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Grading the decay of waterlogged archaeological wood according to anatomical characterisation. The case of the Fiavé site (N-E Italy)

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ABSTRACT

The grading of the anatomical preservation of cells inside waterlogged archaeological wood can be obtained by linking qualitative information obtained by microscopes with quantitative results inferred from physical characterisation. This study shows an approach to grading based on the microscopic analyses of 81 cores coming from poles of the pile-dwelling of Fiavè (IV–II millennia B.C.). Observations and physical analyses were undertaken on three different sectors of each core, taking into account their different state of preservation. In total 239 samples were analysed. The analyses identified 38 cores of larch (*Larix decidua* Mill.), 24 of spruce (*Picea abies* Karst.) and 19 of silver fir (*Abies alba* Mill.). Based on the morphology of decay, clear signs of biological attacks were identified: soft rot cavities, pointed ended, moving along the micro-fibrillar orientation of the cell wall, and "V"-shaped notches of eroded cell wall beyond the granular aspect in cross sections of heavily degraded cells. Results of classification (5 classes) according to micromorphological observations were compared to MWC values, for validation. This way we could verify an increase of average MWC with increasing micromorphological decay class, thus proving the reliability of the grading process. However, the micromorphological grading appeared more informative about the first stages of the attack.

1. Introduction and aims

According to the Italian standard UNI 11205 (2007), archaeological wood is "wood having a recognised archaeological value". It means that this material constitutes remains bearing signs of human action (otherwise the same standard refers to "archaeobotanic remains"). These artefacts witness past cultures (Florian, 1990) and have an archaeological value for which they have to be preserved and sometimes restored (Giachi et al., 2010, 2011). Archaeological or historical wood conservation demands an in-depth knowledge of its state of preservation (Brandi, 1977).

This is affected by a plethora of factors such as wood species, history of finds before burial, and preservation environment (presence of water, oxygen content, minerals like iron, sulphur and salt of the sea water, pressure of ground layers and, mainly, organisms degrading wood) (Jordan, 2001). Often, while being aware of the individual elements involved, it is very difficult to determine the effects of their combined actions (Björdal et al., 2000); for this reason, the general approach is get samples from the material constituting archaeological finds and, as required by standard UNI 11205 (2007), to carry out "several analyses to obtain a good knowledge of wood and the surrounding environment in which finds have been kept".

To characterise the state of preservation of waterlogged archaeological wood, the authors used a previously described multidisciplinary approach (Macchioni et al., 2012) through three analytical steps aimed at characterising wood from the micromorphological, physical and chemical points of view. A similar approach was also utilised by Brunning et al. (2000) with the aim to evaluate the effectiveness of the management regime of the Sweet Track, the oldest trackway in Europe. Through a multidisciplinary approach (physical characterisation, chemical analyses and the investigation of microbiological activity) the authors found a high degradation of wood, mainly of cellulose and hemicellulose; soft rot and erosion bacteria were found to be responsible for the decay.

The micromorphological analysis is aimed at identifying the wood species and at characterising the biological decay at the cell wall level, and it is mainly carried out by means of light and scanning electron microscopes (Daniel and Nilsson, 1986; Donaldson and Singh, 1990; Blanchette et al., 1990, 1991; Kim et al., 1996; Björdal et al., 1999, 2000; Blanchette, 2000; Powell et al., 2001; Schmitt

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et al., 2005; Capretti et al., 2008; Björdal, 2012). By examining the morphology of degradation and which parts of cell walls are attacked, it is possible to identify the microbiological decay agents. The action of these microorganisms leads to important changes of wood anatomical structure, physical properties and chemical composition of the material with profound implications for the dimensional stability of archaeological objects (Schniewind, 1990; Björdal et al., 2000).

Fungi, able to survive in an environment at the same time with very low oxygen partial pressure and rich of water, are grouped as agents of "soft rot" and taxonomically related to Ascomycetes and Deuteromycetes (Eaton and Hale, 1993). They can attack buried wood, and the effect of their action is the transformation of external layers of wood into a soft material (Blanchette, 2003). The chemical effects of soft rot attacks are the preferential depletion of both hemicelluloses and cellulose, and the demethylation of methoxyl groups; a hyphal tunnelling inside the lignified cell walls accounts for such effects (Schwarze, 2007). At the beginning, hyphae move through the "natural openings like rays and pits" already present in the structure (Björdal, 2012). Hyphae then diffuse into the secondary wall forming typical cavities with conical ends: they move in the S_2 layer parallel to the orientation of cellulose microfibrils. Due to the action of enzymes, cavities around hyphae are formed: in transversal section they look like holes in the secondary wall (Björdal and Nilsson, 2008). In a more advanced stage, when holes join together, the secondary wall may appear completely destroyed.

Bacteria are the most adjustable organisms vis-à-vis harsh environments with low or no oxygen partial pressure. They are rods or spherically shaped cocci (Gram-positive and -negative); their dimensions are: 2–8 μm length and 0.5–0.9 μm width (Björdal, 2012). They are distinguished depending on the layer of the wall degraded and the morphology of the lesions. The attack of bacteria begins within the rays against materials stored inside, followed by the decay of parenchyma cells (Rossell et al., 1973). Most common are the so-called erosion bacteria, which are tolerant to low oxygen partial pressure. These organisms degrade the cell wall along the orientation of cellulose microfibrils moving from S_3 into S_2 layer without affecting the most lignified wall. The erosion is due to enzymatic activity around each bacterium (Holt, 1983); moreover extracellular mucilage allows for the motility and adhesion to cell walls (Eaton and Hale, 1993). By microscopic observations, it is possible to highlight the presence of erosion bacteria: starting from the lumen, they produce troughs more or less of the same dimensions as that of the bacterium (Eaton and Hale, 1993). In case of massive attacks, these troughs merge in larger void areas involving the disappearance of the entire portions of a wall. In longitudinal section, these bacteria produce groove-like erosions, more or less along the microfibril angle, and "V" notches (Klaassen, 2008).

The tunnelling bacteria, although rare, are more dangerous because they are able to attack the so-called "compound middle lamella", that is the cell wall layer richer in lignin. The name of this group of bacteria derives from the tunnels originated by the progressing erosion process: inside each tunnel there is only one bacterium at the end. The direction of tunnels is not along the helical orientation of cellulose; they can follow every direction, passing through the S_1 layer and the compound middle lamella. For this reason, they are more aggressive than erosion bacteria. Concave cross walls are visible along tunnels. Several hypotheses have been formulated to explain this finding: they could be the result of a process of start-stop-start-stop advancement (the cross walls indicate each position of the bacterium) (Eaton and Hale, 1993), or these bands are formed by the waste products of bacterial metabolism (Blanchette et al., 1990; Blanchette, 2000; Björdal et al., 2000; Kim and Singh, 2000).

The micromorphological analysis results in a descriptive output that is usually difficult to directly compare with the numerical results produced by physical (MWC,[1] densities) and chemical (percentages of wood cell components) analyses. On the other hand, microscopic observations are pivotal to understanding if the current state of preservation of the material is due to abiotic factors or to the action of microorganisms. This also allows for a better interpretation of the results of quantitative characterisations: for example, Macchioni et al. (2012) reported how it is possible to have samples with a very low MWC value (which should indicate a good state of preservation) in strongly collapsed material (evidenced after observations at microscope).

