Anoxic treatment for the disinfestation of wood cultural heritage: assessment of the effects and harmfulness on different species

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Introduction

Wooden artefacts, wood painted panels and historic furniture represent a significant part of our cultural heritage. Their preservation over time is a challenging task as they can be damaged by several chemical–physical, mechanical and biological phenomena. The last ones are particularly relevant in causing damage, given the organic composition of wood. The microbiological activity of bacteria and fungi results in aesthetic alterations and depletion of mechanical characteristics, but insect infestation still represents a primary cause of loss of cultural heritage artefacts. In the past, several chemical methods mainly based on the use of liquid preservatives or gaseous fumigants have been used for the conservation of wood under insect attack (Unger 2012). Such treatments present numerous negative drawbacks, including a high risk of damaging the objects (Wörle et al. 2012) and serious safety issues for operators due to chemical toxicity. Less dangerous alternatives have, therefore, been proposed and tested, including thermal treatment (using either low or high temperature) (Strang 1995), physical methods (microwaves, X-rays and gamma rays irradiation) (Andreuccetti et al. 1995; Augelli et al. 2007) and the use of controlled atmosphere (Unger et al. 2001).

Starting from the 1990s, interest has grown in a different approach to the preservation of artefacts based on the use of non-toxic and inert gases to produce oxygen-free atmospheres. Anoxic conditions have proven to be effective in pest control and have been extensively applied to the management of museum collections (Gilberg 1991; Koestler 1993). Low oxygen concentrations have also been reported to inhibit biodeterioration due to the development of bacteria and fungi (Tavzes et al. 2001; Valentìn 1990). Besides the preservative treatment of already contaminated objects, oxygen deprivation has been applied for preventive conservation purposes to avoid insect colonization, as part of overall maintenance strategies.

The gases most widely used for treatment include nitrogen, argon and carbon dioxide. In the present work, the evaluation of anoxic treatment achieved using nitrogen is reported. In the current conservation practice, three different approaches are followed for anoxic treatment, depending on available equipment, size of objects to be treated, and economic reasons. The basic and less expensive *static approach* relies on the use of oxygen scavengers in a confined environment (such as barrier-film bags, rigid containers, low-oxygen permeability bubbles) in which the objects are tightly sealed. A more efficient approach is the *static–dynamic* one, in which the objects are kept in a steady nitrogen atmosphere and the oxygen content is further reduced with O_2 absorbers. In the *dynamic* approach, objects are treated in a container in which nitrogen is continuously purged (Hanlon et al. 1992): during the preliminary phase, a high purge rate is used to remove all the air from the container; the flux is then reduced and kept steady to ensure anoxic conditions for the entire duration of treatment. A complete overview of the operative aspects of the treatment has been reported by the Getty Conservation Institute (Selwitz and Maekawa 1998).

The disinfestation effect of anoxic treatment exploits the dehydration induced by oxygen deprivation and the related prolonged stress on insects (Valentìn 1993). As a

matter of fact, low oxygen content progressively enhances the loss of water from the insects leading to accelerated desiccation and death. The efficacy of this mechanism has been confirmed by studies showing that the mortality rate can be increased by reducing the humidity in the treatment environment. Similarly, as the temperature rises, insect dehydration increases, thus achieving a higher mortality rate (Valentìn 1993). Although anhydrous atmospheres and high temperatures actually result in a rapid and complete disinfestation, the treatment of valuable museum objects needs to be fine-tuned according to the most appropriate conservative conditions as indicated by standard preservation protocols (UNI-EN 15757 2010; UNI 10829 1999). In case of storage and conservation of wooden objects, these conditions are 19–24 °C T and 50–60 % RH (a 45 % RH value is suitable only in case of non-painted wooden sculptures). The time required for 100 % mortality may vary considerably within the standard T and RH ranges, as well as according to the insect species, which may respond differently to oxygen deprivation (Gilberg 1989; Selwitz and Maekawa 1998; Valentìn 1993).

It is worth noting that the treatment duration is a fundamental parameter which does not only depend on insects' resistance. The specific characteristics of the wooden objects strongly influence the time for complete disinfestation, as they may delay the achievement of anoxic conditions deep in the bulk of the material. Thus, an additional treatment period may be required. This can be due to both the presence of low permeable superficial finish coatings and the microstructural features of wood species. A reliable estimate of wood permeability is necessary to plan an effective treatment. Wood permeability is highly anisotropic with respect to the direction of growth, as a result of the structural shape and arrangement of wood cells, as well as to cell function in the living tree (Comstock 1970). Methods reported in the literature for the evaluation of the air permeability of wood (Perre 1987), wood fibres (Thoemen and Klueppel 2008) or wood pellets (Yazdanpanah et al. 2010) are mostly based on complex apparatus which measure the mass flux generated by the presence of a pressure gradient through Darcy's law. In this study, a simplified method is proposed which is aimed at estimating the permeability of wood to oxygen in different conditions (as for thickness of the specimens, wood orientation and superficial finishing) during the anoxic treatment, through the measurement of O_2 depletion.

While the efficacy of anoxic treatment with respect to insect mortality is well established and supported by numerous case studies (Chiappini et al. 2009; Valentìn 1993), the evaluation of the non-harmfulness of anoxic treatment for the constituent materials and object finishings under actual conditions still requires thorough investigation.

The conservation of museum objects in an inert atmosphere is known to generally diminish the risk of chemical and photochemical degradations (Beltran et al. 2012) of the constituent materials (i.e. due to surface oxidation); thus, the anoxic treatment has been positively acknowledged as a non-harmful methodology by conservators. In the literature, only a few degradation cases have been reported as a result of a reduction mechanism, which involved unstable dyes and pigments applied to a more delicate substrate than wood, such as textiles and paper (Beltran et al. 2012; Rowe 2004).

Near infrared (NIR) spectroscopy (750–2500 nm; 13,300–4000 cm^{-1}) has often been used for the characterization of different kinds of wood. NIR spectra of wood contain information regarding chemical composition and molecular structure which characterize and influence the physical properties and the performances (Kelley et al. 2004; Schwanninger et al. 2011). With respect to other spectroscopic techniques, NIR has a number of advantages making it an ideal tool for wood analysis. These advantages include minimal sample preparation and rapid acquisition times; furthermore, NIR allows acquisition of spectra in a non-destructive way.

