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Nanoparticle-based consolidants and natural biocides for waterlogged archaeological wood. Challenges in testing new materials with innovative techniques

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Short Abstract

Waterlogged archaeological wood comes from submerged sites (in lake, sea, river or wetland) or from land waterlogged sites. Even if the wooden object seems to have maintained the original size and shape, the wood is more or less severely decayed because of chemical and biological factors which modify the normal ratio of cellulose and lignin in the cell wall. Drying procedures are necessary for the musealization but potentially cause severe shrinkages and collapses. The conservation practices focus not only on removing water from wood but also on substituting it with materials able to consolidate the degraded wood cell walls, like polymers, sugars or resins.

As long as wood is kept waterlogged anaerobic or almost anaerobic conditions are maintained and the biological degradation processes are very slow, but during excavation and conservation treatments wooden artifacts can be subjected to faster decay forms. For this reason, biocides are used during conservation and restoration stages to prevent wood degradation.

Aim of the PhD project was to test bio-inspired products as alternatives to the chemicals currently used for the preservation and restoration of waterlogged archaeological wood. For this purpose, three different nano-scale consolidants were tested: lignin nanoparticles (LNPs) obtained from beech wood *via* a non-solvent method involving dialysis; bacterial nanocellulose (BC) obtained from cultures fed with agro-alimentary waste; cellulose nanocrystals (CNCs) chemically extracted from native cellulose. Waterlogged archaeological wood samples of different species (oak, elm, stone pine and silver fir) characterized by different levels of degradation were impregnated with the consolidants. The treatments efficiency was evaluated in terms of macroscopic observation of treated samples, retention of impregnation products (Y), anti-shrink efficiency (ASE), and equilibrium moisture content (EMC). The results obtained for the three consolidants showed substantial differences: LNPs and CNCs penetrated only about a millimeter inside the treated wood, while BC formed a compact layer on the surface of the cell walls throughout the thickness of the samples. In spite of successful BC penetration, physical evaluation of treatment efficiency showed that BC nanoparticles did not obtain a satisfying consolidation of the material. Based on the reported results more focused test protocols are optimized for future consolidation experiments.

Three essential oils (cinnamon, wild thyme and common thyme) were tested as alternative, natural biocides. The effectiveness of the oils was firstly evaluated *in vitro* in term of minimum inhibitory concentration (MIC) on fungal spores of four cellulolytic strains (*Chaetomium* sp., *Fusarium* sp., *Aspergillus japonicus*, and *Stachybotrys chartarum*). Then, their biocidal activity was tested against the fungal mycelium of the same strains grown in plate and the whole microbiota present in waterlogged archaeological wood and storage water. The results showed that 1% can be considered the MIC for the three oils. At this concentration, the EOs allow to obtain a vitality decrease by almost 100% both on plate grown mycelium and on waterlogged wood microbiota. Even if the results obtained for the three oils are very close, the best values in all the tests were registered for cinnamon essential oil. In a second step, this oil was used as a biocide during the consolidation of waterlogged wooden poles recovered from the excavation of the ancient port of Naples. In this test the vitality decrease registered ranged between 99% and 100%.

The oils effectiveness was evaluated quantifying the cellular ATP through bioluminescence and studying the changes produced in the bacterial and fungal communities' composition through Next-Generation Sequencing (NGS).

Keywords

Lignin nanoparticles (LNP), bacterial cellulose (BC), cellulose nanocrystals (CNC), essential oils (EOs), Next-generation sequencing (NGS), ATP bioluminescence

Extended abstract

The European standard EN16873 (2016) (Conservation of cultural heritage - Guidelines for management of waterlogged wood on terrestrial sites of archaeological significance) defines waterlogged archaeological wood as: "wood whose structure has been filled with water through the sustained inclusion in a water saturated environment". This wood comes from submerged archaeological sites (in lake, sea, river or wetland) or from land waterlogged sites. Generally, waterlogged wooden artifacts preserve their original size and shape but

often they undergo severe cell wall decay due to chemical and biological factors. The pH, the salinity of water permeating the sediments and the chemical nature of sediment (Hedges, 1990) together with the action of biological degraders (e.g. erosion and tunnelling bacteria and soft rot fungi) (Björðal et al., 1999) affect the wood causing a more or less severe mass loss and an increased porosity and permeability that leads to a spongy and weakened material. Drying waterlogged archaeological artifacts is necessary for musealization but it is always a high risk procedure because it could cause severe shrinkages and collapses. Thus, the conservation practices focus not only on removing water from wood, but also on substitute it with materials able to consolidate the degraded cell walls with the two main aims of stabilise shape, size and proportion of the artifact and enable it to withstand the future preservation environment (Grattan and Clarke, 1987). By now, the most used consolidants are polymers (e.g. PEG), sugars (e.g. lactitol) or resins (e.g. Kauramin) (Christensen et al., 2012; Imazu and Morgôs, 1997; Unger et al., 2001). Nano-based consolidants have rarely been tested before on waterlogged archaeological wood and only the work by Christensen et al. (2012) showed the potentiality of cellulose nanoproducs.

As long as wood is kept waterlogged, anaerobic or almost anaerobic conditions are maintained and the biological degradation processes are very slow, but during excavation and conservation treatments wooden artifacts can be subjected to faster decay forms (Björðal and Nilsson, 2002). For this reason, biocides are used during conservation and restoration stages to prevent wood degradation. Nowadays the biocides most frequently used are chemical preservatives (e.g. quaternary ammonium salts, derivatives of the isothiazolone class, etc.) (Unger et al., 2001). These substances have environmental sustainability and safety problems and for this reason their use should be limited. The antimicrobial activity of natural products (essential oils and plant extracts) is nowadays well known and many studies showed that some of these substances (e.g. essential oils from lemon grass, rosemary, tea tree, cinnamaldehyde, eugenol, thymol) have effect against wood degrading microorganisms (Singh and Singh, 2012). Even if some studies tested natural products for the conservation of wooden artifacts (Axinte et al., 2011; El-Gamal et al., 2016), they have never been used in protecting waterlogged wood.

Aim of the present project was to test bio-inspired products as alternatives to the chemicals currently used for the preservation and restoration of waterlogged archaeological wood. For this purpose, three nano-scale consolidants (lignin nanoparticles (LNPs), bacterial nanocellulose (BC), and cellulose nanocrystals (CNC)) and three essential oils (cinnamon, wild thyme, and common thyme) were tested.

