Nitrogen cycling by wood decomposing soft-rot fungi in the "King Midas tomb," Gordion, Turkey

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Archaeological wood in ancient tombs is found usually with extensive degradation, limiting what can be learned about the diet, environment, health, and cultural practices of the tomb builders and occupants. Within Tumulus Midas Mound at Gordion, Turkey, thought to be the tomb of the Phrygian King Midas of the 8th century B.C., we applied a stable nitrogen isotope test to infer the paleodiet of the king and determine the nitrogen sources for the fungal community that decomposed the wooden tomb, cultural objects, and human remains. Here we show through analysis of the coffin, furniture, and wooden tomb structure that the principal degrader, a soft-rot fungus, mobilized the king's highly ^{15}N-enriched nutrients, values indicative of a diet rich in meat, to decay wood throughout the tomb. It is also evident from the $\delta^{15}N$ values of the degraded wood that the nitrogen needed for the decay of many of the artifacts in the tomb came from multiple sources, mobilized at potentially different episodes of decay. The redistribution of nutrients by the fungus was restricted by constraints imposed by the cellular structure of the different wood materials that apparently were used intentionally in the construction to minimize decay.

Natural destruction of wooden artifacts in archaeological sites seriously impedes the ability of anthropologists and archaeologists to reconstruct original cultural practices and environmental conditions. This is particularly true when wood is buried over extended time periods with materials such as foodstuffs or human remains where protein-rich tissues supply nutrients to wood-decaying microbes, accelerating degradation of decomposable artifacts. In the 8th century B.C., the mound builders of Phrygia (located in what is now west-central Turkey) buried a great king along with a rich array of furniture and bronzes within a cedar, pine, and juniper tomb, at what is the archeological site of Gordion, in Turkey. The wooden funeral chamber was covered with 53 m of limestone-rich earth by the builders and is now designated as Tumulus MM, for "Midas Mound." The excavators of the tomb surmised that the king buried within the mound probably was King Midas himself (1). The patterns and degree of wood decay observed in the cedar (*Cedrus libani*) log coffin and throughout the tomb are uncharacteristically extensive given the fungal community responsible for the degradation (2) and seem to be controlled by a combination of proximity to potential nutrient sources and the type of wood used in the original construction.

The skill of the carpenters and artisans of ancient Gordion is evident in their workmanship and use of decay-resistant cedar for the coffin and floor timbers of the tomb and huge juniper logs on the exterior to protect the pine walls of the inner tomb chamber. Despite these efforts, however, significant deterioration by an atypical decay fungus occurred in the tomb. The most prolific wood degraders, the *Basidiomycota* wood rot fungi that cause most decay in today's buildings, were not evident in the tomb when it was excavated. Environments that have a high pH or are extremely dry, wet, or cold exclude these organisms (3). The environmental conditions prevailing within the Tumulus MM chamber over the last 2,700 years were dry for the most part but included leachate of alkaline waters that seeped through the limestone overburden. Accordingly, these conditions selected for a distinct type of decay organism, soft-rot fungi, to flourish within its walls (2).

We applied a stable nitrogen isotope test to determine: the sources of nutrients for the fungal community that colonized the tomb after burial, whether series of different microbial decay episodes could be inferred based on patterns of degradation and stable nitrogen isotope values, and whether the paleodiet of the king could be inferred from residual nitrogen mobilized from his body and stored in the degraded wood.

Methods

Wood from the MM tomb was obtained in cooperation with the Department of Antiquities, Ministry of Culture of the Turkish Republic, and the Museum of Anatolian Civilizations, Ankara. Small segments (mm) of samples were obtained from selected areas of the coffin and table tops as well as from regular intervals along two transects that crossed the wooden tomb structure and placed in sterile tubes for transport and storage. Elemental analyses and stable nitrogen isotope composition were obtained from powdered whole woods by using an online C and N elemental analyzer interfaced to an isotope ratio-monitoring mass spectrometer (the EA-ConfloII-Delta XL Plus system). The mean deviation of reported C and N measurements is $\pm 2.0\%$ of the measured value. Isotopic compositions are reported in $\delta^{15}N$ notation and are referenced to the stable nitrogen isotope composition of N_2 in air. The $\delta^{15}N$ values are calculated according to the following equation,