The major components of wood are the three polymers cellulose, hemicellulose and lignin. Absorption bands of wood in NIR regions arise from overtones and combinations of vibrations of C–O, O–H, C–H and N–H bonds. NIR is used mainly to measure overtone and combination bands of the fundamental stretching vibration of different functional groups such as C–H and O–H (Tsuchikawa et al. 2003).

The absorption signals from the various wood constituents are similar and highly overlapping, and it is not possible to distinguish the single band and associate them with specific constituents. Only some classes of substances, such as polysaccharides and aromatics, can be directly assigned (Gierlinger et al. 2004). Owing to the complex properties of wood, most of IR bands cannot be directly assigned to one single component and the interpretation of isolated bands could be misleading at times.

However, when FTIR data are elaborated by means of multivariate statistical techniques, many wood parameters (discrimination among wood species, chemical composition or physical–mechanical properties) can be successfully predicted (Gierlinger et al. 2004; Jones et al. 2006; Kelley et al. 2004; Pizzo et al. 2015; Tsuchikawa et al. 2003; Watanabe et al. 2013). For this reason, a chemometric approach to the data treatment is particularly useful. PCA (principal component analysis), HCA (hierarchical cluster analysis) and PLS (partial least square) regression are among the commonly used methods for NIR analysis. In particular, NIR spectra have been correlated with wood composition using PLS analysis (Jones et al. 2006; Kelley et al. 2004). For example, the use of calibrated near infrared spectroscopy has been used for predicting the chemical composition of wood samples such as loblolly pine (*Pinus taeda* L.) (Jones et al. 2006). Cellulose, hemicellulose, lignin and other wood components were determined by standard analytical chemistry methods, and on the base of these results, calibrations were developed for each chemical constituent using NIR spectra and PLS regression. PCA, HCA and PLS have also been used for qualitative and quantitative analyses of wood samples also in the region of medium IR (400–4000 cm^{-1}) permitting distinction between hardwoods and softwoods (Chen et al. 2010).

One of the aims of this study was therefore to identify spectral differences among wood samples resulting from chemical alteration, before and after anoxic treatment, by submitting infrared spectra to a chemometric treatment such as PCA. A complete colorimetric characterization of the surfaces was conducted as well.

Wood is characterized by hygroscopic behaviour, which affects all its basic properties: shrinkage/swelling, density, and mechanical properties (Tsoumis 1991). Dimensional changes are induced by variation in environmental RH, which may occur during treatment, and the magnitude of these changes may vary significantly depending upon wood direction and size (Hunt 2012). The related differential

volume variation due to shrinkage/swelling may result in the formation of cracks or detachment of the finishing layers, if present. The absence of superficial mechanical damages has also been monitored and evaluated by means of optical and scanning electron microscopy.

Materials and methods

Specimen preparation

Prior to anoxic treatment and between each diagnostic phase, all specimens were stored in controlled environmental conditions at 50 % RH until constant weight (initial weight), according to the standard protocol (UNI 1999). The mass variation in the specimens (related to variation in the moisture content) with respect to the initial weight was measured immediately after the anoxic treatment and repeated after 5 weeks of reconditioning at 50 % RH.

Three wood species, widely employed in the past for the production of artefacts and furniture, were selected for the evaluations: spruce (*Picea abies L. Karst*), poplar (*Populus alba L.*) and walnut (*Juglans regia L.*). $25 \times 20 \times 15$ mm^3 parallelepipeds were cut from the woods along the radial direction. Each specimen underwent a different superficial finishing procedure as reported in Table 1. A total of 26 specimens were prepared and tested: 10 spruce specimens, 10 poplar specimens and 6 walnut specimens.

The finishing materials (supplied by Bresciani Srl) were chosen to be representative of the highly variable finishes used in the cultural heritage and are listed as follows: glue (powder rabbit-skin glue in deionized water, volume ratio 1:14), beeswax, shellac, dammar resin, ketonic resin, gypsum ground (calcium sulphate hemihydrate "gesso di Bologna" mixed with animal glue), egg tempera (mineral ochre pigment with egg yolk), oil tempera (mineral ochre pigment with linseed oil), brown bole, oil gilding (24 k gold leaf applied with linseed oil).

Table 1 List of surface finishing treatments of the specimens

Wood	Finishing treatment	Specimen ref.
Spruce, poplar and walnut	Not treated (reference wood)	Nt
	Glue	g
	Glue + beeswax	Bw
	Glue + shellac	Sh
	Glue + dammar	Dam
	Glue + ketonic resin	Ket
Spruce and poplar	Glue + gypsum ground	Gy
	Glue + gypsum ground + egg tempera	Egg
	Glue + gypsum ground + oil tempera	Oil
	Glue + gypsum ground + bole layer + oil gilding	Gold

Specimens for the evaluation of O_2 permeability were prepared from spruce, poplar and oak wood (walnut specimens with dimensions suitable for the permeability measurement were not available). Three specimens of a purposefully designed shape and size (Fig. 1a, label A) for each different condition (as for wood species and superficial finishing) were prepared. Poplar specimens were cut along the three main directions, whereas oak and spruce specimens were cut only along the radial direction. An additional set of spruce specimens was also prepared with a reduced thickness of 3 mm. The detailed geometry and dimensions of the permeability specimens are reported in Fig. 1. All specimens were tested before treatment and, in the case of poplar and oak, after the application of glue finishing over the surface (1.7 ± 0.1 g of glue/specimen). A more complex stratigraphy was tested for poplar which included the application of a gypsum layer over the glue.

Anoxic treatment

The anoxic treatment was carried out in *dynamic mode* (Selwitz and Maekawa 1998). A high purity nitrogen gas flux generated by a N_2 Micro Vac system (97.5 %purity with max flux 4 NL/min, supplied by Claind srl) and accumulated into a 50-L stainless steel tank was continuously passed through a tightly sealed low-oxygen permeability transparent plastic bag (EVOH barrier film, Nitrex). The treatment was conducted for 6 weeks, which is the longest duration reported in previous studies for particularly resistant contaminating species (Selwitz and Maekawa 1998). An anhydrous N_2 flux was used in order to simulate the most dangerous treatment conditions, in order to enhance any possible alteration or damage of the specimens due to shrinkage.