Materials and Methods

Waterlogged wood consolidation

Three different nanoparticles were tested as consolidants of waterlogged archaeological wood: lignin nanoparticles (LNPs), bacterial nanocellulose (BC) and cellulose nanocrystals (CNCs). LNPs were obtained from beech wood (*Fagus sylvatica* L.) from the Cimini Mountains in Lazio region, Italy (Piangoli Legno SNC, Soriano nel Cimino, VT). They were produced applying the “anti-solvent procedure” on acidolysis lignin as reported in Zikeli et al. (2018). The obtained LNPs are hollow, sphere-shaped particles with diameters ranging between 40 and 120 nm. BC was provided by Biofaber srl (Italy). It was obtained by fermentation of agro-alimentary waste, in accordance with Pal et al. (2017). It had never been studied before, so morphological characterization of BC spray-dried on a stub was performed on a Zeiss (Sigma VP; Carl Zeiss, Germany) FESEM. CNCs were supplied by CelluForce (Montreal, Canada). CelluForce NCC® is a cellulose hydrogen sulphate sodium salt, provided as a spray dried powder, white in color. The CNCs are spindle shaped crystals, 2.3-4.5 nm in diameter and 44-108 nm in length.

Consolidation tests were performed on 47 blocks of waterlogged archaeological wood obtained from artifacts recovered from the two archaeological sites of Isola Sacra (Fiumicino, Italy) and the ancient port of Neapolis (Naples, Italy). Before the beginning of consolidation procedures the wood species were identified and the state of preservation of the material was characterized by micro-morphological and physical analyses (parameters: Maximum water content, MWC (%), Residual density, RDb (%)).

During the consolidation procedure samples were completely soaked in an impregnation bath containing the nanoparticle-based consolidants for one month. LNPs' bath was obtained adding the nanoparticles to deionised water up to a concentration of 5 mg/ml. The BC bath was prepared adding 1% of Tween20 and 1% cinnamon oil to deionised water. The obtained solution was sonicated for 1 h and then 5% of BC was added. To make the suspension stable and homogeneous it was shook on a magnetic shaker for 1 h. To obtain a liquid suspension of CNCs, 3% of powdered crystals were added to deionised water and shook on a magnetic shaker for 1 h. ten blocks were used for each consolidant to be tested, the remaining seventeen blocks were kept as untreated controls, freeze- or air-dried.

After the treatment the samples were froze (-20 °C) and freeze-dried. A first evaluation of the efficacy of the treatments was obtained by macroscopic observation of the samples. Color, shape, presence of openings and consistency were observed. The penetration of the nanoparticles inside the wood tissues was evaluated by examining gold sputter-coated sections of the treated samples under Scanning Electron Microscope (Zeiss EVO 60). Furthermore, the retention of consolidants (Y) was calculated following the procedure reported by Giachi et al. (2010), the dimensional variations were evaluated and the percentage shrinkage, S, and the anti-shrink efficiency (ASE) were calculated. To evaluate the effect of the different consolidation treatments with respect to moisture absorption the equilibrium moisture content (EMC) was determined.

Waterlogged wood preservation

Three essential oils (EOs), provided by Flora srl, were tested during the experimentation: *Cinnamomum zeylanicum* Blume (cinnamon bark, C), *Thymus serpyllum* L. (wild thyme, WT) and *Thymus vulgaris* L. (common thyme, CT).

The effectiveness values of four different concentrations (3%, 1%, 0.7% and 0.5%) of the selected EOs was estimated by Minimum Inhibitory Concentration (MIC). The oils, diluted in a solution 50/50 v/v of deionised water and ethanol, were sprayed on the surface of the culture medium (Malt Extract Agar (MEA) Sigma-Aldrich) inoculated with four different cellulolytic fungal strains: *Chaetomium* sp., *Fusarium* sp., *Aspergillus japonicus* and *Stachybotrys chartarum*. The antifungal activity was assessed by the presence/absence of fungal growth.

The biocidal activity of the established MIC was firstly evaluated by spraying the 1% oil suspension on grown not-sporogenous mycelium of the same strains. Control plates were treated with a solution of deionised water and ethanol. The efficacy was evaluated by quantifying the ATP bioluminescence before the oil application and after seven days from the treatment. Secondly, the biocides effectiveness was tested against the microbiota present inside waterlogged archaeological wood samples. Five subsamples were obtained by cutting a wood block with a sterile blade and were placed in sterile containers with 20 ml of the water in which the wood was stored until the test began. Three of the five subsamples were treated with cinnamon (C), wild thyme (WT) and common thyme (CT) oil: 1% of the EOs dissolved in ethanol was added to the storage water. The fourth fragment's water was treated with the same amount of ethanol used to dissolve the oils (E). The last subsample was kept as an untreated control (NT). The biocidal activity was evaluated through the quantification of ATP bioluminescence and by examining the total genomic DNA extracted from the wood via Next-Generation Sequencing (NGS). The tests were performed both on small pieces of wood and on aliquots of storage water. Subsequently, another experimentation was carried out during an actual restoration intervention on wooden poles, recovered from the excavation area of Piazza Municipio in Naples, which were consolidated into four tanks containing lactitol-trehalose impregnation baths. After one month from the beginning of the consolidation treatment a conspicuous microbial growth was observed in all the containers so it was decided to treat the baths with a biocide. Two of the tanks were treated with cinnamon essential oil (1%) dissolved in ethanol, Preventol RI80, a biocide currently use in restoration, was added to the two remaining tanks, 1% in concentration, as control. The biocidal activity was evaluated through the quantification of ATP bioluminescence and cultural analyses.

The procedures followed for ATP bioluminescence quantification and total genomic DNA extraction and sequencing were defined during preliminary tests.

Results and Discussion

Waterlogged wood consolidation

Five wood species were identified: stone pine (*Pinus pinea* L.), silver fir (*Abies alba* Mill.), cypress (*Cupressus sempervirens* L.), elm (*Ulmus* sp.), and holm oak (*Quercus ilex* L.). Micro-morphological analyses of wood thin sections allowed for observing the microbial decay of the tissue which was mainly ascribed to erosion bacteria, even though fungal hyphae and spores were observed in several sections. The physical parameters used to assess the decay showed different levels of degradation. The highest MWC values were recorded for the holm oak while stone pine was the best preserved of the analyzed woods.

The macroscopic observation of treated samples and the comparison between consolidated and un-consolidated wood allowed to observe that in contrast to air-dried controls, all treated samples and freeze-dried controls apparently maintained the original shape and no dimensional differences were perceivable by naked eye between differently consolidated blocks and freeze-dried controls. On the contrary, the consolidation treatments deeply affected wood color and aspect (Fig. 1). LNPs treated samples resulted in dark brown color

and a fine brown powder was present on the sample surface, even after freeze-drying. BC consolidated blocks were whitish or yellowish in color, throughout the block thickness. Finally, CNCs treated samples showed a color similar to the controls, but a thin, colorless film was present on almost all the surface of the blocks. That film was easily removable by touching the wood.