Thus, in order to transform the decay descriptions produced by microscope observations into numerical outputs, the state of preservation was graded through specific decay classes. The first classifications of decay in waterlogged archaeological wood were based on both a pin test[2] and the MWC value (Brorson-Christensen, 1970; De Jong, 1977). Three classes were distinguished: "Class I, the most deteriorated wood, contains over 400% water and virtually no core; Class II is 185–400% water with a core present; and Class III with less than 185% of water and only surface degraded" (Florian, 1990).

Björdal et al. (2000) proposed a new classification of decay based on Light Microscope observations; 4 levels of decay were described, highlighting the percentage of biological attack inside cells. By studying the decay morphology within the cell wall layers in transversal and longitudinal sections, the spread of bacteria-driven decay inside foundation poles was evaluated and connected with the variations of oxygen availability.

Klaassen (2008) illustrated a classification of bacterial attack that has to be linked to technological properties of the foundation piles (moisture content, specific gravity and the compression strength) for four different wood species. This classification aimed at a thorough knowledge of pattern, causes and processes of degradation of wooden foundation piles in the Netherlands. He concluded that water flow inside wooden piles plays a fundamental role in bacterial spread, thus accelerating the degradation processes in wood.

Aim of this study is to propose a new classification criterion for the decay of waterlogged archaeological wood at cell wall level, through a micromorphological description starting from a factual evaluation of the cells' preservation. An investigation into the state of preservation of the poles of the pile-dwelling of an ancient site (IV–II millennia B.C.) located in Fiavé, in the Italian alpine region, provided the material for the evaluation of the proposed grading criterion. In particular, the grading classes obtained in the Fiavé samples were compared with the classes obtained after grading the same material on the basis of their values of Maximum Water Content (MWC), which is the most widespread parameter for the evaluation of decay in waterlogged wood among those reported in the scientific literature. This way, it was possible to validate the proposed criterion, as a source of reliable information, consistent with other independent grading procedures.

2. Material and methods

2.1. The site of Fiavè pile dwellings

The Fiavè peat bog is situated in northeast Italy, on the Alps. The site is the result of the typical evolution of an alpine lake, which has undergone phases of sedimentation and silting up turning into a

[1] The percentage ratio between the water content of the fully waterlogged sample and the oven dry weight of wood.

[2] A sharp pin to determine the percentage of the internal sound wood core (Grattan and Clarke, 1987; Florian, 1990).

bog. At the end of the nineteenth century, the harvesting of the peat revealed the presence of wooden artefacts (Perini, 1995). During the twentieth century three excavations were carried out, and especially those coordinated by Renato Perini, between 1969 and 1975, enabled us to understand the structure and complexity of the site (Perini, 1995). Some villages both on the lake-shores and pile dwellings were brought to light. They dated back to IV–II millennia B.C. These excavations caused a deep modification of environmental conditions with dramatic effects for the conservation of wooden piles: many organisms (algae, molluscs and plants) colonised piles with detrimental consequences for the integrity of the material. For a better management of the site, a campaign to study the state of preservation of wood has been recently decided.

2.2. Material

With the aim to analyse wood along the depth of piles' structure, a Pressler corer has been used to obtain samples (cores) from 81 piles. In some cases more than one core per pile was collected at different heights, but in most cases it was very difficult because of the sucker effect of water on degraded wood during the extraction of cores (Fig. 1). Collected cores were transported to the laboratories in containers full of water, in order to maintain the waterlogged conditions.

Each core was divided in two halves: one for chemical analysis (not reported here) and the other one for the micromorphological and physical analysis. Each half was subdivided in an inner (A), an intermediate (B) and outer (C) sector (Fig. 2). A pin test was used to distinguish the outer part (C) from the other two (A and B). In fact, the outer part was very easily distinguishable from the two internal ones because the needle often passed from one side to the opposite one. Instead, a geometrical criterion was adopted to separate A from B. When the state of preservation of halves seemed to be as uniformly bad, they were not subdivided: in that case, the letter D was assigned to the sample. In total from 81 cores, 239 different samples were characterised in this study.

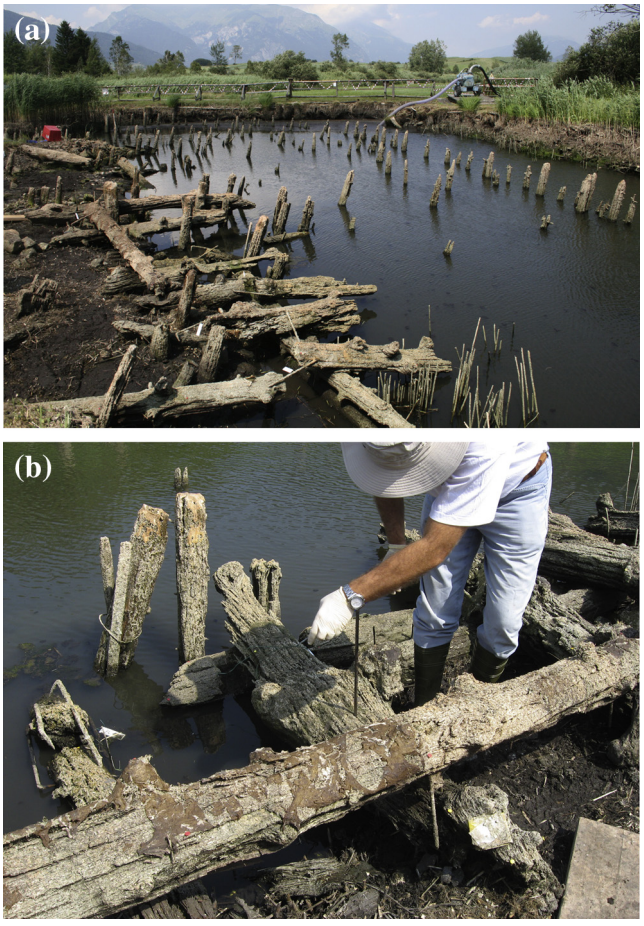

Fig. 1. Sampling phases. (a) Area of the site involved by our investigations as it appeared after water table lowering. (b) Sampling of cores.

Fig. 2. Division of cores. Each core was divided into two halves; one was used for chemical analysis (gravimetric and infrared, letter C) and the other for the physical and anatomical one (letter A + F). Subsequently both halves were each subdivided into three portions taking into account the different state of preservation.

2.3. Microscope observations

Identifications of wood species and morphological investigations were carried out by means of a light microscope on thin wood sections (10–20 μm), along the three diagnostic anatomical directions (transversal, longitudinal–radial and longitudinal–tangential).

Slides were obtained by handmade cutting with a razor blade on frozen specimens obtained from every sector of cores. The characterisation of decay was carried out by a transmission light microscope (DM LB 2, Leica) using also the polarised light to highlight the crystalline cellulose structure (or its possible loss due to the action of fungi and bacteria). The magnifications used were the following: 50, 100, 200, 400 and 630. The first two magnifications were used to have an overall view of the anatomy of samples and were the basis to state the decay grade; magnifications 200, 400 and 630 allowed for an in-depth study of biological decay and of the signs of bacterial and fungal attacks. In such a way, an area of a few μm^2 was studied.

In a few cases, the state of preservation of wood cells was investigated through a scanning electron microscope (SEM Philips XL 20). Small samples, oriented along the three anatomical directions through a manual cut with a common razor blade, were fixed, as described by Björdal et al. (1999), then dried by the critical point drying method (Balzers CPD 030) and gold coated.

