Shu River, and Sishui River basins, and the inland riverine network is densely distributed. The local vegetation is luxuriant, mainly composed of deciduous broad-leaved forests (Zhao, 2015). Paleoenvironmental studies suggest that the Holocene vegetation type in this area was mainly deciduous broad-leaved forest (Tang et al., 1993). Members of the Poaceae and Compositae dominated the community of herbaceous plants. The pollen record and trace element studies indicate that the climate in this region fluctuated several times 5000–4000 years ago, generally trending toward warm and dry (Jin, 1990; Zhao et al., 2014) with warmer average temperatures than at present. The environment of the late Neolithic in the Haidai area provided suitable conditions for human communities to survive and develop and significantly encouraged the Dawenkou Culture's prosperity.

MATERIALS AND METHODS

Human Dental Residue Analysis

Plant microfossil residue analysis has gained increasing popularity in archeology (McGovern et al., 2017; Prebble et al., 2019; Wang et al., 2019; Barber, 2020). Among such studies, dental residue analysis provides direct evidence of human diet and thus becomes an effective avenue for exploring ancient human subsistence, rather than indirect evidence obtained from artifacts or archeological sedimentary deposits. This method was begun in the 1970s, when scholars observed microbotanical remains in both dental calculus and invisible tooth residues, initiating pioneer achievements for research plant residues preserved on fossil teeth. Researchers also extracted plant residues for species identification by processing the residuum collected from tooth surfaces. In the 1970s, Lustmann employed a scanning electron microscope to study the morphological structure of anorganic dental calculus and found that calculus was composed of two components with different patterns of calcification (Lustmann et al., 1976). Dobney and colleagues also used a scanning electron microscope to analyze ancient dental calculus and were the first to discover plant microfossils such as phytoliths and starch granules embedded in dental calculus (Dobney and Brothwell, 1988; Olsen, 1988). Since then, this method has increasingly attracted the attention of scholars, globally. At the beginning of the 21st century, Piperno and Dillehay (2008) applied this method to the question of agricultural origins in Central America and achieved positive results. Based on this work, researchers identified plant species reflected by residues preserved in dental calculus and were able to distinguish wild versus cultivated attributes of plant remains, which played a crucial role in determining the timing of the rise of cultivation in Central America.

Nava et al. (2021) studied microfossils in mineralized dental plaque, and comprehensively analyzed buccal microwear and oral pathology, revealing dietary differences between the last foragers and first farmers at Grotta Continenza in central Italy. This method has subsequently been widely applied in China with great potential. Li et al. (2010) extracted and identified starch grains from dental calculus of Qijia Culture (ca. 4000 BP) people in Gansu Province and discovered that diversified dry farming was the primary subsistence strategy at that time (Li et al., 2010). Tao (2018) used this method to explore human diet at the Peiligang site in Xinzheng County, Henan Province, discovering starch granules from *Quercus*, members of the Fabaceae, tubers, and millets. Tao et al. (2020) also combined stable isotope analysis of human bones and the study of starch granules in human dental calculus from the Laodaojing Cemetery to further investigate the dietary role of wheat and the Eastern Zhou (770–256 BCE) agricultural economy on the Chinese Central Plains (Tao et al., 2020). This method has great potential for elucidating how prehistoric humans utilized plant resources and consequently broaden our insight into the structure of ancient societies and enhance our understanding of our ancestors' subsistence strategies and the process of agricultural development and intensification.

For the Liangwangcheng project, teeth were selected from 31 humans unearthed from 31 Dawenkou Culture graves (Layer 9 in the Liangwangcheng excavation report) for dental residue analysis (**Table 1**). We collected residue samples according to protocols established by Pearsall et al. (2004) and Guan et al. (2014). The residue samples comprise three levels: Level I, sediment attached to the surface of teeth; Level II, a liquid sample obtained by washing the tooth surface with distilled water; Level III, liquid samples derived by ultrasonic cleansing of teeth. A total of 72 residue samples were obtained and processed in the Key Laboratory of Vertebrate Evolution and Human Origins of the Chinese Academy of Sciences in Beijing. The experimental process followed that of Guan et al. (2010), integrating several laboratory operations (Chandler-Ezell and Pearsall, 2003; Pearsall et al., 2004). Processing included the following steps: concentration, deflocculation, and heavy liquid flotation. Starch granule and phytolith extraction slides were scanned with a Nikon Ni-E biological polarizing microscope. One hundred percent of glycerol was used as a mounting medium for starch and phytolith extractions. NIS-Elements D3.2 software was applied to perform two-dimensional (2D) measurements and other examinations. Both phytolith and starch slides were scanned at 200× magnification and photographed at 400× magnification.

Methodology of Geometric Morphometric Analysis of Starch Granules

Starch granule identification is challenging due to the biological attributes of those bodies. For starch granule analysis, researchers have employed an image comparison method, rendering taxonomic identification according to shape, location of the hilum, granule diameter, etc. As part of the development of this method, many modern starch images have been published providing significant comparative reference points for future study. At present, however, researchers have become dissatisfied with such identification techniques and are trying various quantitative methods to reliably identify unknown starch granules (Torrence et al., 2004; Liu et al., 2014; Coster and Field, 2015; Arráiz et al., 2016). The traditional measurement method cannot convey information concerning geometric structure, and radial measurement cannot adequately explain changes in organism shapes.

Geometric morphology, however, avoids the shortcomings of varying data sources, non-repeatability, and size and shape can be calculated altogether (Chen, 2017), which is beneficial to the further study of morphological differences in starch granules. Geometric morphometry analysis has been widely used recently in entomology, aquatic biology, medical science, and archeology (Slice, 2007; Mitteroecker and Gunz, 2009; Addis et al., 2010; Webster and Sheets, 2010; Adams and Otárola-Castillo, 2013; Park et al., 2013; McNulty and Vinyard, 2015; Savriama, 2018). However, it has not thus far been applied in starch granule analysis. For these reasons, we chose to apply geometric morphometry in our study. Thirty-five landmarks were identified for a single starch granule (**Figure 2**). TpsDig2 software was used to obtain landmark 2D coordinate data on starch granules and generating a thin plate spline (TPS) file for each species or unknown group. The plant species name is used as the classifier, and the landmark x and y value are used as variables in the matrix, constituting a grid with 71 columns. Data for one individual starch granule is recorded as one observation, i.e., one row in the data matrix. The TPS file then is imported into MorphoJ statistical software (Klingenberg, 2011) for general procrustes analysis (GPA) and canonical variate analysis (CVA).

General procrustes analysis is a straightforward approach to determining shape correspondence. Additionally, GPA is a multivariate exploratory technique that involves transformations of individual data matrices to provide optimal comparability. This technique scales and rotates each configuration of landmarks so that shape information can be extracted and compared among samples. CVA is used to identify shape features that best distinguish among multiple groups of specimens (Klingenberg, 2011) and is one of the most widespread analytical approaches in morphometrics. In our work, a CVA of the covariance matrix was conducted on the GPA transformed coordinates to reduce dimensionality for further analyses. We imported the TPS files into MorphoJ software for new

TABLE 1 | Liangwangcheng residue sample information.