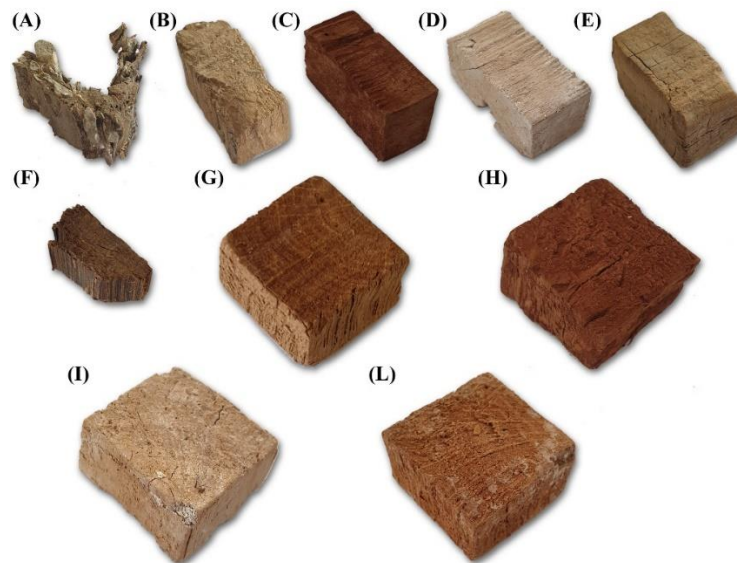


Fig. 1 Aspect of selected treated blocks and controls. (A-E) softwood blocks; (F-L) hardwood blocks. (A) Air-dried control – silver fir; (B) Freeze-dried control - cypress; (C) LNPs - stone pine; (D) BC - stone pine; (E) CNCs – cypress; (F) Air-dried control - elm; (G) Freeze-dried control - holm oak; (H) LNPs- holm oak; (I) BC - holm oak; (L) CNCs - holm oak

SEM analyses of the treated wood samples allowed observing how the nanoparticles had penetrated inside the wood tissues. The analysis of a control un-consolidated sample showed that in the cross section (Fig. 2 A) cells lumen were empty and in several cases the secondary cell wall appeared detached from the middle lamella. In radial section (Fig. 2E), the vessel and fiber walls appeared smooth and the pits were well visible. In LNPs treated samples a thick layer of nanoparticles was observed on the external surface of the sample (Fig. 2B) but only few particles had penetrated inside the vessels up to a depth of ca 200 μm (Fig. 2F). In BC treated wood the secondary detached cell walls were consolidated and appeared as tubes coming out from the observed internal cross section (Fig. 2C). A compact layer of BC was observed on the surfaces of vessels and fiber's walls in the whole length of the sample (Fig. 2G). Finally, CNCs had penetrated inside the wood forming a more or less homogeneous layer on the vessel's walls up to a depth of ca 1.3 mm (Fig. 2D, H).

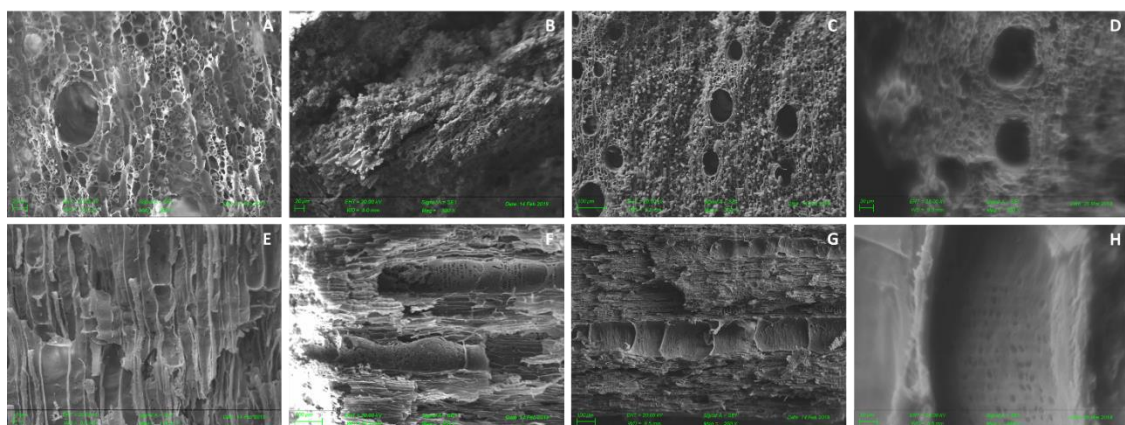


Fig. 2 SEM micrographs of wood sections. (A-D) cross sections; (E-H) internal radial sections. (A, E) control sample; (B, F) LNPs treated wood; (C, G) BC treated wood; (D, H) CNCs treated wood

The retention of consolidant (Y) was lower than 20% for almost all the analyzed blocks and in some cases negative values were obtained. Considering that the parameter Y is calculated as the increase of weight of treated wood with respect to the same untreated wood, the low and negative values for LNPs and CNCs treated

samples can be explained assuming that the change in weight due to the nanoparticles penetrated in the outer part of wood blocks was lower than the method's intrinsic error. In spite of the good penetration observed through SEM, the Y values obtained for BC were very similar to those of LNPs and CNCs. Considering the lightweight of nanocellulose materials, these results can be explained assuming that also in this case the weight increase due to the consolidant was negligible.

The anti-shrink efficiency (ASE) values calculated showed that the predominant anti-shrink efficiency must be attributed to the freeze-drying procedure. The contribution of the consolidants to the shrinkage suppression was not substantial. The ASE values, with only one exception, ranged between a minimum of 2% and a maximum of 46%.

The results obtained for equilibrium moisture content (EMC) showed that the effect of the different consolidation treatments with respect to moisture absorption was negligible. In general, the EMC values obtained for blocks belonging to the same archaeological source but treated with different consolidants varied slightly and not significantly. It is interesting to note that in the RH range used for conservative purposes (35-85%), the average EMC values for all blocks never exceeded the 18% considered as the threshold for the risk of biological attacks.