2.4. Physical characterisation

A small specimen was obtained from each sample resulting from subdivision of cores. Weight and volume (by the water displacement method) were measured first, at the saturated state and then, only the weight, after exsiccation in oven-dried conditions (Macchioni, 2003). The obtained results, allowed calculating some of the most used parameters to evaluate the decay of waterlogged wood (Schniewind, 1990):

1. Maximum Water Content (MWC, %), as the ratio between the mass of water in the specimen and the anhydrous mass of wood, that is:

$$\mathrm{MWC} = \frac{M_W - M_0}{M_0}$$

M_W and M_0 being the wet and anhydrous mass, respectively.

2. Basic Density (BD, g/cm^3), as the ratio between anhydrous mass and wet volume.
3. From BD, another parameter was obtained: the Residual Basic density (RBd, %), calculated as the ratio between the measured density of the archaeological material and the average density of non-degraded wood from the same species, derived from literature. Typically samples with RBd lower than 65–70% are considered degraded, whereas they are heavily degraded if RBd is lower than 40% (Macchioni, 2003).

3. Results

3.1. Micromorphological analysis

From the 81 different cores, 38 larch (*Larix decidua* Mill.), 24 spruce (*Picea abies* Karst.) and 19 silver fir (*Abies alba* Mill.) poles were identified. All these species reflect the forest species of the area.

3.1.1. The decay agents

At the microscope observation, the samples clearly showed signs of microbiological degradation. The organisms which caused this decay were soft rot fungi and erosion bacteria, generally present together both in transversal and longitudinal sections.

In case of soft rot decay, holes in the secondary wall were visible in cross section: if decay was at an initial step, holes were isolated in the wall, while they merged together in highly degraded walls up to

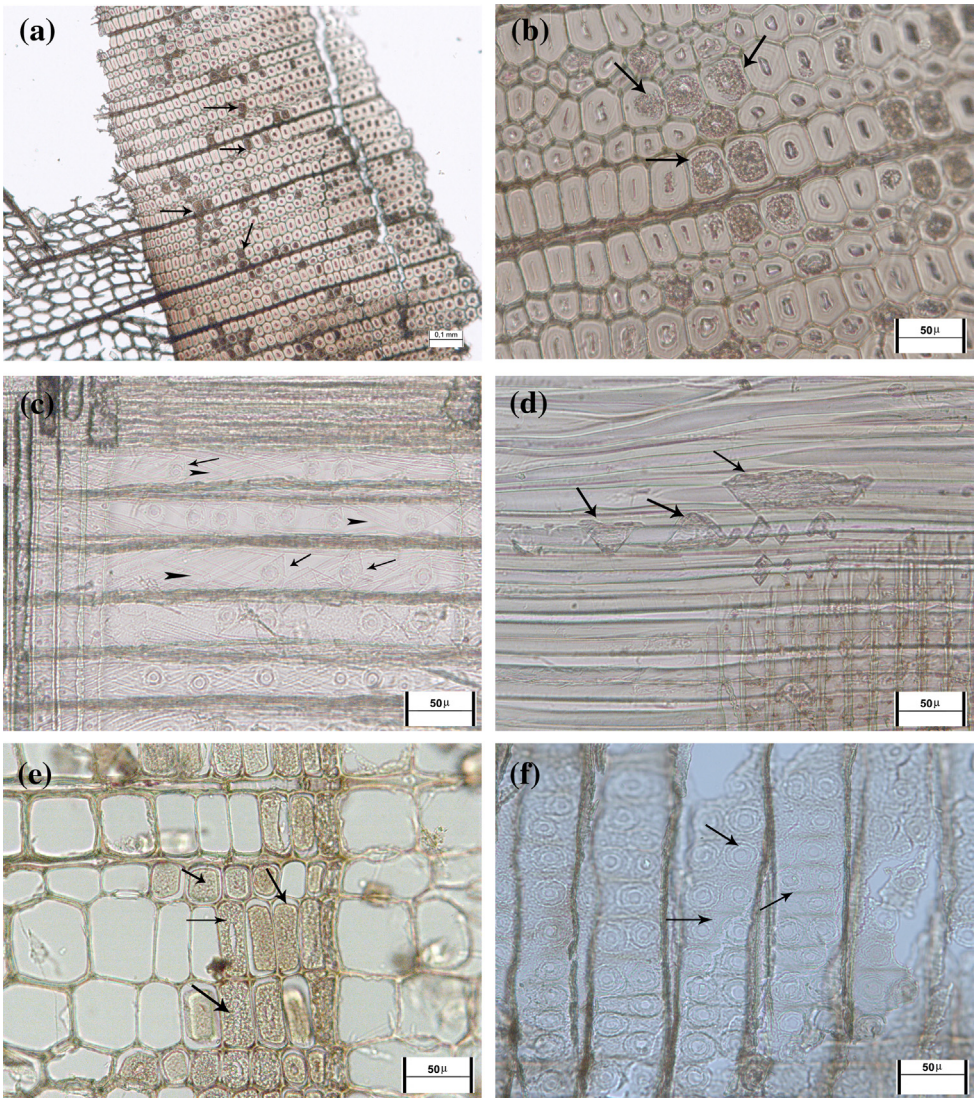

Fig. 3. Characteristic aspect (light microscope) of waterlogged archaeological wood degraded by soft rot fungi and erosion bacteria. (a) Typical pattern of an initial decay by erosion bacteria in cross section. Single degraded cells (arrows) surrounded by healthy cells (larch) (bar 0.1 mm). (b) Thanks to a higher magnifications the effects of erosion bacteria (arrows) at the level of the cell wall are visible. The degradation begins from the lumen and from the S_3 layer bacteria move towards the S_2 layer of the wall transformed into an amorphous material (bar 50 µm). (c) The effects of soft rot fungi and erosion bacteria in the same radial section. Soft rot cavities (arrowheads), pointed ended, moving along the micro-fibrillar orientation of the cell wall are together with conical erosion (arrows) around bordered pits produced by erosion bacteria (silver fir) (bar 50 µm). (d) In another radial section there are many "V" shaped notches (arrows) of eroded cell wall (larch) (bar 50 µm). (e) Severe decay of erosion bacteria of the entire cell wall produce the detachment from the middle lamella and after the total destroying of S_3 and S_2 layers, the remaining granular material (arrows) fills the lumen (cross section of larch) (bar 50 µm). (f) In case of very strong decay in longitudinal section the wall of tracheids takes on a granular appearance: pits (arrows) are hardly visible among several grooves of erosion (larch) (bar 50 µm).

transform the wall itself in a granular material filling the lumen. In longitudinal sections the presence of soft rot fungi and erosion bacteria was evident (Fig. 3). Bacteria were visible because seemingly healthy cells were near to some strongly degraded ones in cross section (Fig. 3a, b); another aspect of the attack of these organisms was the typical conical erosion trough (Fig. 3c) around pit borders. In Figs. 3c and 4 the channels produced by soft rot fungi are visible.

In case of severe bacteria-driven decay (Fig. 3e) cell walls, transformed into a granular material, detached from the middle lamella that instead seemed unaffected. In longitudinal section the same cell walls appeared completely eroded by very thin grooves (Fig. 3f).

3.1.2. Grading of biological decay

From the above observations, with the aim to categorise the effects of biological attacks, the grading approach described in Table 1 was used. This approach aimed at describing the aspect of wood anatomy mainly with the transmission light microscope at rather low magnification (50–100×). As for the decay level, only the aspect of wooden tissues was considered, whereas the identification of decay agents was not regarded as an essential requisite; however, the presence of specific markers of wood degrading organisms was used for the selection of the class.