Analytical techniques

Stereomicroscopic observation of the specimens' surface and polished cross section was made by means of a Leica M205C stereomicroscope, equipped with a Leica DFC290 digital camera.

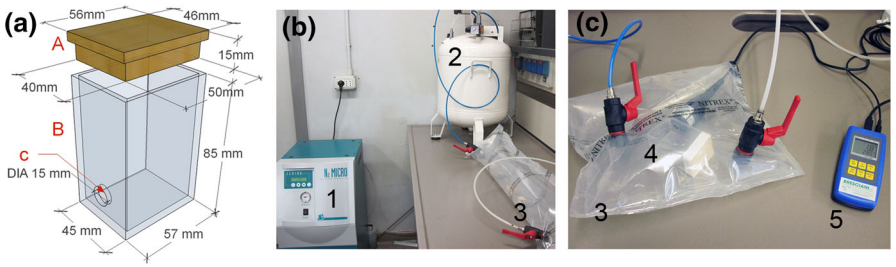

Fig. 1 Experimental set-up of the permeability measurements. **a** Wood specimens (*A*) and prismatic chamber (*B*) equipped with a hole to host the O_2 sensor (*C*). **b** Apparatus composed of N_2 generator (*1*) connected to the anoxic bag (*3*) through an accumulation tank (*2*); detail of the sealed anoxic bag during the test (**c**) of a wood specimen (chamber + wood specimen + sensor (*4*), connected to a data logger (5))

Colorimetric characterization before and after the anoxic treatment was performed (three measurements for each sample) by means of a Konica Minolta 2500d spectrophotometer equipped with a D65 illuminant at 10°, wavelength range between 360 and 740 nm. Measurements were elaborated according to the CIE L*a*b* standard colour system.

FTIR characterization was carried out by a Thermo Nicolet 6700 instrument coupled with a Continuum microscope, using a MCT detector in the spectral range 4000–600 cm^{-1} with a 4-cm^{-1} resolution. Measurements of the specimen's surface were taken in ATR mode using a Ge crystal. NIR analyses were conducted with a Smart NIR integrating Sphere, using an InGaAs detector in the spectral range 10,000–4000 cm^{-1} with a 4-cm^{-1} resolution. Three spectra for each sample were recorded. Data resulting from the FTIR and NIR characterization were analysed by principal component analysis (PCA). V-Parvus 2010 software [www.parvus.unige.it] was used for the calculation of principal component and data loadings.

ESEM observations were performed by an environmental scanning electron microscope Zeiss EVO 50 EP, equipped with an Oxford INCA 200—Pentafet LZ4 spectrometer in secondary electron mode.

Evaluation of oxygen permeability

The wood specimens were applied to a methacrylate prismatic chamber (Fig. 1a), in which the O_2 content was in equilibrium with the atmospheric one. The shape of the specimens was designed to ensure good contact with the chamber, and airtightness was ensured by sealing the specimen/chamber connection with low permeable sealing tape. The chamber was equipped with a circular hole to host an O_2 sensor (GMH 3691 residual oxygen metre, measurement range 0.0–25.0 % O_2 concentration, accuracy $\pm$0.2 %) connected to a data logger. The permeability system (specimen + chamber + sensor) was placed into a low-oxygen perme-ability plastic bag and underwent the anoxic treatment in the same condition previously described. The wood specimens acted as permeable diaphragms through which oxygen was able to flow from the chamber to the N_2-filled bag, according to the concentration gradient. The variation in O_2 concentration of the chamber was recorded over time with 5-min intervals and curves for different wood species, and superficial finishings were recorded accordingly. Each reported permeability curve results from the average values of three specimens (except for the specimen with gypsum-ground finishing which was only tested once). Measurements were taken until a 0.1 % O_2 residual concentration in the chamber was obtained, but, given the tendency of the curves to plateau, once very low oxygen concentration was reached, the 1 % concentration level was identified as the most convenient threshold to better discriminate the different permeability behaviours.

Results and discussion

Evaluation of oxygen permeability

The evaluation of wood permeability as measured by O_2 depletion under dynamic anoxic condition was preliminarily performed on poplar. The results are reported in Fig. 2a in which the O_2 concentration is expressed versus time. As far as the wood specimens are concerned (wood without finishing or before treatment), the curves highlight the considerable anisotropy depending on the wood direction, as a result of the specific cell structure and fibre arrangement. Along the longitudinal direction, the highest oxygen permeability is observed and the 1 % threshold is passed in <30 min. In this condition, permeability is promoted by the presence of elongated vessels, deriving from individual cells joined together, which are characterized by relatively large diameters and thin cell walls and appear as single pores in cross section (Fig. 2b) (Tsoumis 1991). To a minor extent, longitudinal permeability can also be supported by the ray cells, vertical parenchyma and fibres, laterally connected to the vessels through pits (Peralta 2001). Permeability along tangential and radial direction is far less effective as indicated by the time required to reach 1 % threshold, which is equal to 27 and 41 h, respectively. On both directions, the cell arrangement appears more compact after ESEM observation (Fig. 2c, d). The