Waterlogged wood preservation

The Minimum Inhibitory Concentration (MIC) was set at 1% for the three oils. The bioluminescence assays and the cultural analyses carried out to evaluate the biocidal activity of the EOs' MIC on the mycelium of the selected fungal strains and on waterlogged wood microbiota showed that the three oils allowed obtaining a neat decrease in the microbial vitality. The results were very homogeneous for *in vitro* tests (Tab. 1) while slight differences among the oils were registered for the tests on wood (Tab. 2). These could be attributed to a different effectiveness of the oils against complex microbial communities probably linked to their composition.

The NGS showed that the distribution and relative frequencies of bacterial taxa were affected by the different treatments while the fungal community composition was more or less homogeneous for all the analyzed samples. These results let hypothesizing that the biocidal treatments had differential effects on the bacterial taxa but not on the fungal species.

Tab. 1. Percent decrease of the amount of ATP extracted from the fungal mycelia after the treatment with EOs.

	ATP Decrease (%)			
	C	WT	CT	WE
<i>Chaetomium</i> sp.	99.99	100	100	99.94
<i>Fusarium</i> sp.	100	99.99	100	-43.67
<i>Aspergillus japonicus</i>	99.99	99.97	99.99	37.02
<i>Stachybotrys chartarum</i>	100	100	98	93

Tab. 2. Percent decrease of the amount of ATP extracted from storage water and waterlogged archaeological wood samples after the treatment with EOs.

	ATP Decrease (%)				
	C	WT	CT	E	NT
Water	100	95.44	95.38	-116.52	79.25
Wood	99.95	98.18	99.53	17.23	57.85

The test carried out by using cinnamon essential oil during the restoration of wooden artifacts allowed comparing the biocidal potential of this oil with respect to a biocide currently used in the wood preservation (Preventol RI80). The test showed that cinnamon oil is slightly less effective than Preventol (Tab. 3), but the results were very satisfying and laid the base for the possible use of EOs in this field.

Tab. 3. Percent decrease of the amount of ATP and Colony Forming Units (CFU) after the biocidal treatment.

Tank	Biocide	ATP Decrease (%)	CFU Decrease (%)
V2	Preventol RI80	99.97	99.99
V3	Preventol RI80	99.74	100

V4	C	98.97	99.98
V5	C	99.04	98.68

Conclusions and Future Perspectives

Regarding the three nano-scale consolidants based on cellulose and lignin, the obtained results did not allow for discriminating significant differences regarding their efficacy but they laid a profound base for more focused tests in future. The problems in color changing observed for LNPs and BC could be solved by modifying the impregnation baths. Regarding the different penetration of the tested consolidants, it was not possible to identify relations neither with the state of preservation of wood nor with the shape and dimension of the nanoparticles. Thus, the different behavior must be related to chemical interactions between the nanoparticles themselves and between them and the wood. LNPs and CNCs penetration problems could be solved by modifying the impregnation conditions while for BC a better consolidant effect could be achieved by modifying the shape of the nanocellulose or combining filamentous with spherical nanoparticles in order to obtain web-like structures more suitable to fill the voids and to support the cell wall, exploiting the potential of nano-scale consolidants to enter wood ultrastructure.

The excellent results obtained in testing essential oils as biocides for the preservation of waterlogged archaeological wood revealed the potential of these products during the delicate conservation and restoration phases of wooden artifacts. The application of cinnamon essential oil during an actual restoration process of archaeological wooden artifacts confirmed the *in vitro* results.

Finally, the very good results obtained applying biochemical quantification of ATP showed the usefulness of this technique in monitoring the biological contamination of wood after the recovery, during the storage and restoration phases. Furthermore, the analysis of the microbiota composition through NGS allowed to observe the presence of cellulolytic and/or ligninolytic species, underlining the high biological risk to which wood is subjected during these delicate conservation phases, and to highlight the differential effect of the biocides on bacterial and fungal communities.

1. Introduction

The European standard EN16873 (2016) (Conservation of cultural heritage - Guidelines for management of waterlogged wood on terrestrial sites of archaeological significance) defines waterlogged archaeological wood as: “wood whose structure has been filled with water through the sustained inclusion in a water saturated environment”. This wood comes from submerged archaeological sites (in lake, sea, river or wetland) or from land waterlogged sites. Generally, waterlogged wooden artifacts preserve their original size and shape but often they undergo severe cell wall decay due to chemical and biological factors. The pH and the salinity of water as well as the chemical nature of sediments (Hedges, 1990; Pearson, 2014; Unger et al., 2001) together with the action of biological degraders (e.g. erosion and tunnelling bacteria and soft rot fungi) (Blanchette, 2000; Blanchette et al., 1989) affect the wood causing a more or less severe mass loss and increased porosity and permeability which lead to a spongy and weakened material.

Drying waterlogged archaeological wooden artifacts is necessary for the musealization but it is always a high risk procedure because it could cause severe shrinkages and collapses. Thus, conservation practices focus not only on removing water from wood, but also on consolidating the degraded cell walls aiming for stabilised shape and size of the artifact as well as to enable it to withstand the conditions of the future preservation environment (Grattan and Clarke, 1987). A consolidant confers on wood a good stabilisation by using the minimal amount of product while being stable to variations in relative humidity. However, these are not the only features required from an effective consolidant, conservation history highlighted no treatment exists which is able to preserve an artifact forever. Thus, all the materials used in restoration practices must be removable or, at least, leave the object retreatable. Several materials have been tested and used for the consolidation of waterlogged archaeological wood (for a review see Christensen et al., 2012). By now, the most used consolidants are polymers (e.g. polyethylene glycol, PEG), sugars (e.g. lactitol) or resins (e.g. Kauramin) (Christensen et al., 2012; Unger et al., 2001).

The use of nanomaterials in the conservation of cultural heritage expanded during the last few decades (Baglioni et al., 2015). Different kinds of nanoparticles were mainly used to clean and consolidate wall paintings. Tests with nanosols of silica and alkaline nanoparticles were conducted to consolidate wood artifacts

and to deacidify waterlogged archaeological wood (Chelazzi et al., 2006; Mahltig et al., 2008). Anyway, even if Cipriani et al. (2010) tested cellulose derived consolidants and Christensen et al. (2012) report of consolidation attempts made with cellulose whiskers, no additional literature is available about the consolidation of waterlogged archaeological wood with nanomaterials obtained from renewable sources.