$$\delta^{15}N = (R_{sa}/R_{std} - 1) \times 1{,}000(‰),$$

where R_{std} and R_{sa} are the $^{15}N/^{14}N$ ratios of the standard and sample, respectively. Acetanilide was used as a standard for elemental abundance and stable isotope composition. Reported values represent the average of three analyses with average reproducibility of samples within a standard deviation of $\pm 0.4‰$. Other wood samples were prepared for scanning electron microscopy. Segments of wood were infiltrated with distilled water, frozen to $-20°C$, and sectioned with a cryo-cut freezing microtome. Sections of wood then were air-dried and mounted on aluminum stubs, coated with gold, and examined as described previously (4).

Results and Discussion

Soft-rot decay, characterized by cavities formed in the S_2 layer of the secondary cell wall, was found in nearly all the wood of the tomb structure, coffin, and furniture (Fig. 1). The number of

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Abbreviation: MM, Midas Mound.

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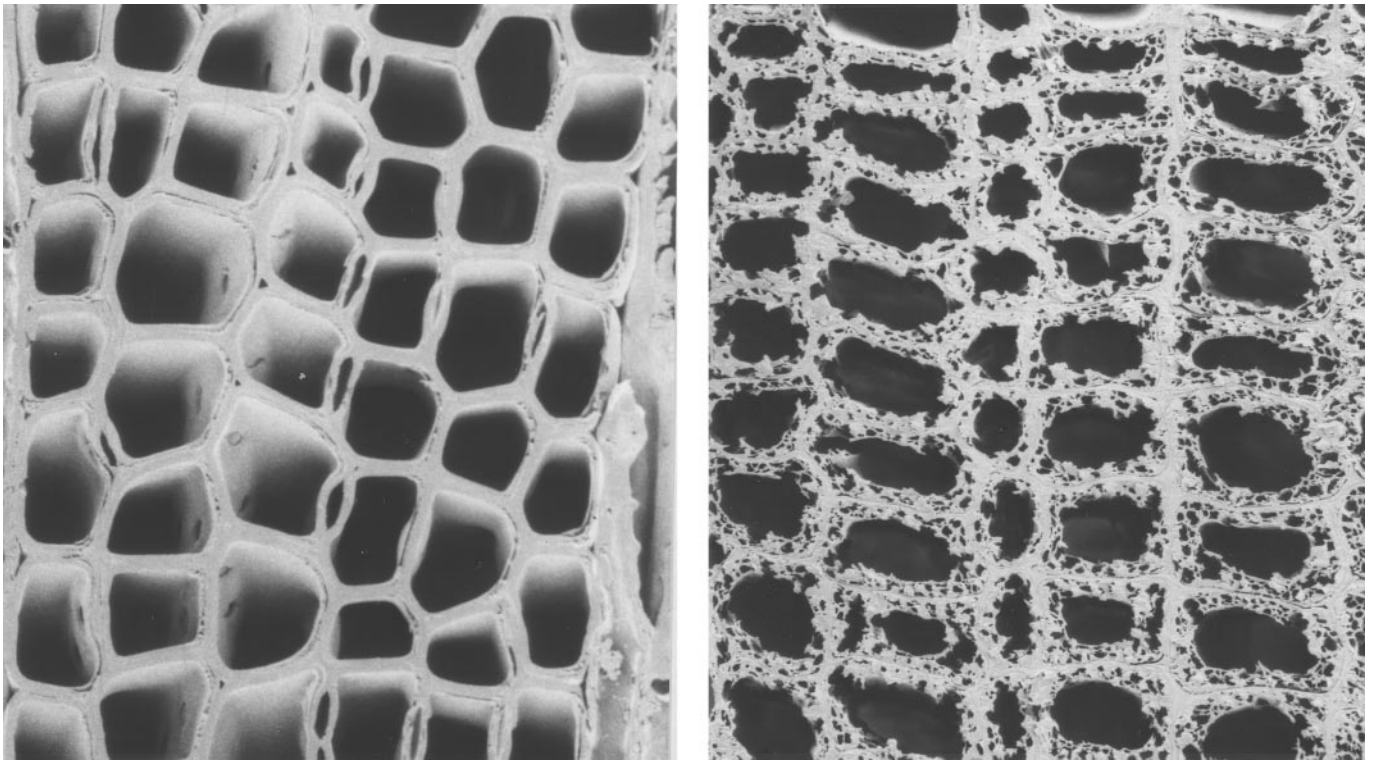