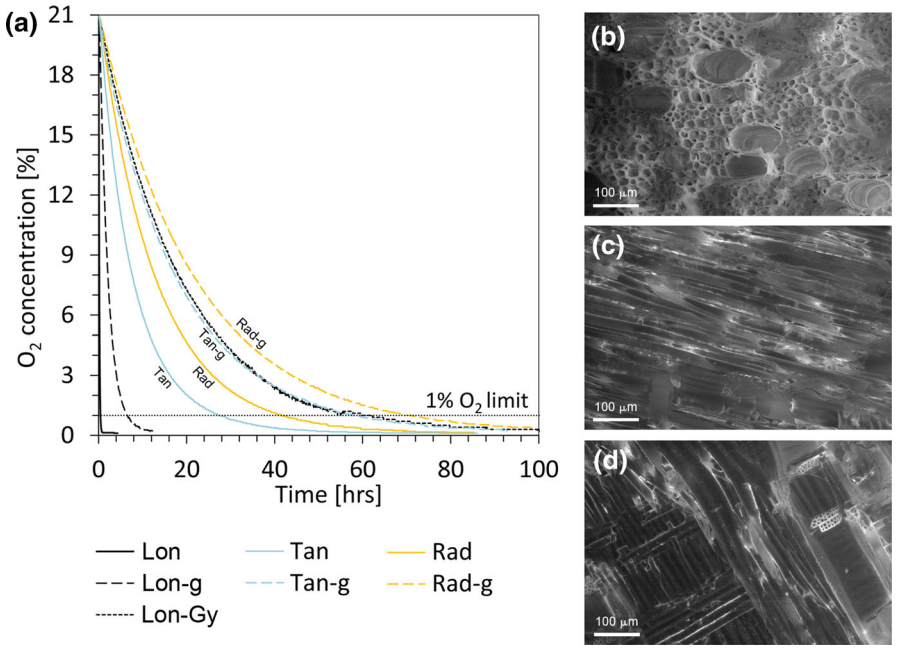

Fig. 2 O_2 permeability curves of poplar (**a**) under different finishing conditions (*g* glue, *Gy* glue and gypsum layer) and according to the different wood directions (*Lon* longitudinal, *Rad* radial, *Tan* tangential); ESEM images of poplar wood along the longitudinal (**b**), tangential (**c**) and radial (**d**) direction

large vessels are no longer available along the O_2 flux direction to sustain the gas migration; the wood microstructure is characterized by the presence of single-row ray cells along the tangential section (Fig. 2c) and of the typical perforation plates between the vessels along the radial section (Fig. 2d).

The O_2 migration velocity is dramatically altered by the application of surface finishings. It is worth noting that the objective of such treatments is indeed to modify the surface characteristics in order to enhance durability, by reducing the penetration of water, dust, pollutants and other environmental contaminants. This task has been traditionally achieved by applying a water-based solution of rabbit-skin glue on the wood surface (Rivers and Umney 2007) in order to partially clog the porosity (the so-called "turapori" treatment); hence, a reduction in wood permeability is expected. This is confirmed by the curves of wood after glue application which in all cases show a slower O_2 migration rate (dashed curves in Fig. 2a). The reduction in permeability is higher for the tangential and radial directions, which both show an almost comparable increase in the time to reach the 1 % threshold of about 30 h in comparison with the non-treated specimens. The glue application is able to reduce the porosity of the longitudinal section as well, but to a minor extent, corresponding to a 5-h increase. The permeability along this direction has also been studied after the application of a gypsum layer which simulates a typical ground preparation for painted panels. This additional thick layer (thickness ranging between 0.5 and 1 mm) is more effective than glue in reducing permeability, providing a final result of 60 h (55-h increase with respect to the glue finishing alone). This result is comparable to those of the tangential and radial surfaces after glue finishing (Fig. 3).

In Fig. 3, the permeability results for poplar are summarized and compared to oak and spruce specimens along radial direction. The bare wood specimens of oak and spruce have a similar final result below 30 h, to the former species characterized

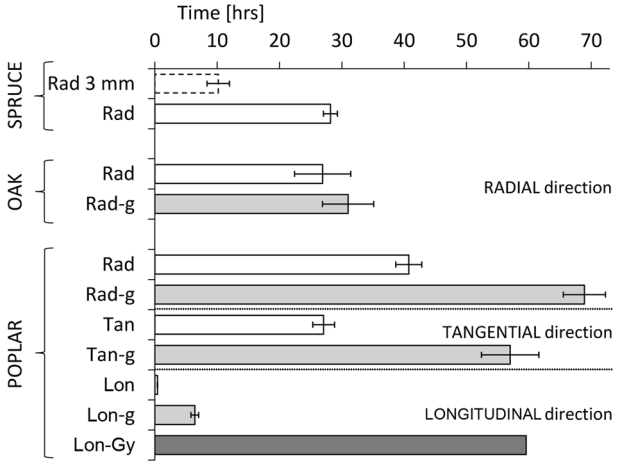

Fig. 3 Time to reach a 1 % oxygen concentration in the measurement chamber, according to the different species, wood directions and superficial finishing (*g* glue, *Gy* gypsum ground)

by a higher variability, and they both display a more permeable behaviour than poplar in the same condition. The application of the glue finishing to the oak specimens results in a very slight permeability reduction (+4 h). Finally, the influence of the specimen thickness was investigated for spruce wood along the radial direction. As a result of the variation from 3 mm thickness to 15 mm thickness, the time required to achieve the required oxygen migration almost tripled. Moreover, a reduced specimen thickness seems to enhance the measurement variability.

The overall result, summarized in Fig. 3, demonstrates that the time required to remove 99 % of environmental oxygen, and thus to bring the wood to actual anoxic conditions up to a 15 mm depth, may vary considerably from <1 h in the most permeable conditions (transverse section of poplar) to 60 h in case of unfavourable conditions with respect to wood direction and presence of pore-clogging finishing (radial section of poplar with glue finishing). It should be recognized that the addition of thick and low permeable layers, as in the case of gypsum ground, will further reduce the O_2 migration and therefore will result in longer treatment intervals.

Evaluation of the side effects and non-harmfulness of the anoxic treatment

A complete FTIR–ATR diagnostic survey was conducted on the specimens before and after the anoxic treatment. The results confirmed the total absence of any chemically induced alteration of the treated materials as a result of the anoxic procedure. The absorption peaks did not show any variation as for wavenumber, spectral features and intensity (spectra not reported). The same conclusions apply for the aesthetic appearance. With respect to colorimetry, the analyses always resulted in global colour variation values (ΔE) lower than 1.5, thus undetectable to the naked eye.

On the other hand, an alteration of the moisture content of the wood specimens is expected as a result of the anhydrous N_2 flux, and this may induce mechanical damage due to the consequent shrinkage of the material. The theoretical equilibrium moisture content of the specimens in laboratory condition is 9–10 %, which drops to 1 % after the treatment (Tsoumis 1991). The results of the related mass variations are reported in Fig. 4. The percentage mass variation is always calculated with respect to the initial weight of the specimens. As a result of the anoxic treatment, all specimens show an expected mass decrease related to the loss of humidity. The average values of the mass variation are −8 % for walnut, −7.2 % for poplar and −7.8 % for spruce (negative values indicate weight loss). As wood tends to reach equilibrium conditions with respect to the environmental conditions to which it is exposed, measurements were repeated after a 6-week post-treatment reconditioning at 50 % RH. The variation in the moisture content of the specimens was confirmed to be reversible for all species, as the values of ΔM % after reconditioning are all below −0.5 % with respect to the initial weight.