As long as wood is kept waterlogged, anaerobic or almost anaerobic conditions are maintained and the biological degradation processes are very slow, but during excavation and conservation treatments wooden artifacts can be subjected to faster decay forms (Björdal and Nilsson, 2002). For this reason, precautions must be taken to limit wood degradation. The supplement to the Official Gazette of the Italian Republic n. 244 (2001), also known as “Museum Standards”, suggests the thermo-hygrometric values recommended for the prevention of microbiological attacks on organic artifacts. For waterlogged wood the state of complete submersion at temperature lower than 4 °C is prescribed and should be maintained during the storage phases preceding the restoration. When it is not possible to respect these guidelines a biocide should be used. Biocidal products are “active substances and preparations containing one or more active substance, applied in the form in which they are supplied to the user, intended to destroy, deter, render harmless, prevent the action of, or otherwise exert a controlling effect on any harmful organism by chemical or biological means” (98/8/EC, 1998; Regulation (EU) No528, 2012). Nowadays the biocides most frequently used in the preservation of archaeological wood are chemical preservatives (e.g. quaternary ammonium salts, derivatives of the isothiazolone class, etc.) (Unger et al., 2001). These substances have environmental sustainability and safety problems and for this reason their use should be limited. In recent years, more and more attention has been paid to identifying biocides of natural origin. Several studies shown the effectiveness of substances extracted from plants and animals in limiting and/or contrasting the growth of organisms and microorganisms and proposed their application in different fields (i.e. food preservation, pharmaceutical). Even in the field of conservation of cultural heritage several attempts have been made in exploring the biocidal potential of natural products for the control of biodegradation of textiles, stone and wooden artifacts (Axinte et al., 2011; Barresi et al., 2015; El-Gamal et al., 2016; Fidanza and Caneva, 2019; Matusiak et al., 2018; Sasso et al., 2013; Stupar et al., 2014). Anyway, these substances have never been used in protecting waterlogged wood.

The PhD project was aimed to find bio-inspired consolidants and preservatives that could substitute the chemicals currently in use. In particular, focusing on the concepts of bioeconomy and circular-economy, rarely applied until now to the field of cultural heritage, the study tested three cellulose and lignin nano-scale consolidants (lignin nanoparticles, bacterial nanocellulose and cellulose nanocrystals) and three essential oils from organic farming (*Cinnamomum zeylanicum* Blume - cinnamon bark, *Thymus serpyllum* L. - wild thyme, and *Thymus vulgaris* L. - common thyme) as alternative biocides.

Lignin nanoparticles (LNPs) can be synthesized from technical lignin obtained as a by-product of paper industry or second generation ethanol biorefineries and have been proposed for a wide range of applications as e.g. active components or fillers in polymer blends introducing lignin properties like anti-oxidant activity or UV protection on a nano-scale level (Beisl et al., 2017).

Nanocellulose can be obtained by processing plant cellulose or as a product of primary metabolic processes of certain bacterial strains (Kargarzadeh et al., 2017). Bacterial cellulose, (BC), is produced by the fermentation of *Gluconoacetobacter* species, which can produce high aspect ratio (length to diameter) nanofibers, with three-dimensional porous networks, retaining the highest purity. Because of its hydrophilic nature (99% of the constituents is water), flexibility, non-toxicity, good biocompatibility, and wide availability BC has been extensively used in diverse fields from food and paper industry to biomedical applications (Kunjalukal Padmanabhan et al., 2017).

Cellulose nanocrystals (CNCs), also known as nanocrystalline cellulose, nanowhiskers, nanorods, and rod-like cellulose crystals, are obtained by hydrolysis with highly concentrated acids (6–8 M) and high-power mechanical or ultrasonic treatments of the crystalline domains of cellulose nanofibrils. Their dimensions and the degree of crystallinity depend on the cellulose source and extraction conditions (Abdul Khalil et al., 2014; Kargarzadeh et al., 2017).

The International Organization for Standardization (ISO) defined essential oil (EO) as “product obtained from a natural raw material of plant origin, by steam distillation, by mechanical processes from the epicarp of citrus fruits, or by dry distillation, after separation of the aqueous phase -if any- by physical processes” (ISO9235, 2013). EOs are volatile, complex compounds produced by plants as secondary metabolites. At room temperature, they are liquid, limpid, rarely colored, soluble in lipid and organic solvents generally with lower density than water. In nature, they play an essential role in protecting plants from bacterial, viral, fungal, and insect attacks as well as against herbivory. They can be present in all plant organs (e.g. buds, flowers, leaves, seeds, twigs, stems, fruits, bark) and are generally stored in secretory cells, cavities, canals or glandular

trichomes. The EOs producing plants are usually known for their numerous useful effects (e.g. antioxidant, analgesic, sedative, spasmolytic, local anesthetic) and are used for food preservation or as part of the traditional pharmacopoeia. The antimicrobial activity of these natural products have been widely studied and several of their biological effects (e.g. cytotoxicity, phototoxicity, mitochondrial damaging) are well known (Bakkali et al., 2008). Because of the great number of constituents, essential oils seem to have no specific cellular targets so they could be considered as biocides with broad-spectrum activities (Carson et al., 2002). As lipophiles, they can pass through the cell wall and cytoplasmic membrane altering the polysaccharide layers structure and permeabilizing the membrane, but they can also target proteins, enzymes and the DNA (Bakkali et al., 2008; Nazzaro et al., 2013; Singh and Singh, 2012).

Several works tested essential oils and/or their components against wood degrading microorganisms (Sadiki et al., 2017; Salem et al., 2016; Singh and Singh, 2012; Temiz et al., 2010; Xie et al., 2017, 2015; Yen and Chang, 2008; Zhang et al., 2016). Cinnamon and thyme EOs were selected for the study because these oils and their main components (cinnamaldehyde, eugenol, thymol, and carvacrol) proved to have a biocidal activity against several wood degraders. In particular, cinnamaldehyde was effective against brown rot fungi *Tyromyces palustris* and *Coniophora puteana* and the white rot fungus *Trametes versicolor* while α -methyl cinnamaldehyde, (E)-2-methylcinnamic acid gave good results against white rot fungus *Lenzites betulina* and brown rot fungus *Laetiporus sulphureus* (Axinte et al., 2011; Cheng et al., 2008; Kartal et al., 2006). Eugenol can cause the lysis of spores and complete inhibition of mycelial growth in seed born fungi, and is active against white rot fungi *Trametes hirsuta*, *Schizophyllum commune*, *Pycnoporus sanguineus*, and *L. betulina* and brown rot fungus *L. sulphureus* (Cheng et al., 2008; Zhang et al., 2016). Singh and Chittenden (2008) by screening 12 essential oils to evaluate their antifungal activity against common mold, stain and wood decay fungi showed that eugenol and cinnamaldehyde were the most effective in inhibiting the growth of test fungi on treated wood blocks. Cinnamon oil was tested against mold and sapstain fungi, proving to be highly effective when used in ethanol, and against brown (*Poria placenta*) and white (*Coriolus versicolor*) rot fungi (Amusant et al., 2009; Li et al., 2008). Finally, the essential oil of *Thymus vulgaris* has biocidal activity against bacterial isolated from deteriorated wood (*Bacillus subtilis* and *Bacillus safensis*) while isolated thymol and carvacrol are active against white rot fungi *T. hirsuta*, *S. commune*, and *P. sanguineus* (Sadiki et al., 2017; Zhang et al., 2016).