No.	Specimen no.	Lab no.	Cultural period	Sex	Sample type
1	M154	S500	Late Dawenkou	Male	Level I
2	M154	S501	Late Dawenkou	Male	Level II
3	M154	S502	Late Dawenkou	Male	Level III
4	M129	S503	Late Dawenkou	Male	Level I
5	M129	S504	Late Dawenkou	Male	Level III
6	M120	S505	Late Dawenkou	Female	Level I
7	M120	S506	Late Dawenkou	Female	Level III
8	M81	S507	Late Dawenkou	Male	Level I
9	M81	S508	Late Dawenkou	Male	Level III
10	M254	S509	Late Dawenkou	Male	Level I
11	M254	S510	Late Dawenkou	Male	Level III
12	M151	S511	Late Dawenkou	Female	Level I
13	M151	S512	Late Dawenkou	Female	Level II
14	M151	S513	Late Dawenkou	Female	Level III
15	M113	S514	Late Dawenkou	Male	Level I
16	M113	S515	Late Dawenkou	Male	Level III
17	M225	S516	Late Dawenkou	Male	Level I
18	M225	S517	Late Dawenkou	Male	Level III
19	M143	S518	Late Dawenkou	Female	Level III
20	M135	S519	Late Dawenkou	Male	Level I
21	M135	S520	Late Dawenkou	Male	Level II
22	M135	S521	Late Dawenkou	Male	Level III
23	M114	S522	Late Dawenkou	Female	Level I
24	M114	S523	Late Dawenkou	Female	Level III
25	M144	S524	Late Dawenkou	Unknown	Level I
26	M144	S525	Late Dawenkou	Unknown	Level III
27	M92	S526	Late Dawenkou	Female	Level I
28	M92	S527	Late Dawenkou	Female	Level III
29	M118	S528	Late Dawenkou	Female	Level I
30	M118	S529	Late Dawenkou	Female	Level III
31	M216	S530	Late Dawenkou	Male	Level I
32	M216	S531	Late Dawenkou	Male	Level III
33	M145	S532	Late Dawenkou	Female	Level I
34	M145	S533	Late Dawenkou	Female	Level III
35	M108	S536	Late Dawenkou	Unknown	Level I
36	M108	S537	Late Dawenkou	Unknown	Level III
37	M147	S538	Late Dawenkou	Unknown	Level I
38	M147	S539	Late Dawenkou	Unknown	Level II
39	M147	S540	Late Dawenkou	Unknown	Level III
40	M226	S541	Late Dawenkou	Male	Level II
41	M226	S542	Late Dawenkou	Male	Level III
42	M125	S545	Late Dawenkou	Female	Level I
43	M125	S546	Late Dawenkou	Female	Level II
44	M125	S547	Late Dawenkou	Female	Level III
45	M111	S548	Late Dawenkou	Male	Level II
46	M111	S549	Late Dawenkou	Male	Level III
47	M127	S553	Late Dawenkou	Female	Level II
48	M127	S554	Late Dawenkou	Female	Level III
49	M256	S555	Late Dawenkou	Male	Level I
50	M256	S556	Late Dawenkou	Male	Level II
51	M256	S557	Late Dawenkou	Male	Level III
52	M252	S558	Late Dawenkou	Female	Level I
53	M252	S559	Late Dawenkou	Female	Level III
54	M251	S560	Late Dawenkou	Female	Level I
55	M251	S561	Late Dawenkou	Female	Level II
56	M251	S562	Late Dawenkou	Female	Level III
57	M130	S563	Late Dawenkou	Unknown	Level I
58	M130	S564	Late Dawenkou	Unknown	Level II
59	M130	S565	Late Dawenkou	Unknown	Level III
60	M116	S566	Late Dawenkou	Male	Calculus
61	M116	S567	Late Dawenkou	Male	Level III
62	M121	S568	Late Dawenkou	Male	Level I
63	M121	S569	Late Dawenkou	Male	Level II
64	M121	S570	Late Dawenkou	Male	Level III
65	M109	S571	Late Dawenkou	Unknown	Level II
66	M109	S572	Late Dawenkou	Unknown	Level III
67	M218	S573	Late Dawenkou	Unknown	Level I
68	M218	S574	Late Dawenkou	Unknown	Level II
69	M218	S575	Late Dawenkou	Unknown	Level III
70	M271	S576	Late Dawenkou	Female	Level I
71	M271	S577	Late Dawenkou	Female	Level II
72	M271	S578	Late Dawenkou	Female	Level III

Procrustes fit and to generate a covariance matrix. CVA assumes that the covariance structure within all groups is the same, therefore, a pooled within-group covariance matrix is used throughout for CVA and for computing Mahalanobis distances between pairs of groups. The Mahalanobis distance matrix and Procrustes distance matrix may help explain group similarities and differences.

Our study also applied a supervised machine learning method for model training to modern starch geometric morphometric data. The supporting vector machine (SVM) algorithm was used for model training. SVM is a supervised learning model and related learning algorithm for analyzing data in classification and regression analysis. This algorithm maximizes the margin between class boundaries and is often used in data mining projects (Boser et al., 1992; Steinwart and Christmann, 2008). At present, this method is widely used in the deep learning field, such as in image classification. The geometrical morphometric data on modern starch is thus trained by SVM to yield more precise identifications of unknown starch granules. The species name is used as the classifier, 35 coordinates of x and y, i.e., 70 values constitute the variables. R programming language (version 3.6.2) (R Core Team, 2020) is used for the SVM computing.

Species identification, and classification of unknown archeologically derived starch granules were based mainly on comparison with a modern starch database established by our laboratory team and with reference to the recent published record. We have thus far compiled a modern reference database of more than 67 Chinese starch-producing species/varietals (see **Figure 3**) including both domesticated and wild taxa. Some plants such as certain species of *Colocasia*, produce extremely small (<5 μm) starch granules, thus inappropriate for the