The occurrence of mechanical damage after the treatment was monitored by stereomicroscopic and ESEM investigations. Selected results of the specimens having the most complex stratigraphic structure are reported in Figs. 5 and 6. The

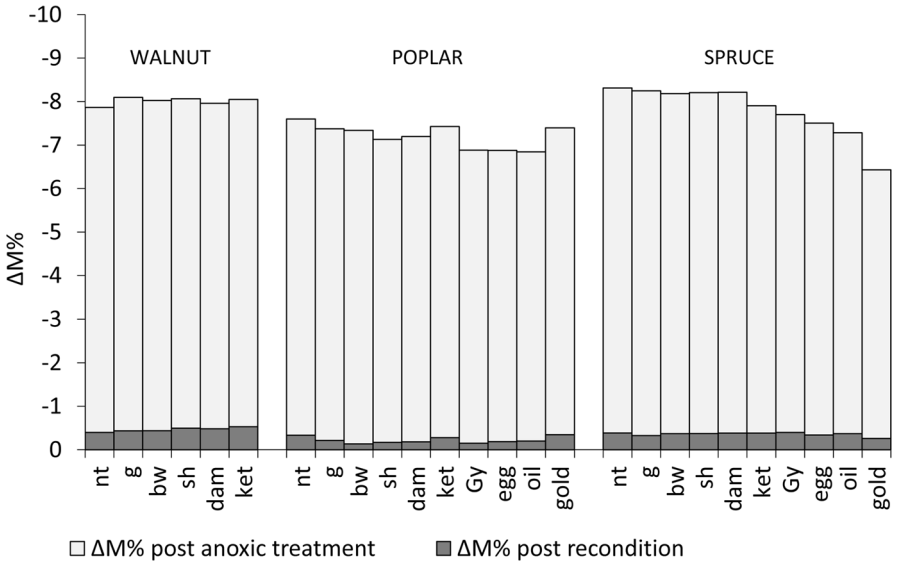

Fig. 4 Percentage mass variation in the specimens after the treatment and after reconditioning with respect to the initial weight in equilibrium condition (laboratory condition 50 % RH)

cross section of the poplar specimens with tempera finishing shows good adhesion of the white preparation layer (gypsum ground) to the wood substrate (Fig. 5a, b). The gypsum ground is characterized by an uneven distribution with several voids and cavities which are all preserved after the treatment. The overlapped ochre tempera layer has a lower thickness, around 100 μm, and a very regular distribution without visible cracks. The anoxic treatment did not cause any damage to the finishing: no evidence of newly formed cracks or fissure can be observed, and the adhesion between the different layers is completely preserved. Similar conclusion can be drawn after the observation of the painted surface (Fig. 5c, d). As the tempera layer ages, a typical pattern of diffuse microcracks (*craqueler*) is formed, which can be used as an indicator of the potential damage (Fig. 5c, d, white arrows). All the surface defects (white spots due to the uneven distribution of the tempera by brush) are unchanged after the treatment. In particular, the observation of the microcrack width and distribution at higher magnification indicates that the mass variation and the related shrinkage/swelling associated with the treatment have not caused any permanent damage (Fig. 5e, f).

The oil gilding represents the most complex finishing, considering the overall stratigraphy and compositional heterogeneity of the different layers. Moreover, the very limited thickness and minimal mechanical resistance of the superficial gold leaf provide an efficient indicator for the damage evaluation. As far as the cross section is concerned (Fig. 6a, b), it can be observed that no significant variation occurred as a result of the treatment, neither to the general adhesion between the layers, nor to the cohesion of the finishing materials. The existing microfissure at the wood/gypsum interface is totally preserved as for its extension and width (Fig. 6a,

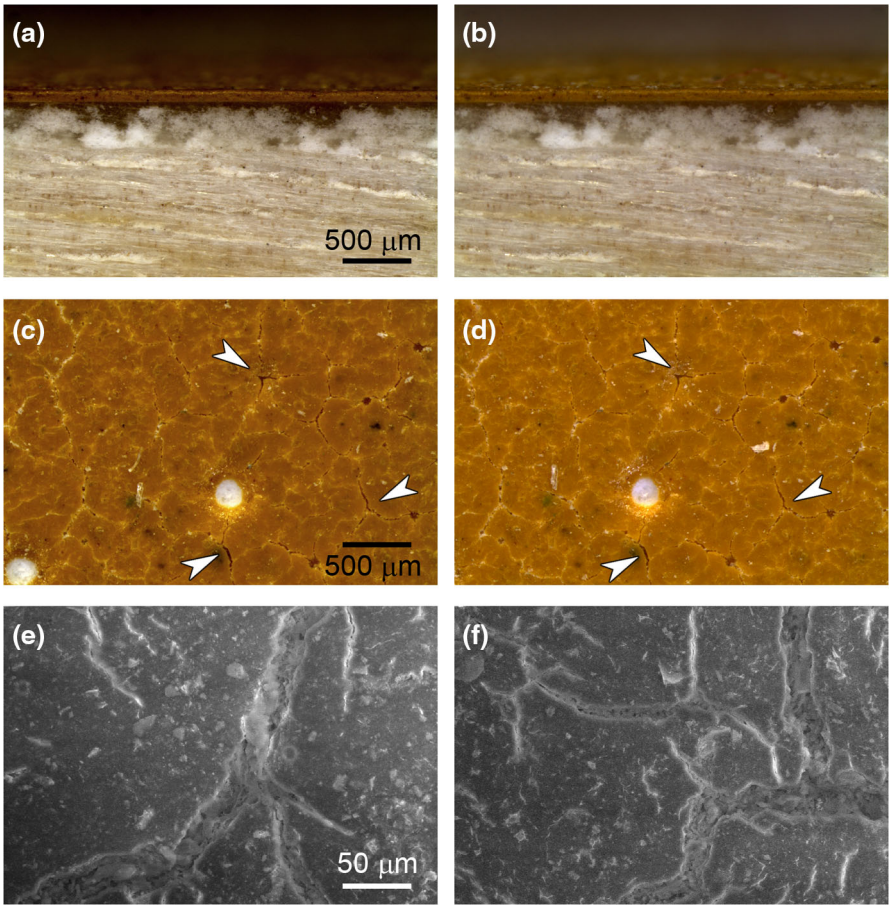

Fig. 5 Comparison of the stratigraphy and surface of the egg tempera specimen before (*left column*) and after (*right column*) the anoxic treatment. Stereomicroscopic (**a**–**d**) and ESEM (**e**, **f**) documentation

b, white arrows). The same applies to the bole layer. The gold leaf is fairly adherent to the substrate, and ESEM observation of the surface confirms the absence of cracks and discontinuities induced by mechanical stress during the anoxic treatment (Fig. 6c, d).