To test the biocidal activity of the selected oils three techniques were used and compared: cultural analyses, ATP bioluminescence and next-generation sequencing (NGS). While cultural tests are routinely used to evaluate the presence of waterlogged wood biodeteriogens, ATP quantification and NGS have almost never been applied to this field.

Adenosine triphosphate (ATP) is the cellular energy currency in all living organisms, it is used for all the energy requiring processes including biosynthesis, motility and reproduction. Upon cell death the cellular ATP content is rapidly degraded to AMP (Shama and Malik, 2013). To evaluate the cell vitality, ATP levels can be conveniently measured by ATP bioluminescence, first described by W.D. McElroy (McElroy, 1947). Here the luciferase enzyme, from the firefly of the genus *Photinus*, reacts with ATP in the presence of its substrate luciferin, oxygen and magnesium ions to produce oxyluciferin, AMP (adenosine monophosphate), pyrophosphate and light. The amount of light generated by this enzymatic reaction is proportional to the ATP in the reaction. ATP-based techniques has emerged as an alternative to conventional culture-based methods which are simple but time consuming and underestimating the actual microbial population because many microorganisms are viable but noncultivable (Bautista et al., 1997; Rakotonirainy et al., 2003a). The ATP quantification methods are rapid, robust, easy to perform, affordable and can detect both cultivable and uncultivable organisms, with high sensitivity from samples with extremely low microbial burden (Van der Kooij et al., 1995; Velten et al., 2007; Venkateswaran et al., 2003). Thus, the quantification of ATP levels is a reliable measure for monitoring contamination and biofilm formation and guide cleanliness and corrosion management in various industries. ATP biomonitoring is widely used in the food processing industry for the detection of microbial contamination (Corbitt et al., 2000; Kunová et al., 2015; Miettinen et al., 2001), in the healthcare sector to monitor the cleaning practices (Amodio and Dino, 2014; Boyce et al., 2009) and the microbiological quality of the water (Arroyo et al., 2017; Costa et al., 2004). The quantification of ATP has also been applied in the field of the conservation of Cultural Heritage. During the years, it has been tested on several artifact (stone monuments, paper and textile) to survey the biological contamination and to evaluate the efficacy of biocidal treatments (Ljaljević et al., 2014; Rakotonirainy et al., 2003a, 2003b, 2007; Rakotonirainy and Arnold, 2008; Rakotonirainy and Dubar, 2013; Unković et al., 2019).

The waterlogged archaeological wood microbiota has been widely studied in order to understand the degradation processes affecting this precious material and to set valuable consolidation treatments. The study

of wood biodeteriogens and of their degradative activity usually involves cultural and microscopic analyses (Anagnost, 1998; Kim et al., 1996; Nilsson et al., 2008; Nilsson and Björdal, 2008a, 2008b; Schwarze, 2007). Since the last decades, molecular techniques have been applied to this field of study. They were mainly based on the amplification and sequencing of DNA extracted from cultured microorganisms and only in few cases directly from wood (Helms et al., 2004; Landy et al., 2008; Palla et al., 2014, 2013). Next-generation sequencing is an innovative technique that was applied to the study of waterlogged archaeological wood conservation only in the last couple of years. Even if five studies regarding the high-throughput sequencing of microbial communities involved in the biodeterioration of waterlogged archaeological wood were published (Li et al., 2018; Liu et al., 2018; Wagner et al., 2018; Xu et al., 2017; Yin et al., 2019) only two of them were aimed to characterize the microorganisms present inside the wood. The other works focused on the analysis of the storage water and the burial environment or were mainly aimed to characterize aDNA in order to perform evolutionary studies.

2. State-of-the-art

The European standard EN16873 (2016) (Conservation of cultural heritage - Guidelines for management of waterlogged wood on terrestrial sites of archaeological significance) defines waterlogged archaeological wood as: “wood whose structure has been filled with water through the sustained inclusion in a water saturated environment”. This wood comes from submerged archaeological sites (in lake, sea, river or wetland) or from land waterlogged sites. Like any organic material wood is decomposed by biological processes in nature, in the aforementioned sites oxygen is limited and only slow degraders like erosion and tunnelling bacteria and soft rot fungi can attack wood (Björdal et al., 1999). As long as wood is kept waterlogged anaerobic or almost anaerobic conditions are maintained and the degradation processes are very slow, but during excavation and conservation treatments wooden artifacts can be subjected to faster decay forms (Björdal and Nilsson, 2002). For this reason, biocides are used during conservation and restoration stages to prevent wood degradation. Nowadays the biocides most frequently used are chemical preservatives (e.g. quaternary ammonium salts, derivatives of the isothiazolone class, etc.) (Unger et al., 2001). These substances have environmental sustainability and safety problems and for this reason their use should be limited.

The antimicrobial activity of natural products (essential oils and plant extracts) is nowadays well known and many studies showed that some of these substances (e.g. essential oils from lemon grass, rosemary, tea tree; cinnamaldehyde; eugenol; thymol; etc.) have effect against wood degrading microorganisms (Singh and Singh, 2012). Even if some studies tested natural products for the conservation of wooden artifacts (Axinte et al., 2011; El-Gamal et al., 2016), they have never been used in protecting waterlogged wood.

Waterlogged wood cannot be exposed in museums as it is but it has to undergo a drying process. To avoid shrinkages and collapses, before drying the wood it is necessary to treat it with a consolidant that substitute the water inside the wood consolidating the degraded cell walls and stabilizing the structure. Several materials have been tested and used for the consolidation of waterlogged archaeological wood (for a review see Christensen et al., 2012). By now, the most used consolidants are polymers (e.g. polyethylene glycol, PEG), sugars (e.g. lactitol) or resins (e.g. Kauramin) (Christensen et al., 2012; Unger et al., 2001).

The aim of this PhD thesis project was to test bio-inspired products for the preservation and conservation of waterlogged archaeological wood.

3. Materials and Methods

3.1. Waterlogged wood consolidation

3.1.1. Nanoparticles

Three different nanoparticles were tested as consolidants of waterlogged archaeological wood: lignin nanoparticles (LNPs), bacterial nanocellulose (BC), cellulose nanocrystals (CNCs).