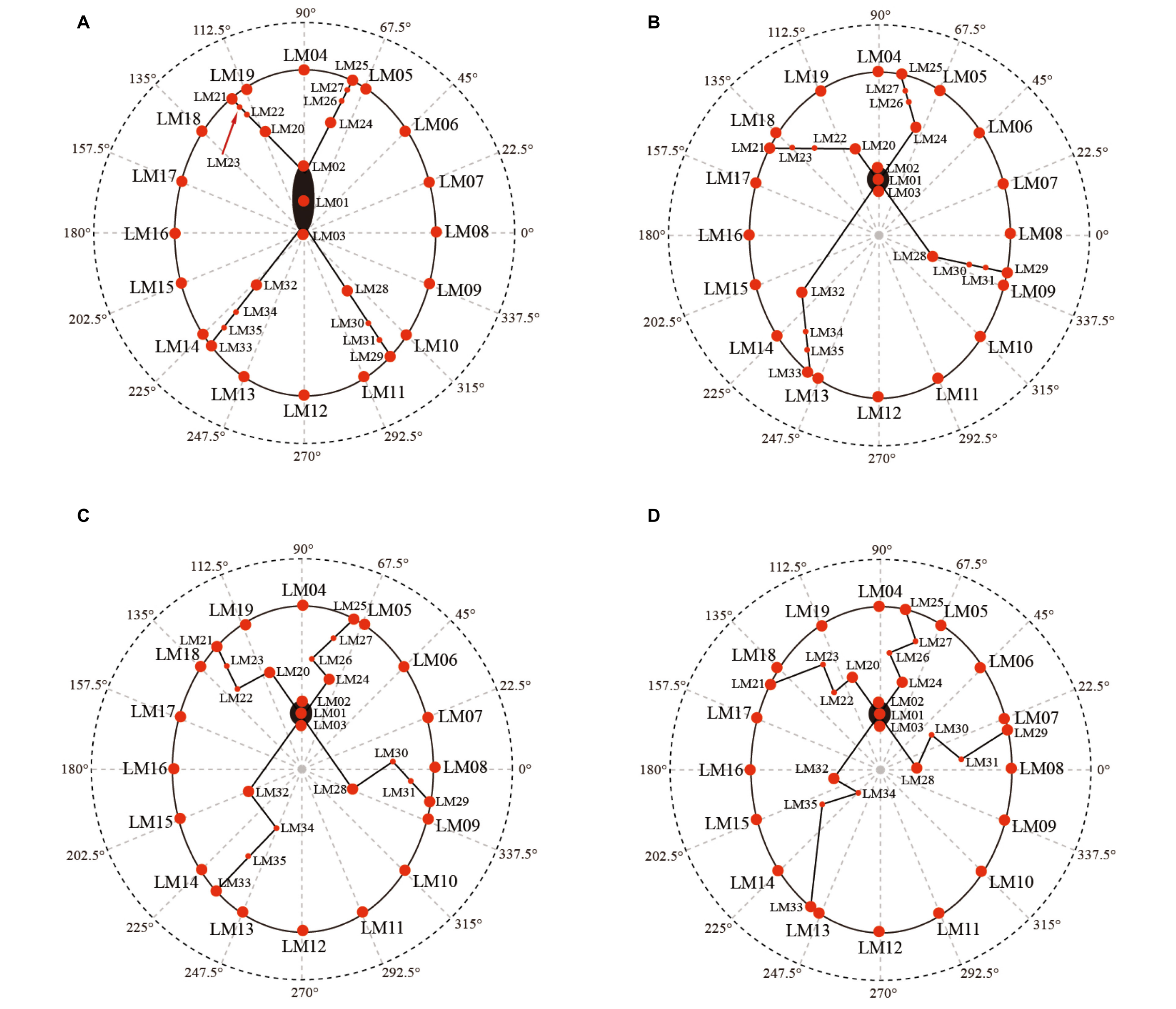

FIGURE 2 | Definition of the 35 landmarks on starch granule. **(A)** Extinction cross with no bend; **(B)** each arm with one bend; **(C)** each arm with two bends; and **(D)**, each arm with three bends.

acquisition of geometric morphometric data. In this case, TPS files were acquired from 41 species/varietals. Phytolith comparisons were based completely on published resources (Yang et al., 2009, 2013; Liu et al., 2011, 2014, 2019; Wan et al., 2011a,b, 2016; Wang S. et al., 2011; Wang et al., 2013; Yang and Perry, 2013; Ma et al., 2019; Li et al., 2020). Plant anatomy references were used to identify other plant organ fragments (Zheng and Wang, 1983).

RESULTS

A total of 1241 starch granules were recovered from all the three levels of residue samples. All teeth yielded starch granules except those from M143. Among these starch granules, 533 were recovered from Level I samples, 335 from Level II samples, and 373 from Level III samples (see **Supplementary Table**). Among these starch remains, 1031 granules (83.07% of the total) are identifiable by comparison with our reference database. The residue samples were taken by levels for several reasons. Level I samples are thought to reflect micro-residue originating in soil, while Level II samples, which are obtained by wet brushing, are thought to contain micro-residues from both soil and the surface of the sampled specimens. The main aim of the wet brushing is to isolate Levels I and III samples, to avoid cross-contamination from Levels I to III samples to the greatest extent possible. In this case, Level I samples are equally as important as Level III samples since both could provide valid indications, while Level II samples are difficult to analyze because they are, by definition, mixtures with multiple points of