Fig. 1. Scanning electron micrographs of transverse sections from sound wood of Cedar (*C. libani*) (left) and decayed wood from the king's cedar coffin showing advanced stages of soft rot (right). Cavities formed within the S_2 layer of the secondary cell wall are diagnostic of soft-rot attack. Tracheids shown in the micrographs are $\approx$50 μm in width.

cavities observed in transverse sections indicated that an extremely advanced stage of soft-rot decay was present. In samples obtained from the floor region near the coffin, the entire secondary wall was riddled with cavities. Often the cavities coalesced together forming large voids in the cell wall. Soft-rot fungi, which consume primarily the cellulose, hemicellulose, and some lignin in woody tissues, function optimally when additional external sources of nitrogen are available beyond that of the degraded wood. Nitrogen sources available to the fungi, i.e., the body of the king, the tomb furnishings, and the tomb structure itself, were investigated by measuring the δ^{15}N values and atomic C/N ratio of artifacts within the tomb (Fig. 2; Table 1). A wide range in C/N (12.2–113.1) and δ^{15}N [$-$4.0–16.2‰] values is evident in the 10 samples depicted in Fig. 2. Undegraded woods, present in only a few regions of the tomb, show C/N consistent with sound woods, a nitrogen-poor tissue with C/N typically greater than 70 (5), whereas the degraded portions of the coffin and tabletops show dramatic nitrogen enrichments shifting the C/N to as low as 12.2. The changes in C/N are controlled by losses of polysaccharides and lignin as well as concentration of nitrogen by the soft-rot fungi. Previous research on the Tumulus MM wood revealed high nitrogen contents within some of the degraded sections of the coffin and a tabletop, leading the authors to speculate that sources of nitrogen other than that naturally found in the wood cells may have supplied the high nutrient levels required for such uncharacteristically extensive soft-rot degradation (6). Soft-rot fungi normally require higher nutrient levels than other wood-rot fungi to cause appreciable amounts of degradation; however, laboratory investigation suggests they can enrich the degraded wood residue in nitrogen derived from external sources such as growth media (7). The degraded wood of the tomb exhibited a low C/N, as would be expected for wood supplemented with an external nitrogen source.

The δ^{15}N values of degraded coffin wood directly under the king are highly enriched in ^{15}N, with a δ^{15}N value of $\approx$15‰ (Table 1). The δ^{15}N values taken from undegraded portions of the inner tomb walls exhibit isotope values in the range of -4 to -1.7‰, values more typical of nitrogen in undegraded wood (8). When the δ^{15}N values of the tabletops and coffin are compared, the results unambiguously demonstrated that the soft-rot fungi used at least three distinct nutrient sources. The nitrogen found in the remains of the tabletops, for example, could not have come directly from the king. The tables were most likely colonized instead by an isolated population of decay fungus that primarily used nitrogen available from either the residual wood or food stuffs placed on the tables at the time of burial. Additional sources may include volatile amines derived from the decayed king and food. The anomalous δ^{15}N value of tabletop 7 [16.2‰], with respect to the other tabletops (see Table 1), is enigmatic, but its similarity to the values at the coffin indicates that in this instance an enriched ^{15}N source of nitrogen (meat or fish) was available to the decay fungus.

Archeological plant specimens have been reported to exhibit enrichment in ^{15}N over time (9). One potential cause of the isotopic enrichment of the nitrogen in degraded residues is the loss of volatile ^{15}N-depleted gasses during microbial decomposition of proteins and other N-containing compounds, with the result being enrichment in ^{15}N within the residue (9, 10). With regard to the present study, alkaline leachate penetrating the tomb would certainly aid in the volatility of protein decay products such as ammonia, putrescine, and cadaverine (11). Such nitrogen-containing compounds have the potential to alter the isotopic composition of the background wood levels in the tomb and lead to an overall ^{15}N enrichment of wood residue. Fungal uptake of remobilized, volatile nitrogen compounds might be an important nutrient source for fungi not able to derive nitrogen directly from the body of the King, as would be the case for the