LNPs were obtained from beech wood (*Fagus sylvatica* L.) from the Cimini Mountains in Lazio region, Italy (Piangoli Legno SNC, Soriano nel Cimino, VT). They were produced applying the “anti-solvent procedure” on acidolysis lignin using DMSO as lignin solvent, as reported in Zikeli et al. (2018). The obtained LNPs are hollow, sphere-shaped particles with diameters ranging between 40 and 120 nm.

BC was provided by Biofaber srl (Italy). It was obtained by fermentation of agro-alimentary waste, in accordance with Pal et al. (2017), in the form of hydrogel pellicles that were washed with distilled water and boiled in 0.5 M sodium hydroxide solution in order to purify the samples. Pellicles were dried at 60 °C to get dried BC membranes and then were grinded with a rotary mill in order to obtain cellulose nanospheres.

The BC prepared for the test had never been studied before, so morphological characterization of BC spray-dried on a stub was performed on a Zeiss (Sigma VP; Carl Zeiss, Germany) FESEM.

CNCs were supplied by CelluForce (Montreal, Canada). CelluForce NCC® is a cellulose hydrogen sulphate sodium salt, provided as a spray dried powder, white in color. The CNCs are spindle shaped crystals, 2.3-4.5 nm in diameter and 44-108 nm in length (from CelluForce product specifications).

3.1.2. Wood samples

Wood samples were obtained from two archaeological sites: Isola Sacra (Fiumicino, Italy) and the ancient port of Neapolis (Naples, Italy). The wood coming from Fiumicino belongs to the shipwreck named Isola Sacra 1 (sample P2550), an horeia-type vessel dated before the first half of the 3rd century AD and excavated in 2011 (Boetto et al., 2017). The wood from Naples pertains the shipwreck F (samples FTdis 4, FTdis 9 and FTdis 48), and a wooden pier (2514.28, 2514.30 and 2514.34) both dated to the roman age (Di Donato et al., 2018). The remains were recovered from the excavation area of Piazza Municipio in 2015 and 2018 respectively. After the recovery, the wood was stored in the biology laboratory of the Istituto Superiore per la Conservazione e il Restauro (IsCR). During all the storage period the fragments were left completely soaked to maintain the state of maximum water content.

From the wood samples 47 blocks were obtained with dimensions 2.5 x 2.5 x 1.5 cm and 3.0 x 1.5 x 1.5 cm, depending on the availability of the material. Ten blocks were used for each consolidant to be tested, the remaining were used as not-consolidated controls, freeze- (7) or air-dried (10).

Each sample was marked with an acronym indicating the consolidant (L = lignin nanoparticles, BC = bacterial cellulose, CNC = cellulose nanocrystals) or the drying method for controls (F = freeze-drying, A = air-drying), and the wood type (S = softwood, H = hardwood) followed by sequential numbers (Tab. 1).

Tab. 1 The utilised samples, type of wood, archaeological sample and consolidant or drying method

Sample name	Wood	Archaeological sample	Consolidant/drying method
LS1	Softwood	P2550	Lignin nanoparticles
LS2		FTdis 4	
LS3		FTdis 48	
LH1 – LH2 – LH3	Hardwood	FTdis 9	
LH4 – LH5		2514.28	
LH6		2514.30	
LH7		2514.34	
BCS1	Softwood	P2550	Bacterial cellulose
BCS2		FTdis 4	
BCS3		FTdis 48	
BCH1 – BCH2 – BCH3	Hardwood	FTdis 9	
BCH4 – BCH5		2514.28	
BCH6		2514.30	
BCH7		2514.34	
CNCS1	Softwood	P2550	Cellulose nanocrystals
CNCS2		FTdis 4	
CNCS3		FTdis 48	
CNCH1 – CNCH2 – CNCH3	Hardwood	FTdis 9	
CNCH4 – CNCH5		2514.28	
CNCH6		2514.30	
CNCH7		2514.30	
FS1	Softwood	P2550	Freeze-drying
FS2		FTdis 4	
FS3		FTdis 48	

FH1	Hardwood	FTdis 9	Air-drying
FH2		2514.28	
FH3		2514.30	
FH4		2514.34	
AS1 – AS2	Softwood	P2550	
AS3		FTdis 4	
AS4		FTdis 48	
AH1 – AH2	Hardwood	2514.28	
AH3 – AH4		2514.30	
AH5 – AH6		2514.34	

3.1.3. Wood characterization

Before the beginning of consolidation procedures the wood species were identified and the state of preservation of the material was characterized by micro-morphological and physical analyses according to the Italian standard and to the most used protocols (Capretti et al., 2008; Jensen and Gregory, 2006; Romagnoli et al., 2018; Schwarze, 2007; UNI11205, 2007).

The identification of wood species and micro-morphological examinations were carried out on thin sections (10–20 µm) cut in the three anatomical planes (cross, longitudinal-radial and longitudinal-tangential) by means of transmission light microscopy (DMRB, Leitz) as prescribed by Schniewind (1990). Samples were cut by hand with a razor blade or using a cryo-microtome (Cryostat CM 1900, Leica). To identify the wood species the observed anatomical features were compared to literature works (Jacquot, 1955; Jacquot et al., 1973; Schweingruber and Baas, 1990).

To highlight the presence of micro-organisms the sections were stained with an aqueous solution of 1% w/v methylene blue in 50% lactic acid. Microbial decay was evaluated using both bright-field and polarized light microscopy in order to identify decay patterns and highlight the loss of crystalline cellulose.

To assess the decay by means of physical tests, wood blocks were weighted and their volume was defined by the water displacement method. Weight was measured again after the samples were dried in an oven at 103°C ± 2°C up to a constant weight. The physical parameters used for the characterisation are reported in Tab. 2. Reference values for basic density of non-decayed wood were taken from the literature (Capretti et al., 2008; Giordano, 1986; Macchioni et al., 2012).