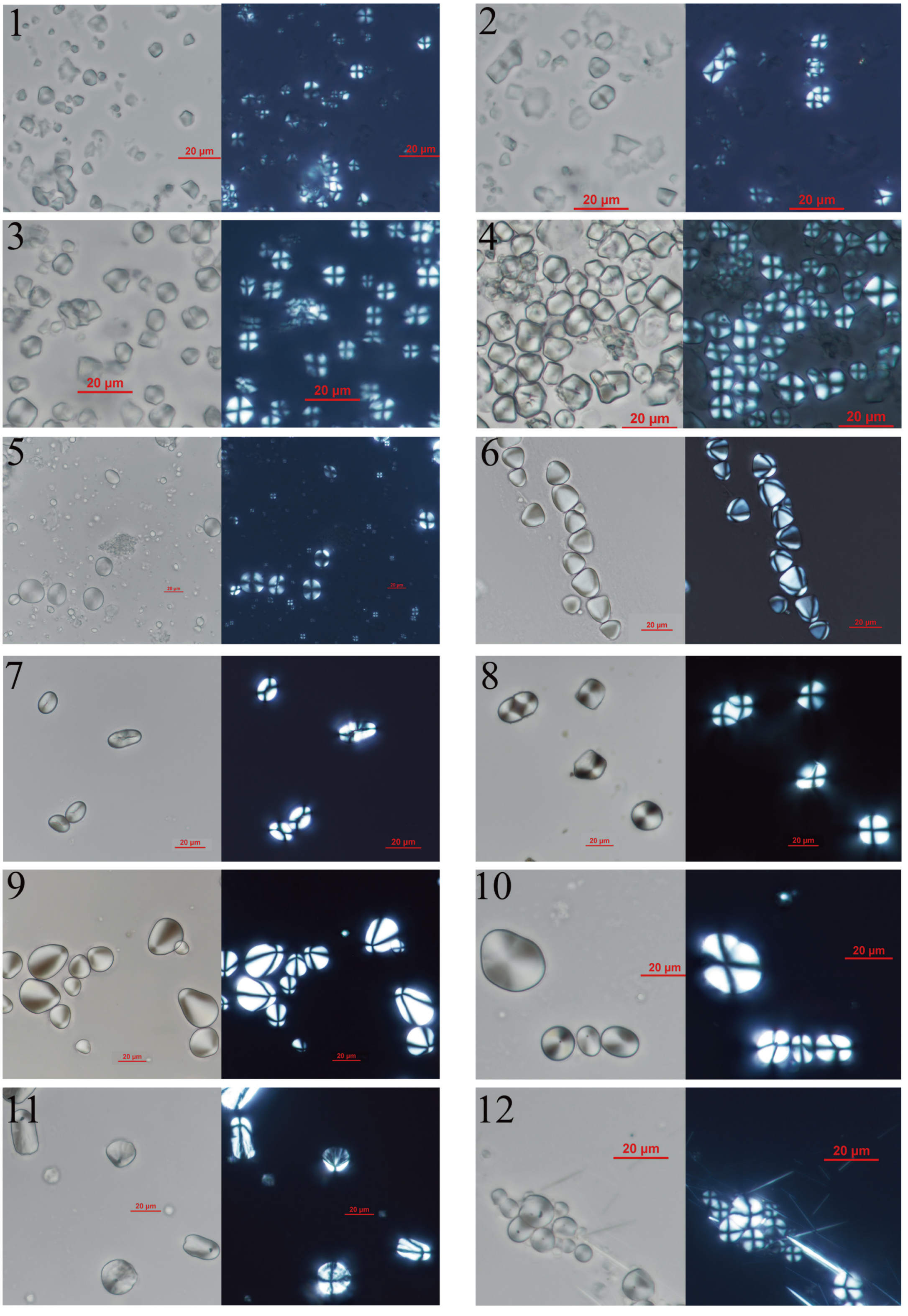

FIGURE 3 | Modern plant starch references included in this paper. [**1–2**, *Oryza sativa*; **3**, *Panicum miliaceum*; **4**, *Setaria italica*; **5**, *Triticum aestivum*; **6**, *Dioscorea opposita*; **7**, *Vigna vexillata*; **8**, *Smilax china*; **9**, *Bolbostemma paniculatum*; **10**, *Trapa bispinosa*; **11**, *Nelumbo nucifera* (root); and **12**, *Pinellia ternate*].

origination. Therefore, micro-residues from Level II samples are not discussed in our work.

We compared starch granules yielded by Level I samples with those recovered from Level III samples by direct image comparison and geometric morphology. In the images, different morpho-types could be observed between starch granules from Levels I and III. CVA results revealed substantial differences between Levels I and III samples, reflected by the peak values of canonical variates appearing in different positions (**Figure 4**). The Mahalanobis distance also showed apparent differences between the groups ($P < 0.001$). As a result, we conclude that the starch granules contained in Level III samples are not contaminated by layer deposits and can be regarded as direct evidence of the Liangwangcheng ancient human's plant utilization.

In addition to starch granules, phytolith remains were also discovered, although in a much smaller proportion, and were mostly not diagnostic to the species level. A small number of unidentifiable plant fibers, pollen grains, and fungi were observed.

Typology of Starch Granules

Archeological starch granules were classified into five types according to the system established by Guan et al. (2020) based on morphology and size (**Figure 5**):

Type 1, polyhedral body, possibly produced by foxtail millet (*Setaria italica*) (**Figure 3: 4**), broomcorn millet (*Panicum miliaceum*) (**Figure 3: 3**), or rice (*Oryza sativa*). The 2D shapes are mostly polygonal or spherical, with invisible lamellae and centric to slightly eccentric hila. Furthermore, many granules classified in this type exhibit pronounced fissures. Among these polyhedron bodies, ones with smaller diameters and sharper edges are likely to derive from rice according to our reference database (**Figure 3: 1–2**) and the published literature (Liu et al., 2011; Wang S. et al., 2011).

Type 2, lenticular body, probably produced by members of the tribe Triticeae. The 2D shapes of these starch granules are nearly round, elliptical, or lenticular, with closed and centrally located hila and fuzzy lamellae. Fissures are absent in most individual grains and the extinction crosses are primarily straight. It should be noted that the Triticeae tribe includes more than 10 genera in China, and both domesticated and wild species. These starch granules resemble the morphology of modern specimens from *Aegilops*, *Roegneria*, *Secale*, and *Triticum* according to our reference database (**Figure 3: 5**) and published literature (Wei et al., 2007; Hart, 2014; Wan et al., 2016, 2020).

Type 3, ellipsoidal, semi-ellipsoidal and elongated ellipsoidal body, maybe produced by plant underground storage organs, and probably including members of the Nymphaeaceae (water lilies), Trapaceae (water chestnuts), and Araceae families such as lotus root (*Nelumbo nucifera*), water caltrop (*Trapa bispinosa*), and *banxia* or crow-dipper (*Pinellia ternata*), the latter used in Traditional Chinese Medicine and identified by comparison with our reference database (**Figure 3: 6**, **8–12**). This type exhibits primarily fuzzy lamellae and eccentric hila, and the extinction crosses are mostly bent, making it easier to distinguish.

Type 4, kidney bean shaped body, which includes starch from legumes (Family Fabaceae) according to our reference database (**Figure 3: 7**) and published literature (Wang et al., 2013). Most exhibit visible fissures and lamellae, but the hila are mostly invisible. The center of the extinction crosses appears as a dark linear area.

Type 5, includes compound and damaged starch granules. Compound granules are not separated, and damaged starch granules are broken or incomplete; therefore, we were unable to specify their morphological characteristics and identify them to the species level.

Phytolith Remains

A total of 79 phytoliths were extracted from the Level III residue samples (**Figure 6**). These phytoliths could be classified into six types based upon criteria provided by Lu et al. (2006) including Elongate, Saddle, Bilobate short cells, Bulliform cells, Dendriform phytoliths from hulls and short cells. Bilobate short cells appeared most frequently. The small number of foxtail and broomcorn millet husk phytoliths nonetheless suggest that these millet varieties were used by the Neolithic occupants of Liangwangcheng.

Other Plant Organ Fragments and Remains of Fungi

Bordered pits, fibers, cells of unknown biological origin, and other fragments were discovered in all the residue samples, including Level I, Level II, and Level III. However, these organ fragments lack biological attributes and are, thus, not identifiable. In addition, these remains are extremely fragmentary, and were recovered in only minor quantities, therefore, such fragments were not included in this paper.

One discovery is particularly noteworthy. In addition to starch granules, phytoliths, and other tissue fragments, we also observed some biological granules. These smaller granules are spheroidal, oval, and radial in shape and display extinction crosses under polarized light. Initially, these granules were identified as damaged starch. However, after comparison with relevant published data (Haslam, 2006; Torrence, 2006; Krull et al., 2013; Atsatt and Whiteside, 2014; Walsh et al., 2018; Shen et al., 2019), we consider these granules to be molds; even hyphae were observed in some individual specimens. These mold granules have smaller average diameters than regular starch granules; generally <10 μm. Their extinction crosses caused initial misidentification, further emphasizing the importance of ensuring the precision of plant starch identification.

In Level III samples, 19 such mold bodies were observed. These fungi remains can be classified into three types based on their morphological features (**Figure 7**). Because of the integrity of their spore structure, we believe these fungi are generated later in the samples. However, it is a difficult issue to adequately evaluate. We hope that a more comprehensive examination can be made in the future to facilitate discussion of the origin of these fungi.