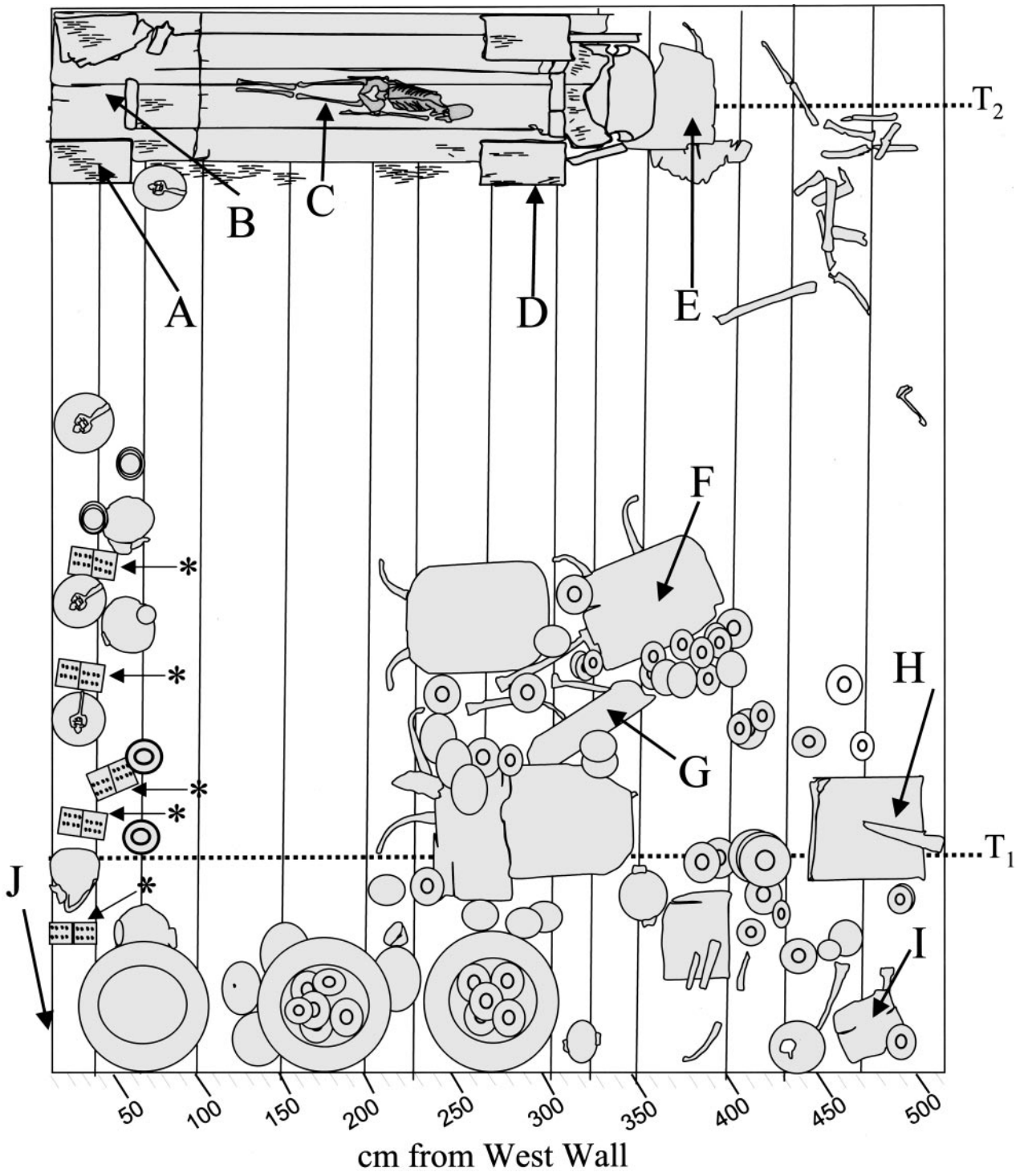

Fig. 2. Sketch plan of tomb with principal wooden and bronze artifacts drawn (redrawn from ref. 1). Letter designations correspond to items analyzed for their ^{15} content and atomic C/N ratio (see Table 1 for values). The transects T_1 and T_2 show regions from which floorboards were sampled for analyses after artifacts were removed from the tomb. Asterisks indicate the location of degraded leather belts.

tabletops. We do not believe, however, that the high level of ^{15}N enrichment in the degraded wood of the coffin and tabletop 7 is due to this process. If the enrichment in ^{15}N observed in these items were solely due to a fractionation caused by volatilization of microbial decay products rather than a ^{15}N-enriched source, one would expect the enrichment phenomenon to occur in all degraded regions of the tomb. This clearly is not the case. The elevated ^{15}N of the wood in the coffin is derived from the king's body that was placed in it.