FTIR–NIR characterization

For what concerns the NIR region, at first, in order to highlight the differences among the finishings applied to the three species (as reported in Table 1), the spectra acquired before, after the anoxic treatment and after the reconditioning period in the range 10,000–4000 cm^{-1} were considered. It is worth noting that the

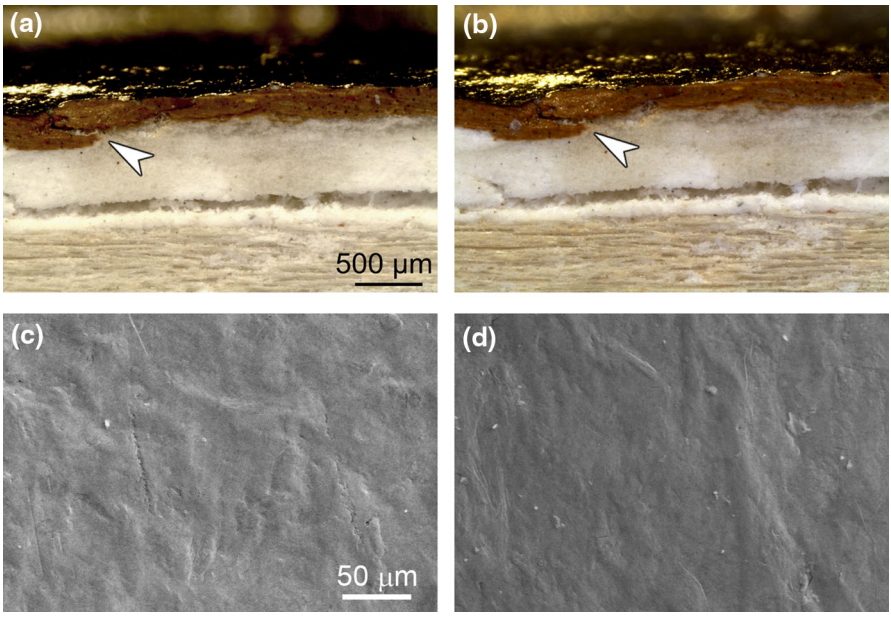

Fig. 6 Comparison of the stratigraphy and surface of the oil gilding specimen before (*left column*) and after (*right column*) the anoxic treatment. Stereomicroscopic (**a**, **b**) and ESEM (**c**, **d**) documentation

most distinctive spectral information of the first overtone and combination bands is concentrated in this region (Fujimoto et al. 2012).

Since it has been demonstrated (Jurado-López and de Castro 2004) that NIR spectroscopy in combination with chemometric techniques (PCA, principal component analysis, and HCA, hierarchical cluster analysis) is a powerful tool in order to distinguish among binding media in the cultural heritage field (Sarmiento et al. 2011; Sciutto et al. 2014), this approach was preliminarily applied to the entire specimen set (considering all untreated woods, finishing and the three treatment conditions). It is worth noting that the resulting first two PCs together account for 89 % of total variability. The score plot (Fig. 7, PC1 vs PC2,) allows for qualitatively distinguishing among different groups of samples according to the type of finishing. In particular, the three different untreated wood species are clearly identified as well as separated groups by the score plot (Fig. 7, dotted circles). Each group contains three specimens corresponding to all the treatment conditions (before anoxia, after anoxia and after reconditioning), but PCs are not able to discriminate between them. The finishings based on resins or glue usually form a very thin superficial layer, as they tend to penetrate within the wood porosity. As a result, in the score plot, such specimens are all grouped and not well separated from the untreated woods. A distinctive group of samples comprises all the specimens treated with beeswax as a result of the increased thickness of this finishing. For this group, the information related to the wood species is retained as well (Fig. 7, dashed circles). As previously reported for the cross-sectional observation, the specimens with gypsum layer alone or with the overlapped egg/oil tempera are characterized

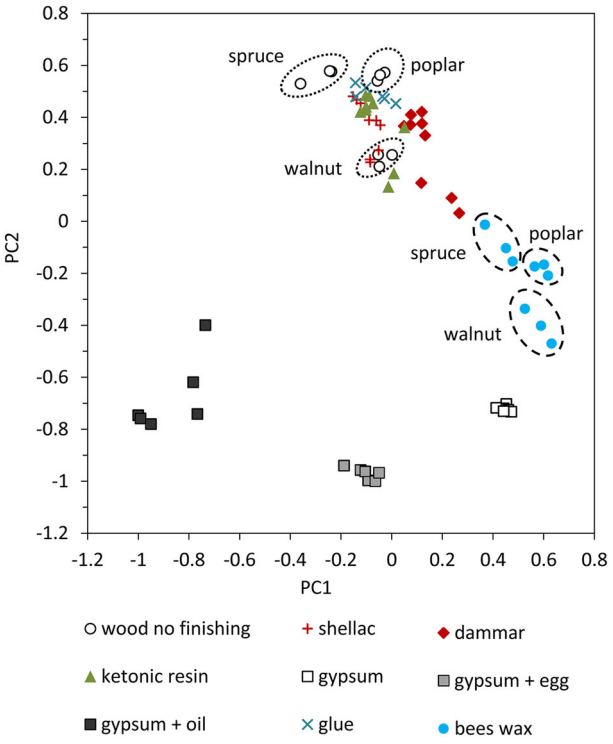

Fig. 7 Score plot of NIR spectra of all specimens before the anoxic treatment (PC1 accounts for 73.09 % and PC2 accounts for 15.87 % of total variance, respectively)

by the highest thickness. The spectral features of these organic materials prevail over the typical signals of wood. These specimens are therefore well separated, and in the case of gypsum and egg tempera layers, the information related to the wood species cannot be retrieved.