Results of Geometric Morphometric Analysis of Starch Granules

The Liangwangcheng CVA procedure identified eight significant canonical variates. Eigenvalues indicate that canonical variable

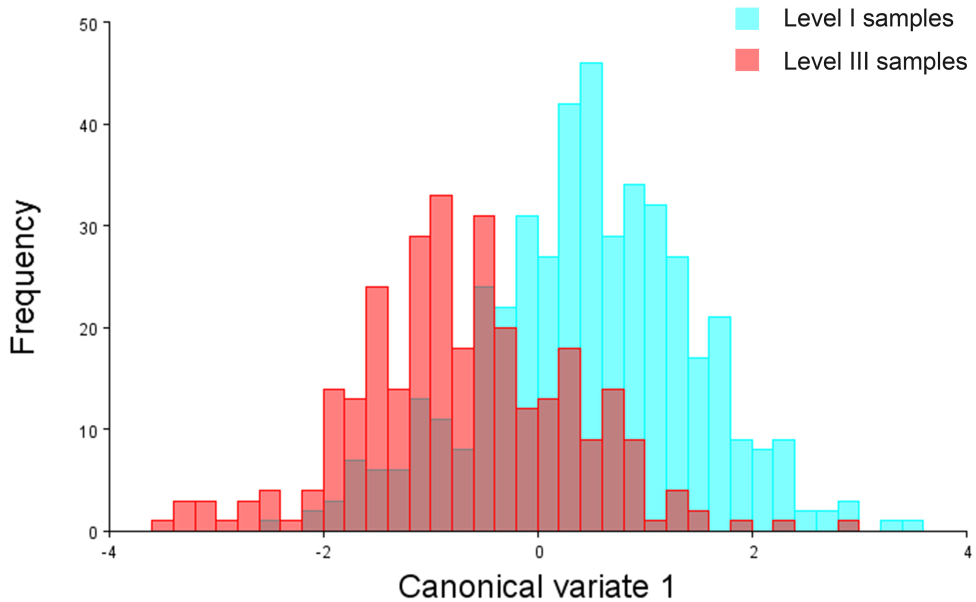

FIGURE 4 | Canonical variable frequency bar chart of Levels I and III samples.

(CV) 1 accounted for 45.83%, 34.04% for CV2, and 10.91% for CV3, representing 90.78% of the total variations. According to the CVA scatter plot, Liangwangcheng starch overlaps with about eight control groups. Among these control groups, *O. sativa*, *S. italica*, and *P. miliaceum* are closer to the Liangwangcheng group and the other four groups are more distant (**Figure 8**). In other words, the CVA shows that starch granules from Liangwangcheng derive mainly from rice, foxtail millet and broomcorn millet, and a small portion derives from legumes, members of the Triticeae tribe, roots and tubers, and some aquatic plant fruits. **Figure 9** displays the confidence ellipses (probability = 0.9) for each group. Moreover, the Mahalanobis distances (**Table 2**) and Procrustes distances (**Table 3**) further describe the canonical variate scores and support our conclusion.

SVM Prediction of Geometric Morphometric Data

The SVM model suggests that the Liangwangcheng starch granules (n = 301) possibly derive from several plant species. **Table 4** displays predicted data groups with percentages >2%. This prediction suggests that cereal crops (millets and rice) (37.21%), roots and tubers (33.56%), aquatic plant fruit (7.64%), and legumes (4.32%) are present in the Liangwangcheng starch remains.

DISCUSSION

Plant Resources Revealed by Residue Analysis

In this study, starch granules and other plant microfossils extracted from human dental remains inform vital issues concerning human diets and the development of agriculture-based subsistence. Plant starch and phytolith residues indicate that the Neolithic inhabitants of the Liangwangcheng site cultivated millet and rice as the primary source of their vegetal food and simultaneously exploited a variety of edible wild plants including legumes, the fruit of aquatic plants, and underground storage organs. In the Haidai region, rice, foxtail millet, and broomcorn millet coexisted since the Houli period (ca. 6500–5500 BCE), indicated by carbonized plant remains recovered at the Yuezhuang site (Gary et al., 2013), and a relatively stable rice–millet mixed agricultural pattern was established during the Middle and Late Dawenkou periods (ca. 3500–2600 BCE) (Zhao, 2020). The rice and millet starch and millet phytoliths extracted from Liangwangcheng dental residues further supports this perspective, and has helped fill in the blanks of late Stone Age subsistence studies in northern Jiangsu. The total proportion of cereal starch within the whole assemblage is much greater than those of other plant types, indicating that the Liangwangcheng people probably carried out large-scale farming activities. In other words, agricultural production may have become an increasingly stable means for them to acquire edible plant resources. Cultivated crops played a vital role in the Liangwangcheng subsistence base, and occupied a dominant position in the diet of the site's Neolithic inhabitants.

Although comprising a secondary and auxiliary role, wild plant resources were also crucial to the prehistoric occupants of Liangwangcheng, who gathered and utilized wild plant resources such as roots and tubers, legumes and aquatic plants, indicating the extensive use of diverse plant resources. Roots and tubers are generally easy to gather and process, and contain rich energy, thus they are considered one of the most common food resources in both prehistory and the present. Legumes include various