From the residual N in the coffin the ^{15}N isotope composition of the King was probably close to, but slightly less than, the 13.6–15.4‰ observed now. The difference in ^{15}N content between the wood and the king is the result of selective enrichment in ^{15}N that accompanies increasing trophic levels

Table 1. Results from nitrogen elemental and stable isotope analysis of wooden artifacts from the MM tomb

Sample	Description	δ^{15}N, ‰	C/N
A	Coffin support block interior: minimal degradation	−4.0	82.8
B	West ledge of coffin	13.6	12.2
C	Coffin under body remains	15.4	13.5
D	Coffin support block: significant degradation	14.2	17.4
E	Degraded table top 9	2.7	17.0
F	Degraded table top 5	2.5	18.8
G	Degraded table top 4	4.3	21.9
H	Degraded table top inlaid table	4.8	24.5
I	Degraded table top 7	16.2	14.8
J	Undegraded exterior SW wall	−1.7	113.1

Letter designations correspond to artifacts shown in Fig. 1.

within a food web, i.e., primary photosynthate to herbivores to omnivores to carnivores (12, 13). For this reason, the ^{15}N isotope composition of ancient human remains, and in this case the residual nitrogen mobilized from a body by microbes and stored in degraded wood, can be an important indicator of paleodiet (14, 15). The high ^{15}N content remaining in the coffin may indicate that a significant contribution of the king's diet was derived from animals that grazed in water-stressed environments (15), consistent with the location of Gordion in the arid plateau region of central Turkey. Recently, McGovern *et al.* (16) used mass spectrometry and Fourier transform infrared spectroscopy to analyze organic contents from food vessels found in Tumulus MM to reconstruct the mourners' funerary meal. Their analyses indicated that the meal was dominated by barbecued meats, which is consistent with the ^{15}N values presented here from in and around the coffin and tabletop 7 and the suggestion that the king's diet was derived substantially from meats.

In many sections of the tomb, damage caused by the soft-rot fungus was severe and unquestionably aided by the biomass of the king's body. An understanding of the degree to which this fungal degradation depended on the king can be gained by observing the profile of δ^{15}N values and C/N of the floorboards taken along transects under the coffin (T_2) and through the region that includes degraded and collapsed tables and a degraded leather belt that fell from its mounting on the west wall (T_1) (Figs. 3 and 4). The analyses along T_2 illustrate how the nutrients of King Midas were transported by the fungus through the wooden coffin and into the floorboards as the soft-rot fungus spread through the tomb, mixing the ^{15}N-enriched signal of the king's biomass with the lower δ^{15}N values present in the cedar. The exponential decrease in the δ^{15}N values and the concomitant increase in C/N values moving toward the east wall on T_2 indicate that the farther the fungus spread from the king the less of his nitrogen they stored in the degraded wood. The C/N and δ^{15}N values on T_1 exhibit an irregular pattern and most likely represent utilization of food from containers and leather ornaments, as can be seen for the elevated δ^{15}N values where the leather belt crosses the transect at 50–90 cm from the west wall.