The decay grade must be evaluated through the observation of both transversal and longitudinal sections: only through the interpretation of several signs (holes, channels, wall removal, detachments) seen on different sections, the right decay grade can be identified.

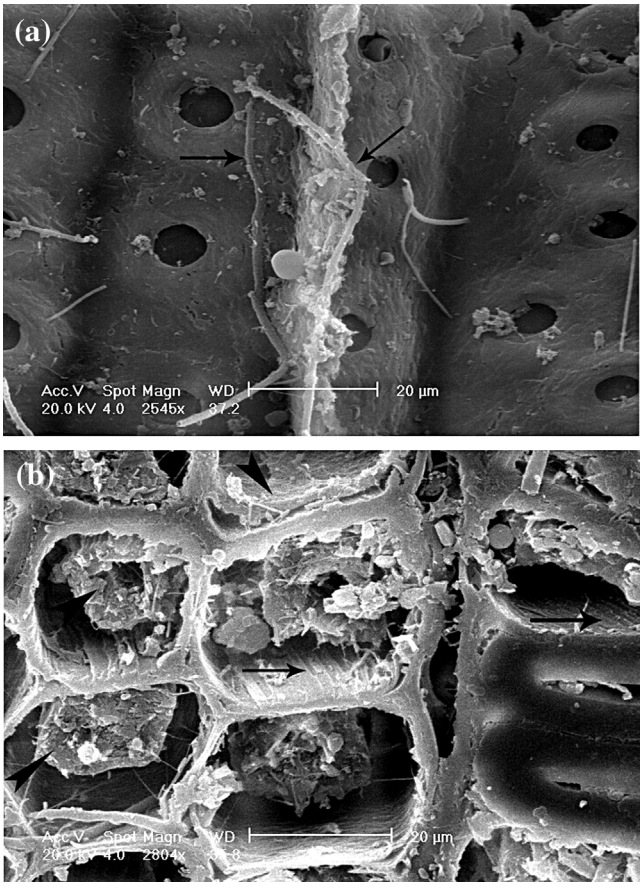

Fig. 4. Biological decay under the Scanning Electron Microscope (larch) (bar 20 µm). (a) Fungal hyphens (arrows) along the tracheids wall. (b) Soft rot decay is visible by the presence of channels pointed ended (arrows) and the aspect of secondary wall is typical of the bacterial decay.

Table 1
The grading of different levels of decay.

Decay grade	Description
0	No sign of decay
1	Decay almost completely absent. There are only some isolated attacks on few cell walls. Extremely rare attacks visible on longitudinal cell walls.
2	Initial decay characterised by some degraded cell walls surrounded by sound cells. Degraded cells are only partially eroded; in few cases the decay is extended to the entire wall, which has been transformed into granular material.
3	The majority of cell walls are decayed: often the lumen disappears because it is filled by products of decay. In longitudinal sections signs of fungal and bacterial attacks are evident and widespread.
4	The identification of wood species can be very difficult. The detachment of S_2 layer from the compound middle lamella is very common, and cells are extremely distorted, also in longitudinal sections.

Apart from the first (class 0, where wood appears unaffected) for the other classes the distinction was based on the extent of degradation on the examined sections: specimens belonging to class 1 showed sporadic degraded cells (holes produced by soft rot fungi and erosions of the cell wall caused by the erosion bacteria activity), visible in transversal and longitudinal sections; in samples of class 2, healthy cells were always predominant in number but, in the degraded ones, attacks were in an advanced stage and involved the entire wall. Specimens were attributed to class 3 when a widespread granular and amorphous aspect of cell walls could be observed, although some elements of structure were intact, and it was still possible to identify the species. Often in class 3 and particularly in class 4 the distinction between soft rot and bacterial attacks was very hard because both holes and erosion troughs join together. In this latter 4th class, detachments of cell wall from the compound middle lamella are very common and these detachments together with collapses and distortions (visible both in cross and longitudinal sections) can make the identification of wood samples very difficult.

From the 239 samples, 4 were graded as 0, 61 as 1, 74 as 2, 53 as 3 and 48 as 4, and Fig. 5 reports the percentage distribution of the samples in each micromorphological decay class, differentiated by the three wood species. Moreover, the average decay index for each core sectors (A, B, C, D) after micromorphological grading is reported in Fig. 6.

Both Figs. 5 and 6 indicate how the distribution of decay among species is not uniform. Larch samples were the majority of the examined specimens (146 out of 239), and Fig. 5a shows that most of them were distributed in classes 0–2, that is those describing a lower biotic attack. Also Fig. 7 confirms that the decay, in this species, was mostly occasional (Fig. 7a, b), even if some samples showed biological decay and detachments (Fig. 7c, d). In particular, sometimes the decay of cell walls caused the filling of lumen and the total detachment of S_2 layers from the middle lamella. In these samples, the observation of the cell walls on longitudinal sections showed a lot of signs of erosion that occasionally led to the disappearance of bordered pits.

Spruce samples were quite few (54). Most of them were distributed in the classes describing the worst decay (3 and 4), whereas there were no samples in class 0 and only 2 in class 1 (Fig. 5c). Consequently, most samples belonging to this species evidenced a high level of decay. Fig. 6 additionally shows how within spruce piles the degradation was concentrated in outer portions (C). Some sections showed only sporadic signs of degradation (Fig. 7e, f), while in other cases detachments, holes and distortions of cell morphology were very common. In many cases

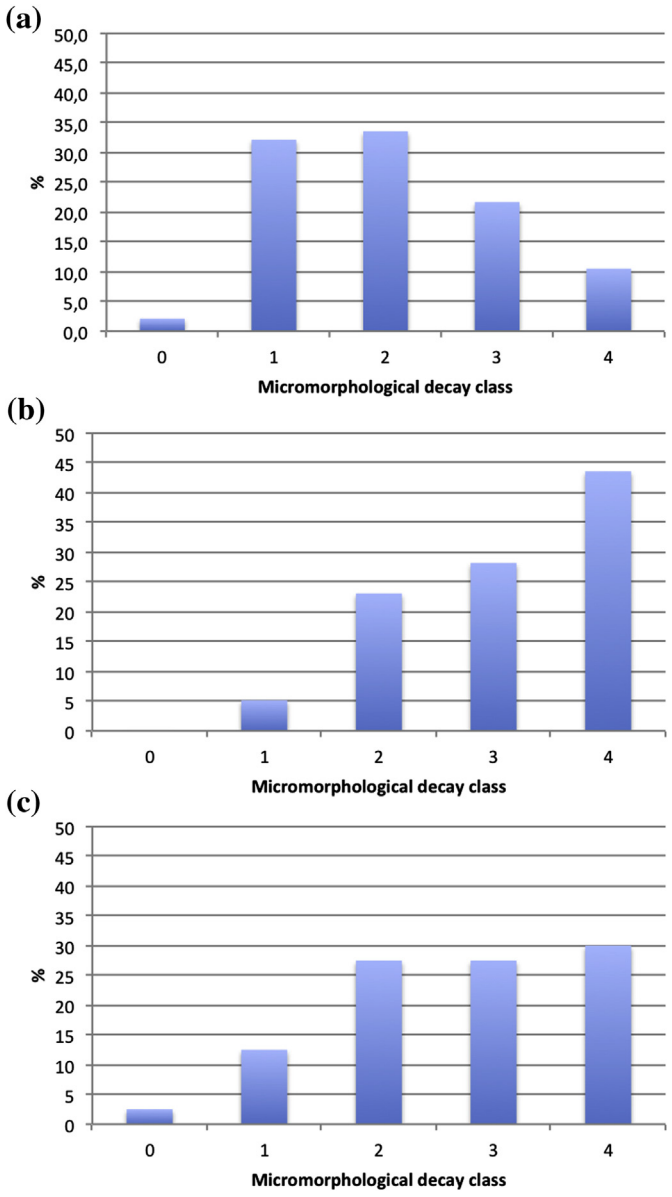

Fig. 5. Percentage distribution of samples among decay classes: (a) larch; (b) silver fir; (c) spruce.