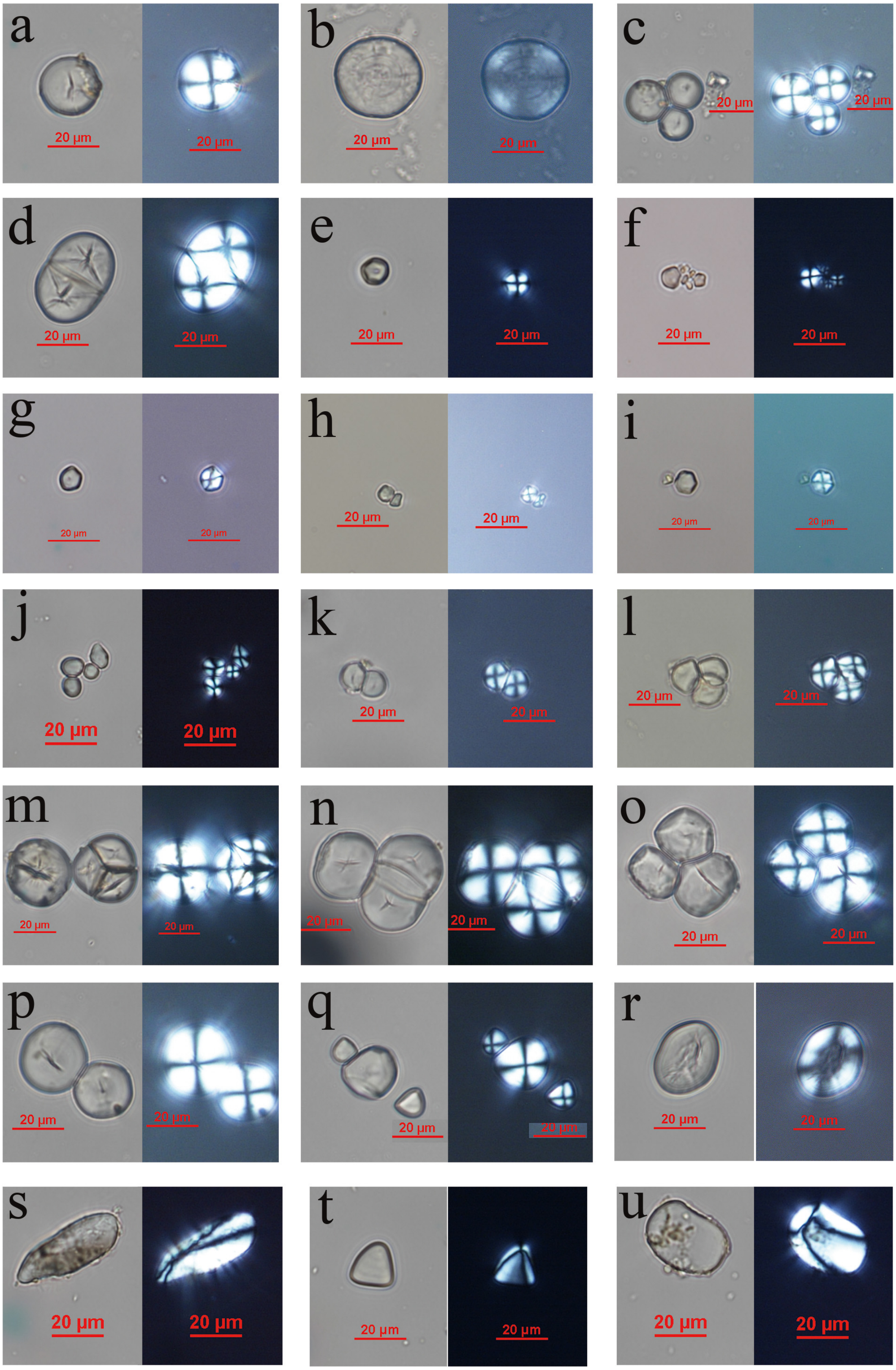

FIGURE 5 | Starch granules from Level III Liangwangcheng dental residue samples. (**a,j,m,s,u**: starch granule from M125; **b,r**: starch granule from M121; **c**: starch granule from M127; **d,h,k,l,n,p,q**: starch granule from M147; **e**: starch granule from M226; **f**: starch granule from M129; **g,i**: starch granule from M144; **o**: starch granule from M251; **t**: starch granule from M111).

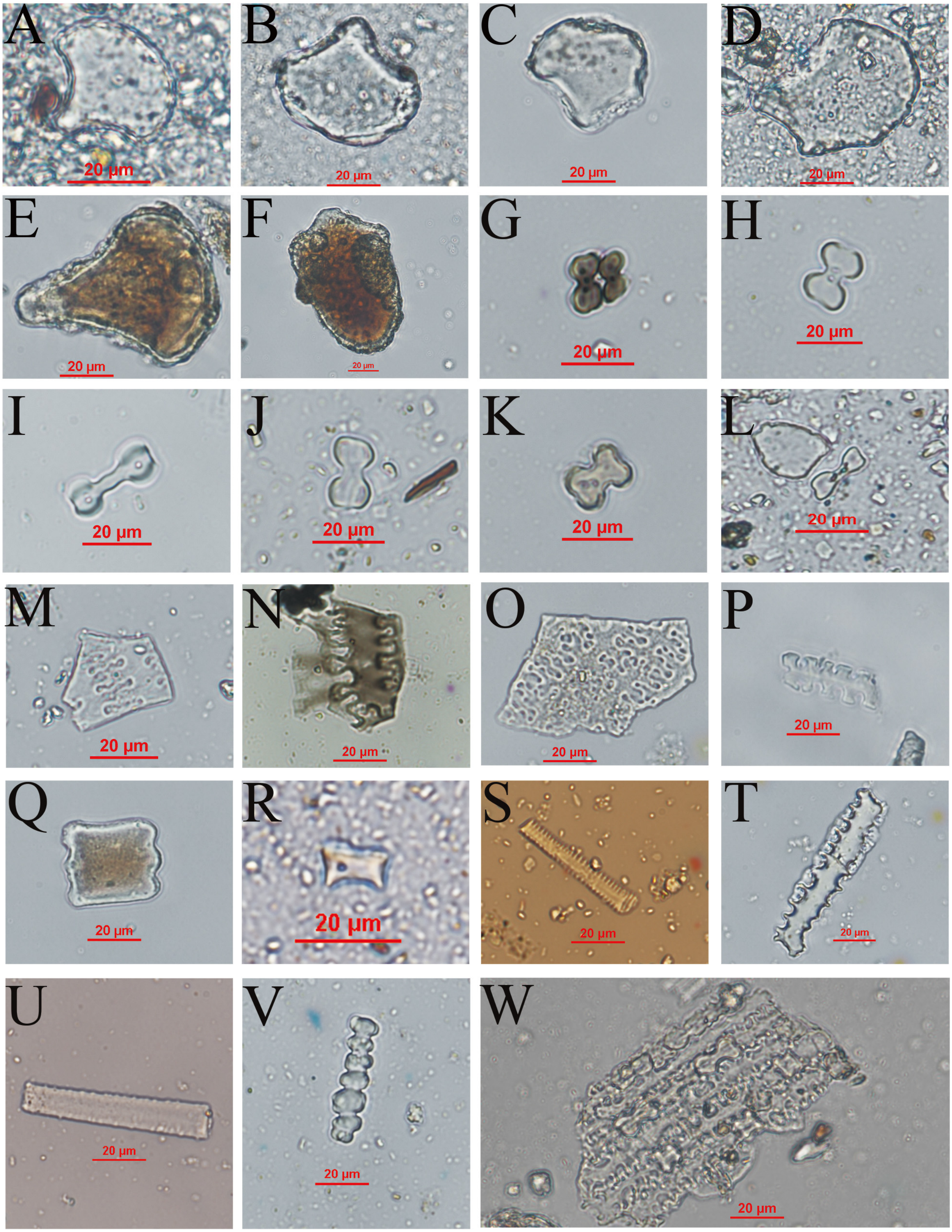

FIGURE 6 | Phytoliths recovered from Liangwangcheng dental residues. (**A–F**, Bulliform cells; **G–L**, Bilobate short cells; **M**, broomcorn millet husk; **N–P**, foxtail millet husk; **Q**, Wavy-trapezoid; **R**, short cell; **S**, Rondel; **T–V**, Elongate; **W**, Bilobate short cells).

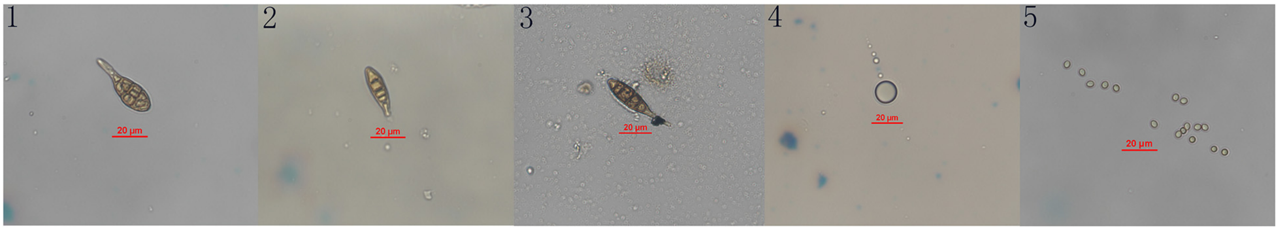

FIGURE 7 | Fungi remains recovered from Liangwangcheng dental residues.