The δ^{15}N and C/N values are different in the floorboards compared with values of degraded tabletops that had collapsed on them. Tables 4 and 5 and the inlaid table are located in the center and eastern part of the tomb, respectively, in an area where floorboards showed less decay than that found under the coffin. Tabletop no. 9 (δ^{15}N value of 2.7‰ and C/N of 17.0) was found at the north of the tomb fallen under the degraded east ledge of the coffin and resting on top of floorboards. The floorboards in that area exhibit a range in δ^{15}N values from 9.8 to 14.5‰ and a range in C/N values from 30 to 40. The relatively

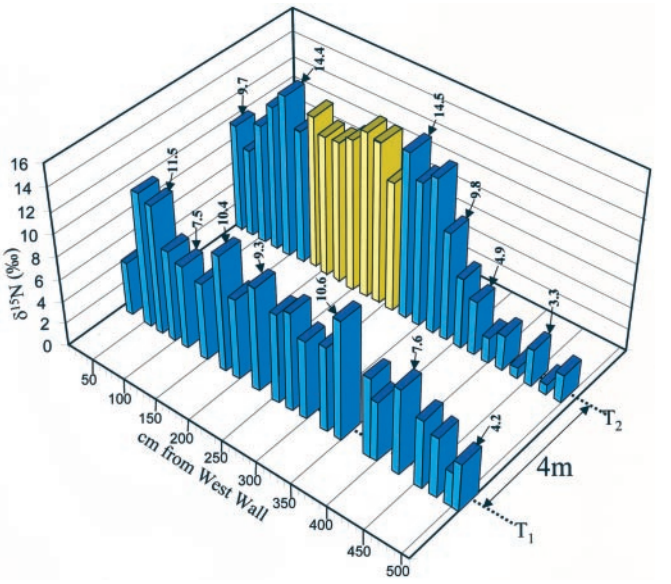

Fig. 3. Stable-N isotope analysis of transects T_1 and T_2 shown in perspective view. The yellow bars indicate position of the remains of the king within his coffin.

depleted δ^{15}N values of the degraded tabletops indicate that the fungus did not use the king's nitrogen during its degradation. The legs of the tables were made of a dense, more decay-resistant boxwood (17, 18) and may have helped to exclude or impede the fungus, that derived N from the king, from moving up from the floorboards to the tabletops. Additionally, the ^{15}N-depleted tabletops, which were found collapsed on the floor upon excavation, indicate that the tables must have collapsed long after active degradation of the floor was complete; otherwise, the ^{15}N-enriched nitrogen would have been mobilized into the tabletops.

Although no gold was found in Tumulus MM, the wooden tomb, ornate furniture, and chemical information locked in the

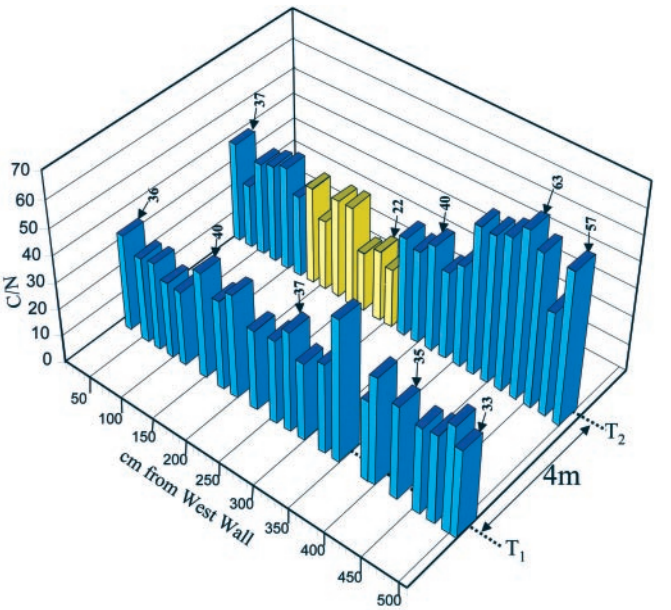

Fig. 4. Atomic C/N ratio of transects T_1 and T_2 are shown in perspective view. The yellow bars indicate position of the remains of the king within his coffin.

decomposed residues constitutes a potentially more valuable source of information with respect to the preservation of wooden antiquities. Our results elucidate the tenuous nature of archeological wood in Tumulus MM and demonstrate the fine balance that must be maintained between the environment, nutrient supply, and microbial community to permit preservation. How long the fungal community was feasting actively on the body of the King and using this rich resource to gradually colonize most of the tomb structure is a matter for speculation, but the extensive decay found throughout the huge cedar and pine timbers suggests the soft-rot fungus may have lingered in the tomb for centuries or possibly millennia. The application of stable isotope techniques to other archeological sites at which wood is found may prove invaluable in accessing decay episodes and providing important information to aid in reconstructing past events.

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