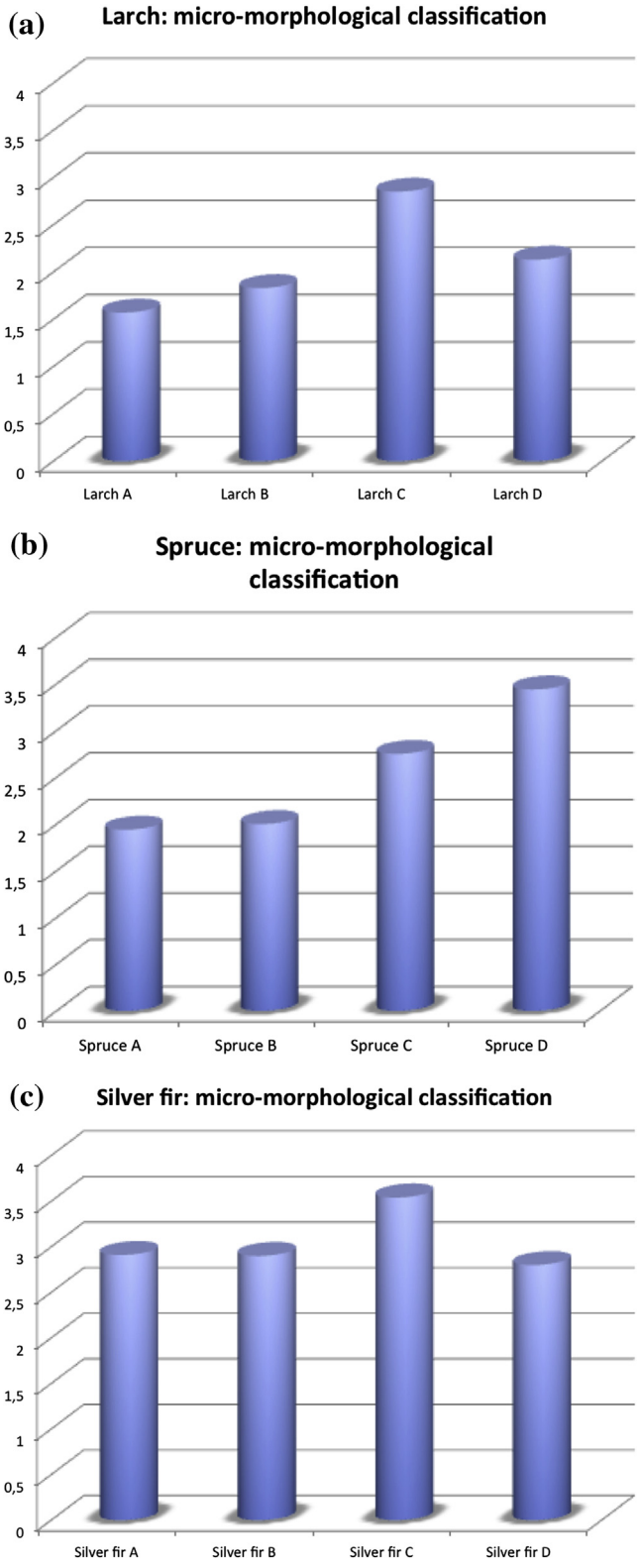

Fig. 6. Distribution of the average value of micromorphological decay index according to species (a: larch, b: spruce, c: silver fir) and portions of cores.

the traces of soft rot attacks (holes inside the cell walls) and erosion bacteria (the wall transformed into an amorphous material) were visible in the same portions of material.

The grading of the 39 silver fir samples evidenced a distribution similar to spruce samples: most of the material was decayed (classes 2–4), whereas only 1 sample belonged to class 0, and 5 to class 1 (Fig. 5c). However, it is worth noticing how silver fir showed a higher percentage of samples in class 4 compared to spruce. Therefore, silver fir showed a high degradation uniformly distributed along the diameter of cores, as also evidenced in Fig. 6. Samples were frequently poorly preserved (Fig. 8a, b): sections often pointed out collapses, and cell walls were transformed in a swollen amorphous mass.

The grading of different core sectors (A, B and C) allowed evaluating the distribution of decay along the diameter of each element.

In most cases, as expected, the inner part (A) was less degraded than the intermediate one (B) which, in turn, was less degraded than the most external one (C). In some cases (8 samples for larch, 6 for silver fir, and 6 for spruce) the level of decay was uniform within the diameter, without any difference between the portions (A, B and C).

In some cases, grades among neighbouring sectors were not perfectly consequential: for example inside core C10, sectors A and