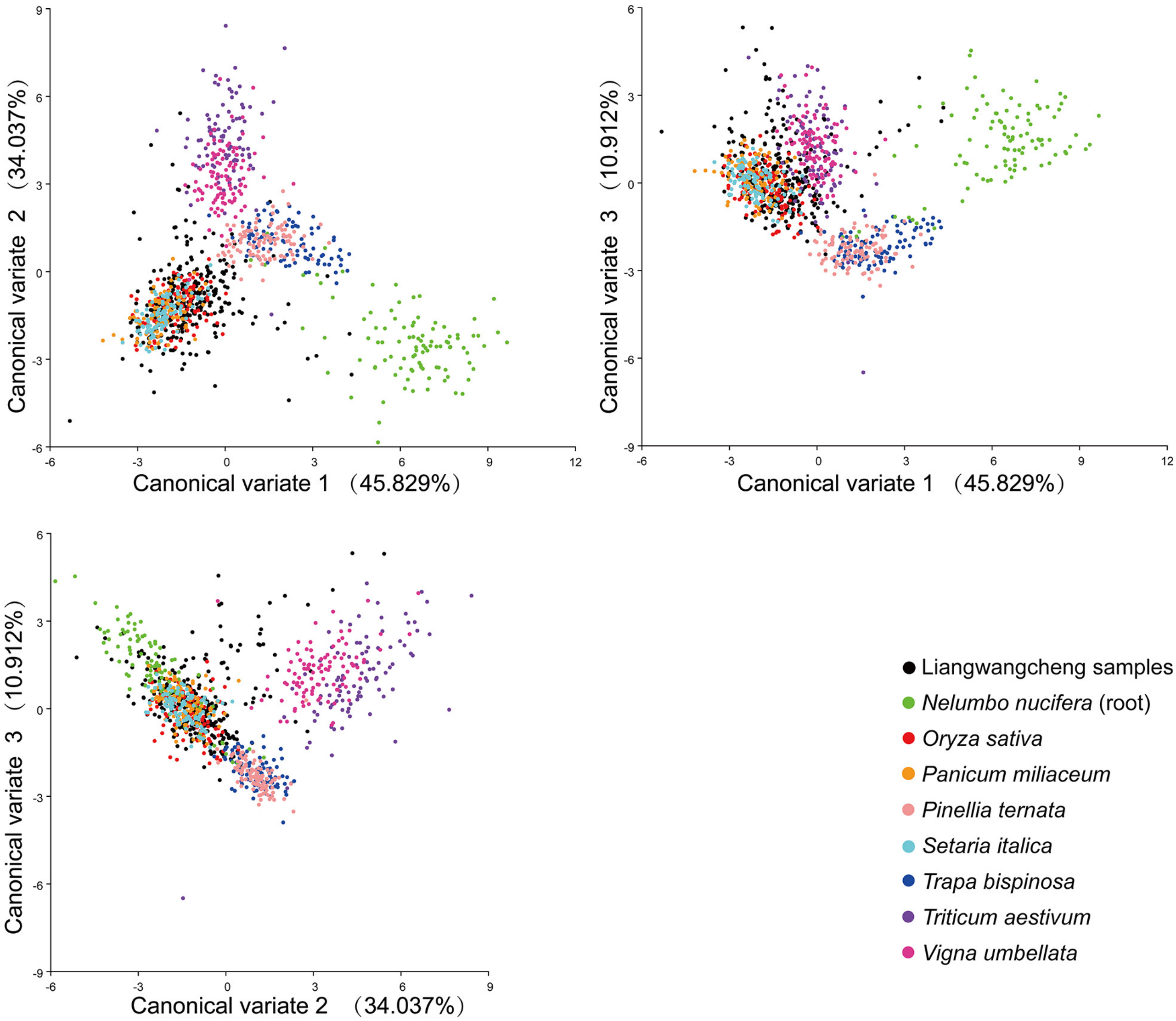

FIGURE 8 | Canonical variate scatter plot of CV1, CV2, and CV3 accounting for approximately 90% of variation.

species and are widely distributed. Due to their high protein content, they have comprised a part of the human diet for eons (Wang et al., 2009). Beans are still an indispensable component of the daily diet in northern Jiangsu today. Aquatic plants such as water chestnut and lotus root, widely distributed in the North Temperate and Subtropical Zones, are nutritiously rich. Our analyses suggest that the hydrothermal conditions and natural environment of the Liangwangcheng area fostered a level of

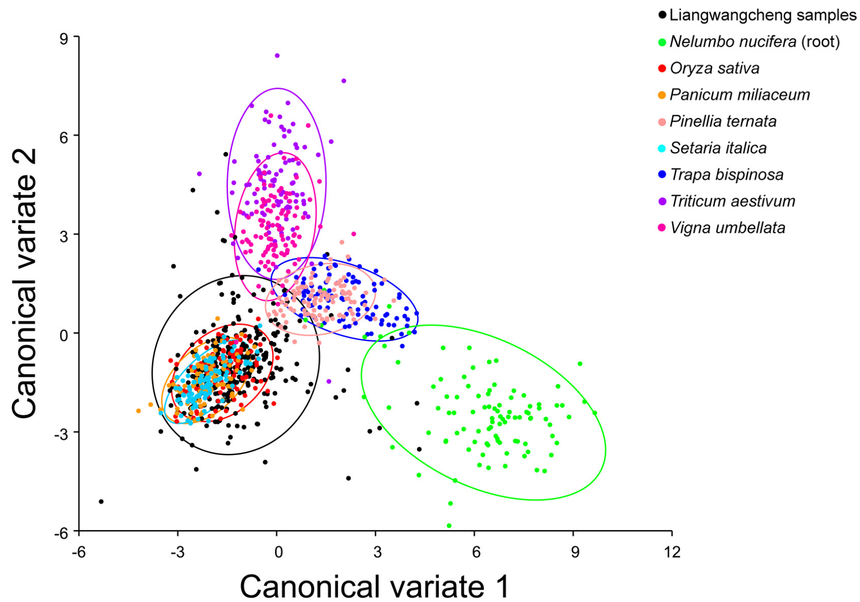

FIGURE 9 | Confidence ellipses (probability = 0.9) based on canonical variate scatter plot of CV1 and CV2.

biodiversity that gave the region's ancient inhabitants ready access to diverse food sources.

Human–Environment Interaction in the Liangwangcheng Area

Flotation work has not been conducted thus far at the Liangwangcheng site, considerably limiting our understanding of the subsistence pattern preserved at this site. Therefore, our study provides crucial evidence facilitating discussion of which plant species were utilized and consumed by the site's inhabitants. Specifically, dental residues provide direct and conclusive evidence essential for reconstructing the diet of Liangwangcheng's prehistoric inhabitants. Routine and sustainable cultivation of cereal plants may provide a stable dietary source. The selection of long-term edible plant resources was closely related to the natural environment and the subsistence productivity developed by ancient humans. According to published paleoenvironment data for the site, in the Late Dawenkou period, the climate in this area was warm and humid, and herbaceous and woody plants flourished. Prehistoric humans seized upon those climatic advantages and actively engaged in agricultural production (Zhao et al., 2014). Many residential houses, a pottery production complex, and a road paved with clay were exposed during excavations at Liangwangcheng (Nanjing et al., 2013) and well-established pottery typologies and evidence of the production of tools made of bone and stone illuminated. These artifacts suggest large-scale settlement during the Dawenkou period, which encouraged residents to engage in reliable agricultural production to support their burgeoning population. Anthropological analyses also indicate that skeletal lesions apparent on human bones were likely caused by heavy agricultural labor activities (Zhu et al., 2013). It seems that Liangwangcheng had favorable social and natural conditions for developing an agricultural economy in the Late Dawenkou period. Environmental conditions at the time were sufficient to support a rich and diverse array of foodstuffs, establishing a subsistence system based on agriculture while retaining a gathering and foraging economy as supplemental.