Since, as stated before, wood is a complex material (due to its chemical composition and to its anisotropic nature) and considering that the NIR region is particularly rich of information, it is not easy to assign all the signals of a specific spectrum and to distinguish the different wood components. Actually, the NIR spectra of the three woods considered in this study (walnut, poplar and spruce), without any finishing and before the anoxic treatment, appear rather similar (Fig. 8). Peak assignment of wood components has been recently summarized in a review (Schwanninger et al. 2011).

It is important to emphasize that the present work was mainly focused on the bands related to water, since one of the aims is to try to distinguish differences among the specimens before and after anoxic treatments, resulting from variation in the moisture content. Two main peaks due to water can be highlighted in Fig. 8. According to Zhang et al. (2011), these two peaks can be related to the combination of O–H stretching and O–H deformation of water:

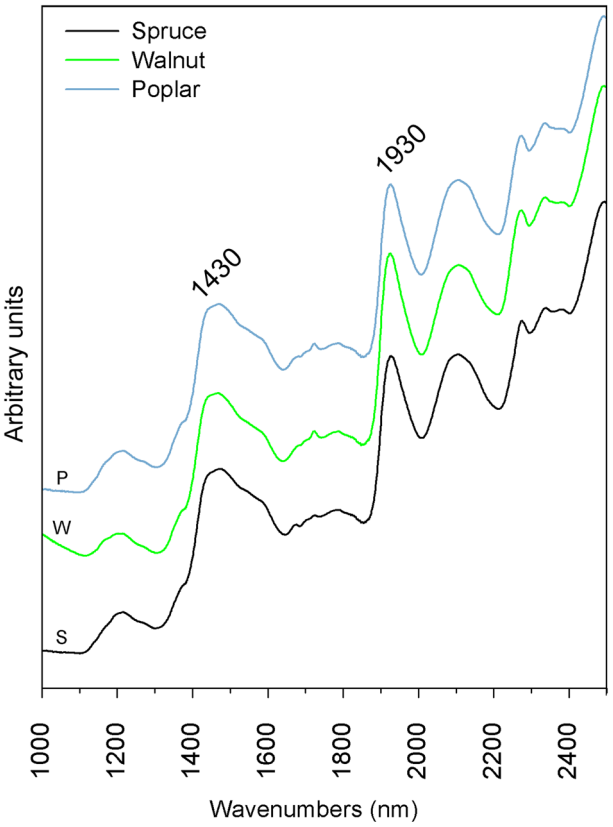

Fig. 8 NIR spectra of the three different wood essences examined in the study (*S* spruce, *W* walnut, *P* poplar) before any treatment

- At about 1430 nm (7000 cm^{-1}), the first overtone of the fundamental O–H stretching vibration due to amorphous region in cellulose and to water (Fujimoto et al. 2012; Schwanninger et al. 2011);
- At about 1930 nm (5100 cm^{-1}), an absorption band due to combination of O–H stretching and O–H deformation.

Another peak observable at 1220 nm (about 8200 cm^{-1}) is due to a O–H stretching vibration, while the quite intense peak at about 2100 nm is due to O–H stretching + O–H deformation of cellulose and also to C–H stretching and C=C stretching due to lignin and extractives (Schwanninger et al. 2011).

Most of the work done on NIR analysis of wood samples and wood products has shown that a simple spectral preprocessing of the data, such as the first or second derivatives (Mehrotra et al. 2010; Riggio et al. 2014), allows a better discrimination of the absorption bands (Fujimoto et al. 2012). This approach has been applied to study the variation in the moisture content induced by the treatments. In Fig. 9, the second-derivative NIR spectra of a single species (bare specimen of spruce) before

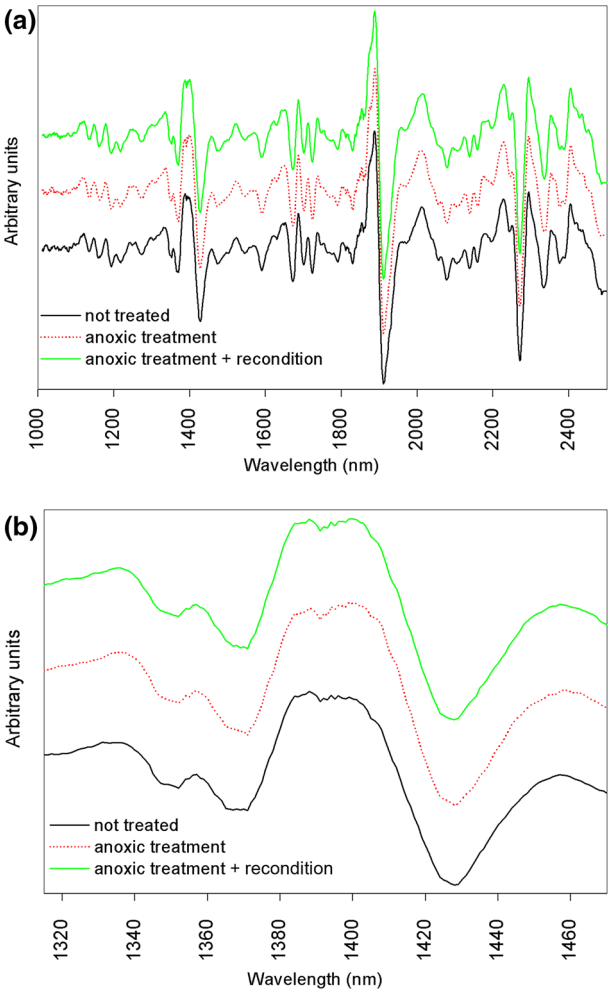

Fig. 9 Second-derivative NIR spectra for a single species: spruce (**a**) and detail of the 1320- to 1460-nm region (**b**)

the treatment, after the anoxic treatment, and after the anoxic treatment plus a reconditioning period are reported.