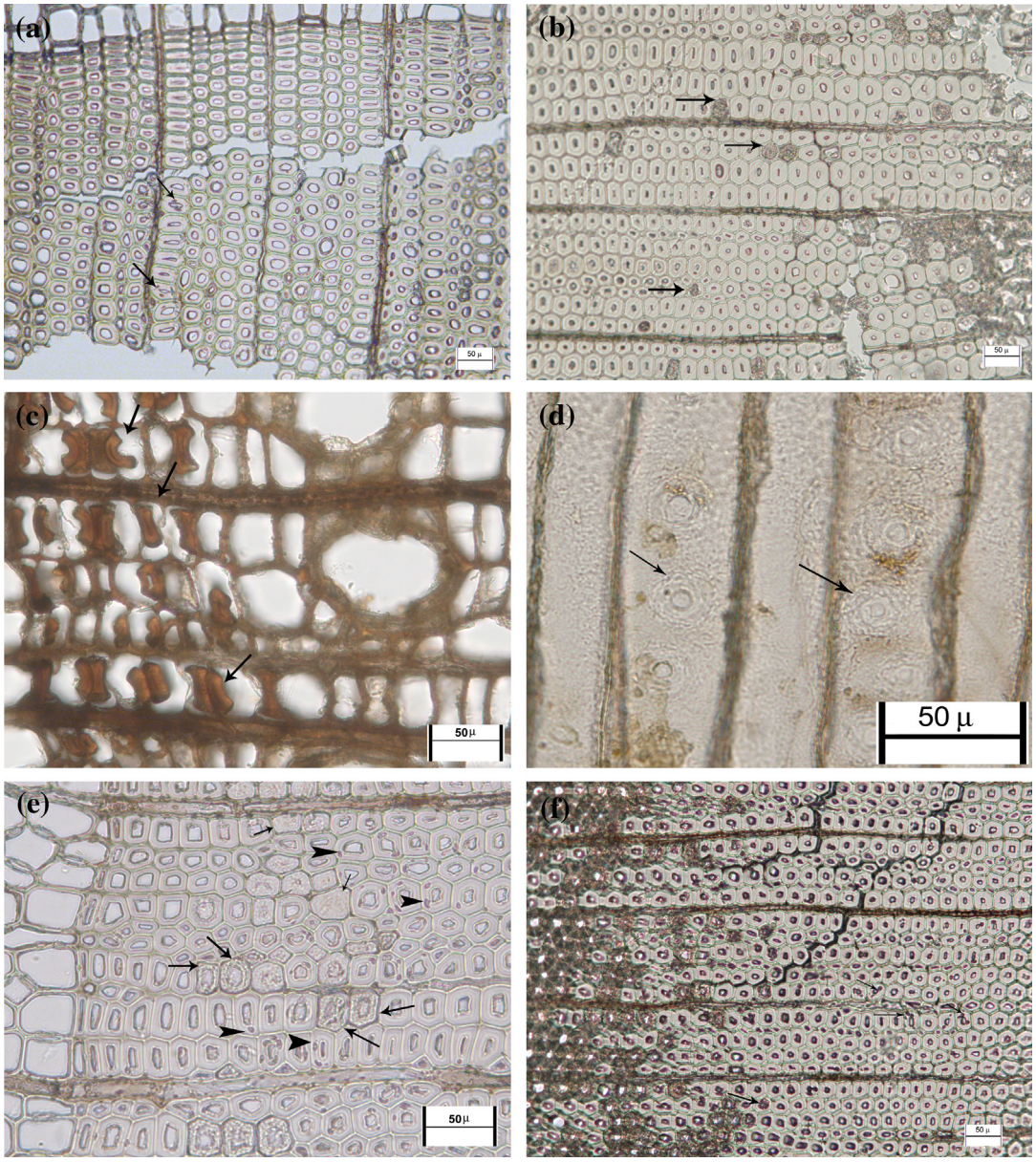

Fig. 7. The aspect of different classes as viewed under light microscope (bar 50 µm). (a) Transversal section of larch from sector number 42B, classified in Class 1. (b) Transversal section of larch from sector 70A classified in Class 1. In both pictures very few degraded cells are visible (arrows). (c) Transversal section of portion 4C classified in Class 4 (larch). In latewood the biological decay caused the complete detachment of the wall from the middle lamella and the consequent collapse of S_2 layer (arrows). (d) Radial section of the same portion 4C. Degradation has been so strong that wall and especially pits are almost completely disappeared (arrows). (e) Transversal section of spruce from sector number 90A, classified in Class 1. In this section the most part of cells appear healthy but there are also walls at different levels of decay: in some there are some holes produced by soft rot (arrowheads), in others the wall looks like a "granular and amorphous material" due to the action of erosion bacteria (large arrows) and finally in few cases the lumen is completely disappeared (small arrows). (f) Transversal section of spruce from portion number 92B, classified in Class 2. Also in this case the distribution of decay is not homogeneous (arrows).

B were graded as 1 while sector C was graded as 3. Therefore, that sample passed from a preserved core to a decayed outer layer.

Only in four cases there was an unexpected distribution of decay along sectors A-B-C: within sample C66 the grades were 2-1-2, respectively; within core C46: 2-2-1; within core C61: 3-1-0; within core C5: 2-1-3.

3.2. Physical characterisation

Tables 2–4 report the MWC values obtained for each core sector from the samples of silver fir, spruce and larch, respectively.

The decay parameters obtained from water content measurement confirmed the results obtained by anatomical analyses. Larch wood seemed to be less affected by decay, and silver fir samples were, on the contrary, the most decayed ones. Spruce samples were in-between.

The distribution of the decay within the cores underlined a high degradation in the external portions, in direct contact with the water of the artificial lake, while the inner parts were better preserved; sectors A and B of the larch samples showed a quite low MWC that, in its minimum values, was very close to the water content of waterlogged unaffected wood (between 150 and 200%, according to the anatomical characteristics of the species). The good state of preservation of the inner sectors of larch cores is also demonstrated by the low standard deviation values in Table 4.

The obtained RBd values were also in agreement with MWC: they were particularly low in the external parts of piles and in silver fir (Fig. 9). However, the very high values of RBd in sectors A and B

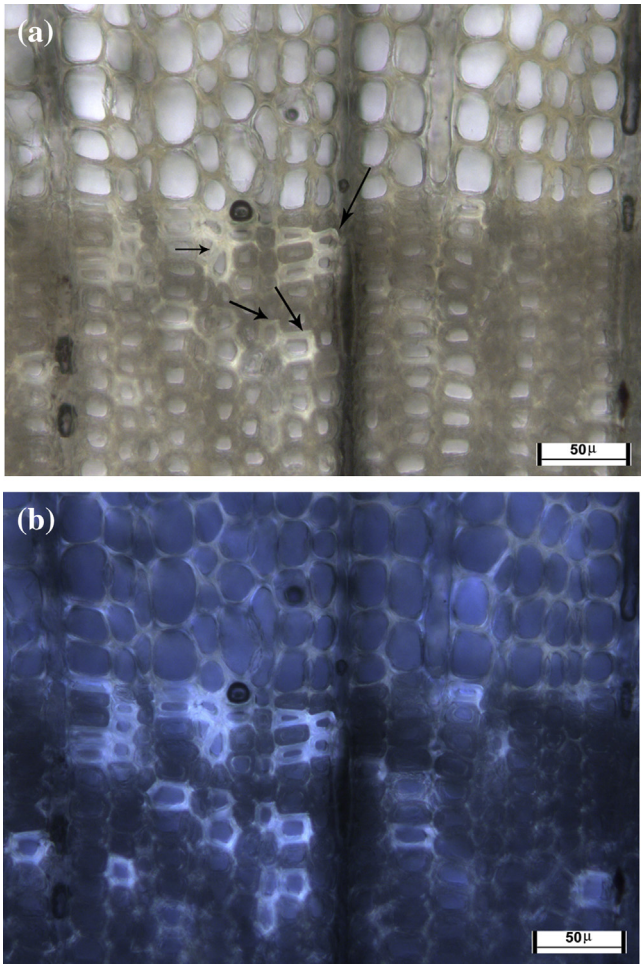

Fig. 8. Severe decay at light microscope (bar 50 µm). (a) Transversal section of sample 101, portion C (silver fir) of Class 4. (b) Transversal section of sample 101, portion C (silver fir) of Class 4: the same LM-photo but with polarised light that allow to highlight the possible absence of crystalline cellulose due to the biological decay. In fact only few cells (arrows, a) retain a strong birefringence while most of cells in spite of a form and a size that seem usual, no longer have sound walls.

of spruce were even higher than in larch. Probably, the explanation is that the RBd reference value of literature is poorly suited to the density of spruce grown in the Fiavé area. Even at present the spruce wood growing in the region is renowned to have on average a lower density, compared with other European areas.

4. Discussion

The micromorphological decay (and its related pattern) found in the Fiavé pile-dwelling is in agreement with other results obtained in similar contexts (Brunning et al., 2000). However, it should be stressed that some of our findings are contrasting with what is shown by Björdal and Nilsson (2008), who observed a clear spatial arrangement of decay between soft rot fungi (and tunnelling bacteria) and erosion bacteria, which was traced back to the different needs of oxygen. Instead, in Fiavé samples these two types of organisms had degraded the same parts of wood (like already reported in Björdal et al., 1999). Moreover, soft rot decay was also found in portions B of piles, and even in the most internal part of piles (A). Considering that the activity of erosion bacteria is strongly affected by the environmental oxygen content (Björdal and Nilsson, 2008), that has to be very low or absent, the explanation for this could be a different distribution between fungi and bacteria over a long time.

In fact, during their history, piles had a rather short period of use, after which they were abandoned and silted up. A very long period (at least 2–3 thousands of years) followed, during which piles remained sealed into the peat bog. Finally, they underwent 40 years of immersion in fresh, oxygen-rich water. This allowed for the observed succession of organisms, which is different from what can be usually seen on waterlogged archaeological wood coming from a shipwreck, which instead sinks rapidly.