The rice–millet mixed agriculture pattern was a unique configuration in the Haidai area (Zhao, 2020), in which the cultivation of both rice and two varieties of millet were established and developed by ancient humans due to salubrious local environmental conditions. In the late Neolithic, agricultural patterns were generally consistent over the whole Haidai area; however, due to differences in geographical locations and environmental fluctuations, specific agricultural adaptations were also adjusted according to the local circumstances, thus temporal and regional differences emerged. No rice remains were found in the Jianxin (He and Liu, 1996) or Jiaojia sites (Wu, 2018) which apparently practiced typical dry farming, being located in inland areas and at higher latitudes north of Liangwangcheng. On the other hand, the Yuchisi (Institute of Archaeology, 2007) and Yangpu sites (Wu, 2018), located in Anhui at a lower latitude, practiced mixed rice–millet farming. The Liangwangcheng site is located on the lower reaches of the Huai River, as are Yuchisi and Yangpu in terms of hydrothermal and environmental conditions, with low-relief terrain and a dense fluvial network. Compared with Jianxin and Jiaojia, this region provides more abundant sources for irrigation and, thus, more suitable environmental conditions for propagating and cultivating rice.

The plant macrofossil and microfossil remains could present what specific plant foods were available to a community, while stable isotopic analytical data directly reflect a long-term configuration of which species had been consumed over many decades, which creates an efficient method for scholars to

TABLE 2 | Mahalanobis distance matrix of Liangwangcheng and control groups.

	Liangwangcheng	***Nelumbo nucifera***	***Oryza sativa***	***Panicum miliaceum***	***Pinellia ternata***	***Setaria italica***	***Trapa bispinosa***	***Triticum aestivum***	***Vigna umbellata***
Nelumbo nucifera	7.7988	0							
Oryza sativa	2.2237	8.2928	0						
Panicum miliaceum	2.1603	8.6368	1.9248	0					
Pinellia ternata	4.3466	7.1142	4.6282	5.1825	0				
Setaria italica	1.9577	8.6145	2.1293	1.7366	5.0945	0			
Trapa bispinosa	4.7390	6.5699	5.1041	5.5989	2.1658	5.5206	0		
Triticum aestivum	5.8538	9.4249	6.4180	6.5914	5.3110	6.6476	5.3937	0	
Vigna umbellata	4.8272	8.5861	5.2456	5.5567	4.6709	5.5536	4.9076	2.8238	0

The P-values from permutation tests (10,000 permutation rounds) for Mahalanobis distances among groups are all <0.0001; P-values < 0.0001 indicate that all groups are significantly different.

TABLE 3 | Procrustes distance matrix of Liangwangcheng and control groups.

	Liangwangcheng	***Nelumbo nucifera***	***Oryza sativa***	***Panicum miliaceum***	***Pinellia ternata***	***Setaria italica***	***Trapa bispinosa***	***Triticum aestivum***	***Vigna umbellata***
Nelumbo nucifera	0.3279								
Oryza sativa	0.0404	0.3439							
Panicum miliaceum	0.0593	0.3704	0.0688						
Pinellia ternata	0.0334	0.3276	0.0430	0.0743					
Setariaitalica	0.1026	0.3952	0.1157	0.0513	0.1175				
Trapa bispinosa	0.1608	0.2435	0.1602	0.2064	0.1507	0.2493			
Triticum aestivum	0.2207	0.3103	0.2088	0.2644	0.2097	0.3115	0.1022		
Vigna umbellata	0.1145	0.3028	0.1020	0.1584	0.1023	0.2056	0.0802	0.1129	0

TABLE 4 | Prediction result in Liangwangcheng starch granule dataset.

Plant taxon	Number of granules	Percentage (%)
Setaria italica	77	25.58
Smilax china (root)	31	10.30
Panicum miliaceum	28	9.30
Trapa bispinosa	23	7.64
Pueraria lobata	21	6.98
Nelumbo nucifera (root)	18	5.98
Eleocharis dulcis	18	5.98
Pinellia ternata	17	5.65
Amorphophallus virosus	14	4.65
Vigna umbellata	13	4.32
Oryza sativa	7	2.33
Total	267	88.71

establish a fuller picture of dietary practices in ancient China (Liu et al., 2020). According to Dong's radiocarbon dating and stable isotopic studies (Dong, 2013), the Liangwangcheng, Fujia, and Huating sites are contemporaneous, and the contemporaneity of these sites make synchronic comparisons of diet composition possible. The stable isotopic evidence also suggests that the diet of different contemporary sites in the Haidai area were not precisely the same. At Fujia, human diets were dominated by millets and millet-fed pigs, while at Huating and Liangwangcheng, people had more diverse diets, including many C3 plants such as rice and aquatic resources. Liangwangcheng and Huating are located in northern Jiangsu, further south than the Fujia site in Shandong province. In brief, millet is a plant resource that existed widely in the subsistence pattern of the Haidai area and has been relied upon for millennia. On the other hand, the rice and other C3 plants consumed by ancient humans were dramatically impacted by the natural environment, especially prevailing hydrothermal conditions.

CONCLUSION

In this study, we detected several species of starch granules in residue samples from 31 Late Dawenkou Culture burials, providing evidence of plant foods consumed by the Neolithic inhabitants of Liangwangcheng, Jiangsu. Geometric morphometric analysis indicates that a portion of these starch grains may be derived from domesticated cereal crops such as foxtail millet, broomcorn millet and rice; and part of them may derive from roots and tubers, legumes and aquatic plant fruits. Based upon CVA results, millets and rice are present in dental samples at the highest percentages. Millet and rice likely became the major consumed crop food sources beginning in middle Neolithic times in the Haidai area. Underground storage organs, aquatic plant fruits, and beans are also present at Liangwangcheng, but are represented at quantitatively lower levels.

Our study indicates that agricultural production was the primary means by which the Neolithic inhabitants of Liangwangcheng obtained plant-sourced foods, supplemented by gathering wild plant resources, and that the composition of the plant food spectrum was broad and diverse. The prehistoric inhabitants of Liangwangcheng would have employed locally appropriate subsistence strategies. Based on archaeobotanical research conducted at surrounding sites in the Haidai area, we conclude that in the late Neolithic period, the subsistence economy in northern Jiangsu was dominated by mixed rice–millet agriculture, and that a foraging and gathering economy persisted in a supplemental role. The plant microfossils discovered at this site provide essential information about the utilization of plant species and the development and florescence of agriculture in the late Neolithic of northern Jiangsu. This is of great significance in reconstructing prehistoric subsistence patterns in the middle and lower reaches of the Huai River, and for exploring interactions between prehistoric human activities and the paleoenvironmental context in which they were situated. These results also contribute to our understanding of crop diversity and the level of agricultural development attained in the late Neolithic period in the Haidai region of China's Pacific seaboard.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

XZ: conducting a research and investigation process, specifically performing the experiments, or data/evidence collection, and writing the initial draft. XtZ, YH, and ZZ: provision of study materials. JO: critical review, commentary, and revision. YG: development and design of methodology, creation of models, and oversight and leadership responsibility for the research activity planning. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.722103/full#supplementary-material

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