Second-derivative spectra show interesting features in particular in the range 1315–1470 nm (7600–6800 cm^{-1}) (Fujimoto et al. 2012). The band at 1366 nm (7320 cm^{-1}) can be assigned to the combination of CH stretching and deformation vibration due to cellulose (Fujimoto et al. 2012; Schwanninger et al. 2011), while the band at about 1430 nm (about 7000 cm^{-1}), as already observed, is due to both cellulose and water and is particularly evident in the second-derivative spectra (Fujimoto et al. 2012). It is worth noting that after the anoxic treatment in the most stressing conditions (anhydrous anoxic atmosphere), no evident difference in water

content is detected by NIR and the same happens after the reconditioning, thus confirming that the moisture content variation is limited and reversible.

In the literature, some authors (Gierlinger et al. 2004; Jones et al. 2006; Tsuchikawa et al. 2003) have demonstrated the usefulness of FT-NIR analysis for the classification of wood species, used in combination with multivariate statistical treatments. In this study, in order to better evidence the differences among the samples in particular as concerns water content, the NIR spectra acquired on the three woods without anoxic treatment, after anoxic treatment and after anoxic treatment/reconditioning were submitted to PCA (3 replicas of each species/ treatment were tested). The differences among the species, which could not be retrieved in Fig. 7, here can be better evidenced since the specimens with the finishing treatments have been excluded.

The score plot (PC1 vs PC2) in Fig. 10a reports the second-derivative results considering the entire spectral range. The PCs allow to particularly evidence the difference among spruce and the other two woods. This is due to several factors, including the fact that while spruce is softwood, poplar and walnut are hardwoods. This corresponds to some differences in the chemical constituents of wood, as highlighted by Schwanninger et al. (2011), who state that hardwoods have a higher content of hemicellulose. In Fig. 10a, the first two PCs together account for 62 % of total variability.

Subsequently, in accordance with what is suggested in the literature, the second-derivative spectra were submitted to PCA treatment, selecting the two following more informative spectral ranges with respect to the moisture content: 1300–1500 nm and 1800–2000 nm. The first two PCs in this case account for 74 %. From Fig. 10b, it is observable how a separation among the samples depending on the treatment is now present: the three essences not treated are grouped on the bottom, while the samples

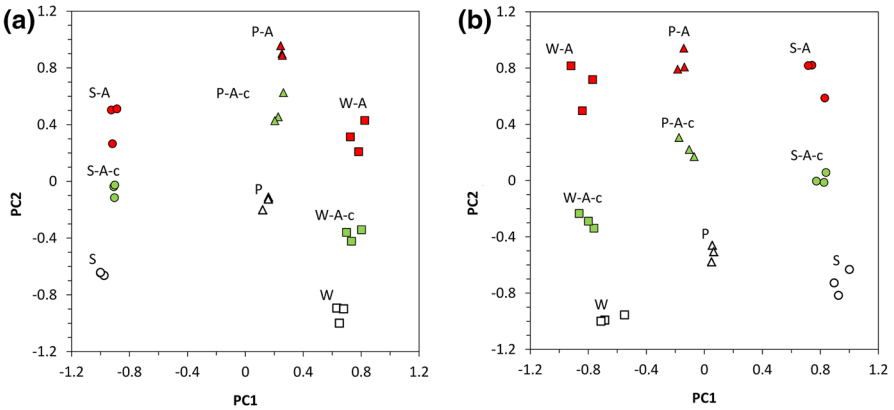

Fig. 10 Score plot of second-derivative of NIR spectra of poplar (P), spruce (S) and walnut (W) specimens with no finishing in the entire spectral range (**a**) and in selected regions (**b**). *Markers* indicate wood specimens: before treatment (no suffix); after the anoxic treatment (suffix "A"); and after the treatment and reconditioning period (suffix "A–c"). PC1 accounts for 49 %, and PC2 accounts for 12 % of total variance, respectively (**a**); PC1 accounts for 48 %, and PC2 accounts for 27 % of total variance, respectively (**b**)

which appear to be more distant are those after the anoxic treatment; finally, the samples after anoxic treatment but reconditioned are closer to the non-treated samples demonstrating that these woods tend to regain equilibrium conditions similarly to that of their initial status. These results further confirm that wood undergoes a limited and almost completely reversible dehydration as a result of the anoxic treatment. Moreover, selected regions of the NIR spectra allow a better discrimination of the woods that are clearly identified by PC1.

Conclusion

The use of anoxic atmosphere for the preservation of wooden objects from insect infestation proved to be non-harmful to the treated surfaces from the aesthetic, chemical and microstructural points of view. Despite the extremely stressful anhydrous testing condition, unlikely to be used in the conservation practice but adopted in order to highlight any possible degradation mechanisms, no significant alteration of the specimens was observed. Surface colorimetric measurements of the different specimens confirmed the absence of fading and discolouration effects. Even in the presence of a complex stratigraphy, the finishing material showed neither signs of detachment between the applied layers and the wood substrate, nor crack formation as a result of possible shrinkage after the variation in moisture content. The chemical stability of the finishing materials has been confirmed.

The data analysis of NIR spectra successfully discriminated among the species belonging to softwoods (spruce) and hardwoods (poplar and walnut), and if the most informative spectral regions are considered, single species discrimination can also be achieved. Moreover, NIR allows the monitoring of the treatment effects with respect to the variation in moisture content. PCA showed that, although humidity variations occurred as a result of the anoxic treatment, their magnitude is limited and they are almost completely reversible after a reconditioning period in standard RH conditions.

A simplified method for the evaluation of wood permeability (with respect to oxygen migration) has been set up and tested. The O_2 permeability is confirmed to be strongly influenced by the wood microstructural features, as a result of their highly oriented directional distribution, with the longitudinal values being much greater than those belonging to the tangential and radial directions. The presence of superficial layers which may delay the migration of oxygen has been simulated, and it is proven that a strong reduction in permeability, up to 60 h in the test, occurs in the presence of complex and thick finishings, thus retarding the achievement of anoxic conditions in the bulk of the material. These results highlight the need to properly evaluate the permeability of real objects while defining the treatment conditions, in order to ensure the most effective disinfestation result. It is worth noting that the permeability test was performed on small-sized and non-aged specimens. This may lead to underestimation of results with respect to actual objects which are characterized by the presence of hollow spaces, cracks and discontinuities resulting from ageing and degradation. The influence of larger dimensions should be taken into account as well, as the potential mechanical damage is influenced by the

high anisotropy. The study of naturally aged wood objects as well as of larger wood specimens should be further investigated.

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