When the micromorphological grading results were analysed through an ANOVA statistical test related to the various factors under study (micromorphological decay class, wood species, and pole sector) the obtained data confirmed the significance of the differences among the micromorphological decay classes and among the wood species, whereas the observed differences related to sectors were not statistically significant, at least when data from all species were grouped together (Table 5). Moreover, a correlation trend between MWC values and micromorphological decay class was found: Fig. 10 shows the distribution of MWC values for the various samples belonging to each one of the 5 classes, and the trend to an increase of water content by increasing the class of decay is quite clear.

Nevertheless, the variability within each class was very high. This high variability could be related to the different scales of the two types of analyses: in fact, the microscopic analysis is carried out on a very small part of the sample, each section being a few microns in thickness and 1–2 mm^2 in surface. In contrast, the physical characterisation is a "bulk" analysis involving several mm^3 of the analysed wood.

A thorough analysis of the results led to some more considerations: the boxplots describing the values showed that from class 0 to class 2 the MWC median values were similarly low (approximately 200%), whereas they started having markedly higher values in classes 3 and 4. This implies that the micromorphological grading is more detailed in the description of the first stages of the

Table 2
MWC (%) values from all silver fir samples.

Core sector	Mean	Max	Min	St. dev.
A	409	582	245	131
B	396	625	217	146
C	503	700	346	115
D	386	596	249	146
Total average	433	700	217	136

Table 3
MWC (%) values from all spruce samples.

Core sector	Mean	Max	Min	St. dev.
A	329	777	171	177
B	291	702	179	152
C	361	883	177	201
D	477	724	243	170
Total average	354	883	171	183

Table 4
MWC (%) values from all larch samples.

Core sector	Mean	Max	Min	St. dev.
A	239	463	90	91
B	234	491	129	91
C	340	1062	124	167
D	270	444	96	115
Total average	271	1062	90	130

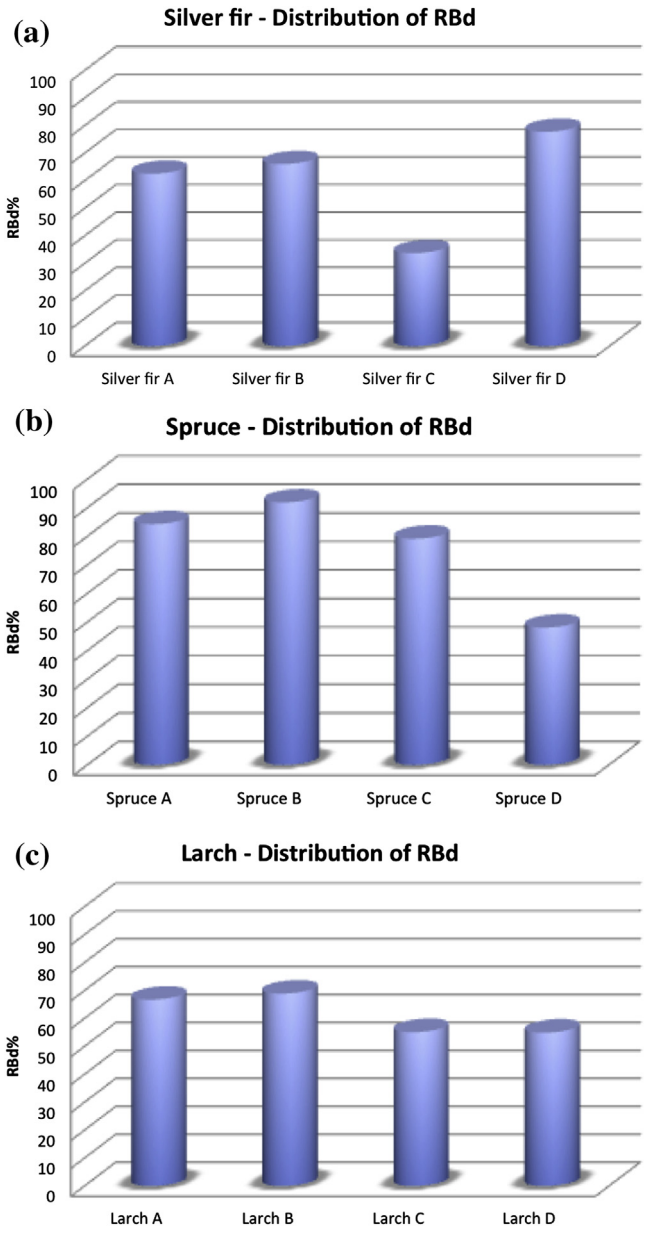

Fig. 9. Distribution of Residual Basic density (%) according to species (a: silver fir, b: spruce, c: larch) and portions of cores.

attack, classes 1 and 2 (see Table 1), and those anatomical changes do not induce any appreciable difference at the level of physical evaluations. In fact, in classes 1 and 2 most cell walls are still intact or their low degree of erosion does not provoke an appreciable

Table 5
ANOVA test, all the factors and interactions.

Factors	Df	Sum sq	Mean sq	*F* value	Pr (>*F*)
Decay class	4	24.9695	6.2424	76.4346	<2.2e-16***
Species	2	1.8149	0.9075	11.1114	2.865e-05***
Sector	3	0.2458	0.0819	1.0033	0.3927
Decay class: species	7	0.6283	0.0898	1.0990	0.3658
Decay class: sector	10	0.5536	0.0554	0.6779	0.7441
Species: sector	6	0.3487	0.0581	0.7116	0.6407
Decay class: species: sector	14	0.5314	0.0380	0.4648	0.9488
Residuals	175	14.2921	0.0817		

***Pr < 0.001, **Pr < 0.01, *Pr < 0.05.

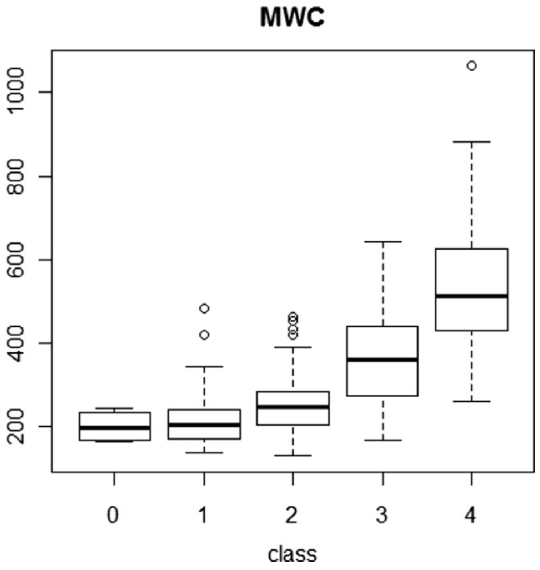

Fig. 10. Boxplots of MWC values within each decay class identified after micromorphological grading.

increase of MWC. The Tukey test (a single-step multiple comparison procedure used to find means that are significantly different from each other) confirmed the statistical significance of the differences between MWC values for classes 3 and 4, and between MWC values for classes 0, 1 and 2, which instead were not different among them (Table 6).

In addition to considering all together the MWC values of samples classified according to the micromorphological grading, as reported above, it was also tried to directly classify samples according to their MWC value, in order to compare the MWC-class with the micromorphological-class for each sample. To such purpose, the MWC-based grading criterion proposed by De Jong (1977) was modified to pass from the original 3 to 5 classes (in such a way the two approaches could be directly compared). More specifically, an amount of 40–50% for the first class, and an amount of 50–100% for the second class was added or subtracted to the thresholds selected by De Jong. In such a way, the following ranges of MWC values were obtained: less than 135% for the class 0-MWC; 135–225% for the class 1-MWC; 225–350% for the class 2-MWC; 350–500% for the class 3-MWC; more than 500% for the class 4-MWC. The general distribution of samples among the various classes is reported in Fig. 11, where the results of both MWC-grading and micromorphological grading are compared. As shown, the distribution of samples for the two different grading criteria was roughly similar.

After that, the value of the MWC-class of each individual sample was subtracted by the value of the micromorphological-class. For

Table 6
Tukey multiple comparison of means of MWC among decay micromorphological classes.

Classes	diff	lwr	upr	*p* adj
1–0	0.05683335	−0.33954039	0.4532071	0.9948562
2–0	0.20731073	−0.18598314	0.6006046	0.5959770
3–0	0.57668246	0.18030872	0.9730562	0.0008145***
4–0	0.96236990	0.56316151	1.3615783	0.0000000***
2–1	0.15047738	0.01041043	0.2905443	0.0283284*
3–1	0.51984911	0.37135391	0.6683443	0.0000000***
4–1	0.90553655	0.74963265	1.0614404	0.0000000***
3–2	0.36937173	0.22930479	0.5094387	0.0000000***
4–2	0.75505917	0.60716073	0.9029576	0.0000000***
4–3	0.38568744	0.22978354	0.5415913	0.0000000***

*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

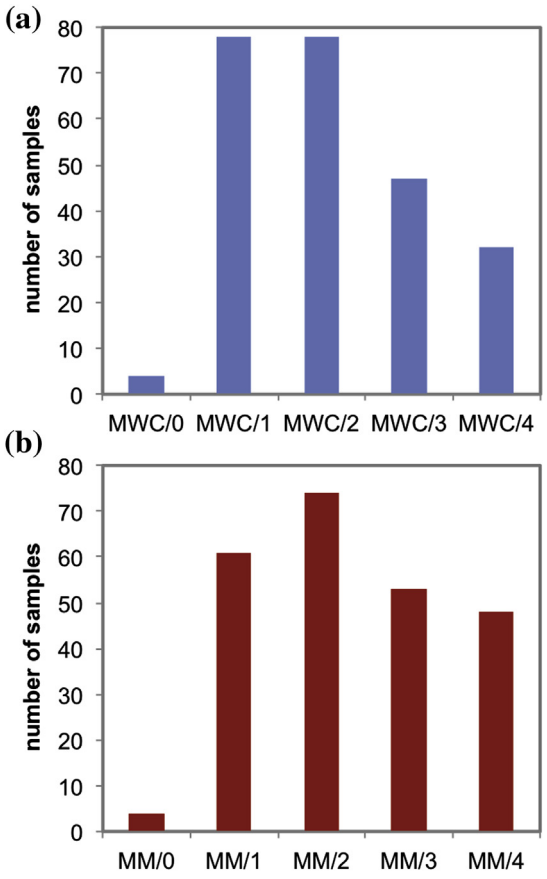

Fig. 11. Distribution of samples for the MWC grading (a) and the micromorphological (MM) grading (b).

instance, sample 2A (larch) was classified as 1 according to micromorphological grading, and 2 according to MWC grading: the difference for that sample was 1 (in absolute value). This way, a difference of 0 identified samples belonging to the same class according to both criteria, a difference of 1 (absolute value) was related to samples in which the difference between the two approaches was of 1 class, and so on.

The calculated differences for the various samples evidenced that 51% of them were assigned to the same class by both criteria (difference equal to 0, Fig. 12), and that for more than 42%, 1 class difference was found. Therefore, only 7% of samples differed by 2 or more classes. The distribution among wood species was practically equivalent to the general distribution, with the relevant exception of silver fir samples, which did not show any sample different for more than 1 class (data not shown).

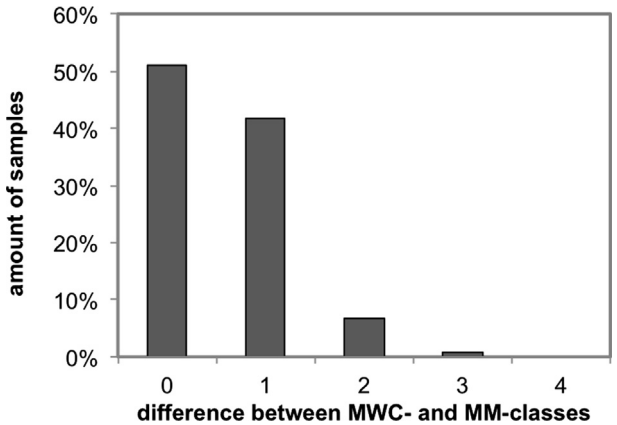

Fig. 12. Distribution of samples in the new classes resulting from the difference between MWC and micromorphological (MM) values.

Our results confirm that our grading procedure according to microscopic analysis gives information consistent with that obtained by the well-known parameter MWC. Of course, the present approach needs to be extended to other samples from different excavations and belonging to different wood species (for instance, angiosperms were not found in the excavation of Fiavé) before it can be reliably adopted. However, the present grading criterion sets the conditions for a quantitative multidisciplinary approach to the assessment of preservation. In fact, possible discrepancies (for instance due to an excessive amount of inorganic fillers or cell collapse) among the three methods of evaluation currently adopted (namely micromorphological, physical, and chemical analyses) could be better identified if all the three methods gave numerical results (as shown in Pizzo et al., 2013, although limited to the case of chemical results). Analogously, a more general criterion of grading samples according to the true level of decay could be eventually adopted in the future, based on the numerical values obtained in all the three analyses. This could reliably assess the true state of preservation and, in perspective, even tailor a method of conservation according to the observed class of decay.

5. Conclusions

This study has illustrated the state of preservation of the different poles left in the pile dwelling of the Fiavè site, which was excavated from peat bog mostly during the seventies of the last century and then kept under the water table. It is possible to confirm the presence of new attacks developed after the transformation of the site from a peat bog into a small lake, and the presence of new attacks by soft rot fungi on portions already attacked by bacteria.

The observed decay was different between species: silver fir samples appeared to be more decayed than both spruce and, mostly, larch samples. In several cases larch samples showed physical parameters very close to those of the sound wood, and almost no attacks from the micromorphological observations. Moreover, the distribution of the decay showed higher concentrations of bacterial and fungal attacks on a thin external layer of the poles, while the inner portions were less attacked. On the contrary, silver fir poles were more attacked even in the inner parts.

A micromorphological grading approach was used for the first time on those samples, which grouped samples into 5 decay classes (0–4). The results presented here show that the grading approach was well correlated with the Maximum Water Content, i.e., the most utilised parameter to describe the decay of waterlogged archaeological wood. It means that this grading approach is mostly suited for multidisciplinary studies on waterlogged archaeological wood, and (although it is partially based on a subjective evaluation) it provides numerical results about wood preservation from an anatomical point of view.

The high number of samples underlays a reliable statistical evaluation. This showed that classes 0, 1, 2 cannot be distinguished on the basis of physical parameters, whereas classes 3 and 4 are significantly separated according to the MWC values. Moreover, when samples were individually classified according to their MWC value, for more than 90% of them the established classification differed by less than 1 class: this confirms the consistence of the proposed grading criterion.

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