Tab. 2 Physical parameters used to assess the wood decay

Physical parameter	Unit	Formula
Basic density (D_{bd})	$\text{g} \times \text{cm}^{-3}$	$D_{bd} = \frac{M_0}{V_f}$
Maximum water content (MWC)	%	$MWC = \frac{M_f - M_0}{M_0}$
Residual density (RD_b)	%	$RD_b = \frac{D_{bd}}{D_b}$

M_0 = anhydrous mass

M_f = mass at maximum water content

V_f = volume at maximum water content

D_b = basic density of non-decayed wood

3.1.4. Methods development of impregnation and wood drying

Impregnation baths containing the nano-scale consolidants were prepared. LNPs' bath was obtained adding the nanoparticles to deionised water up to a concentration of 5 mg/ml. BC suspended in deionised water tended to precipitate, so to obtain a stable suspension several attempts were made. Following the suggestions of the Biofaber team, Tween 20, sucrose, polyethylene glycol 400 (PEG400), oil or a mix of them were added to the BC (concentration 5%), the suspensions were homogenised with the help of a magnetic shaker and/or a

sonicator. The details of the performed tests are reported in Tab. 3. At the end of every test a more or less thick layer of BC had precipitated at the bottom of the container, except for the suspension obtained with procedure reported as T2 in Tab. 3 (0.5% Tween 20 + 1% Cinnamon oil + deionised water, sonication 50% amplitude - 1h, 5% BC, magnetic shaking 500/600 rpm - 1h), which in consequence was selected as the procedure to prepare the impregnation baths.

Tab. 3 Details of the tests performed to obtain stable BC suspensions

Test name	Procedure*
T1	• 0.5% Tween 20 + 1% Cinnamon oil + deionised water • Sonication 1h • 5% BC • Sonication 1h
T2	• 0.5% Tween 20 + 1% Cinnamon oil + deionised water • Sonication 1h • 5% BC • Magnetic shaking 1h
T3	• 0.5% Tween 20 + 1% Cinnamon oil + deionised water + 5% BC • Sonication 1h
T4	• 0.5% Tween 20 + 1% Cinnamon oil + deionised water + 5% BC • Magnetic shaking 1h
T5	• 0.5% Tween 20 + 1% Cinnamon oil + deionised water • Magnetic shaking 30 min • 5% BC • Magnetic shaking 1h
T6	• 0.5% Tween 20 + deionised water • Sonication 1h • 5% BC • Magnetic shaking 1h
T7	• 1% Tween 20 + deionised water • Sonication 1h • 5% BC • Magnetic shaking 1h
T8	• 1% Tween 20 1% Cinnamon oil + deionised water • Sonication 1h • 5% BC • Magnetic shaking 1h
P1	• 1% PEG 400 + 5% BC + deionised water • Sonication 1h • Magnetic shaking 1h
P2	• 1% PEG 400 + 5% BC + deionised water • Magnetic shaking 1h
Z1	• 1% sucrose + deionised water + 5% BC • Sonication 1h
Z2	• 1% sucrose + deionised water + 5% BC • Magnetic shaking 1h
Z3	• 1% sucrose + 5% BC + deionised water • Sonication 1h
Z4	• 1% sucrose + 5% BC + deionised water • Magnetic shaking 1h

*for all the tests sonicator amplitude was set at 50% and magnetic shaker speed at 500/600 rpm

To obtain a liquid suspension of CNCs 3% of powdered crystals were added to deionised water and shaken on a magnetic shaker for 1 h. By adding a greater amount of CNCs the suspension became a gel and thus not suitable for the consolidation treatment.

To perform the consolidation tests, the waterlogged archaeological wood blocks were completely soaked in the baths for one month at room temperature. After the treatment, the blocks were frozen (-20 °C) and then freeze-dried. The lyophilizer (LIO 2000PNS) settings were: chamber temperature -30 °C; condenser

temperature -50 °C; chamber vacuum 2 Pa. The wood temperature was monitored through the use of thermocouples positioned on the samples' surface. The pressure was raised up to room pressure and the blocks were removed from the lyophilizer when reaching a temperature of 18-20 °C. The freeze-drying procedure was selected for this study as it is often used in the context of restoration works on archaeological wooden artefacts.

Control blocks were kept completely soaked in deionised water and were successively frozen and freeze-dried under the same condition as treated samples. Control samples were air-dried at room temperature.

During the consolidation treatments different problems arose for the three tested consolidants. Soon after the beginning of consolidation procedure LNPs and BC precipitated on the bottom of the container. Therefore, to keep the nanoparticles suspended the impregnation baths were gently shaken on a magnetic stirrer for the rest of the test. Additionally, the wood blocks were periodically rotated in order to avoid nanoparticles deposition on their upper surface. In spite of all the precautions taken, at the end of the treatments a more or less thick layer of LNPs and BC was present on the wood (Fig. 1A, B). Those nanoparticles were removed with a soft brush before freezing the blocks.

After one week of consolidation test, the CNCs impregnation bath turned into a gel (Fig. 1C). A test carried out on a small aliquot of the gel showed that to regain a liquid bath the addition of the double volume water was necessary. This solution was not considered in order to avoid an excessive dilution of the consolidant. At the end of the treatment, the wood blocks were extracted from the gel and the residual CNCs on the wood surface were removed with a soft brush.

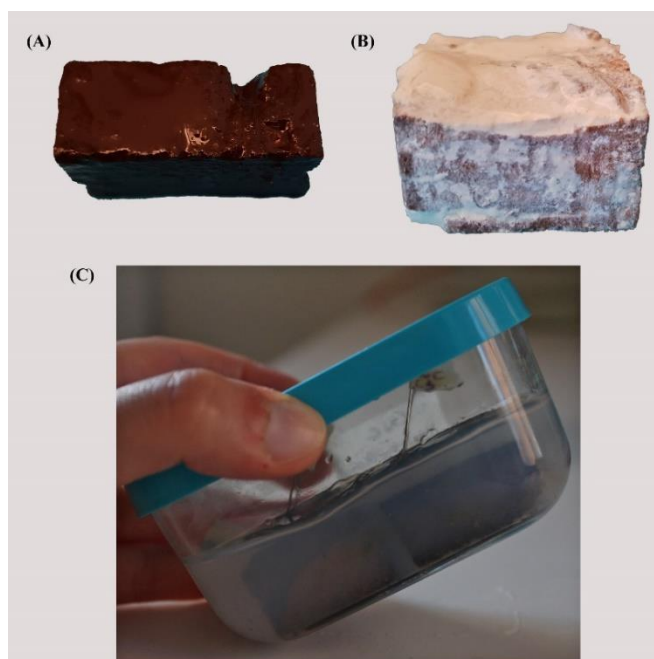


Figure 1. LNPs (A) and BC (B) treated blocks at the end of the consolidation treatment, a layer of nanoparticles is present on wood surface; (C) CNCs impregnation bath turned into a gel.

3.1.5. Evaluation of the efficacy of the treatments

A first evaluation of the efficacy of the treatments was obtained by macroscopic observation of the samples. Color, shape, presence of openings and consistency were observed.

The penetration of the nanoparticles inside the wood tissues was evaluated by examining gold sputter-coated sections of treated blocks (LH5, BCH5 and CNCH5) and freeze-dried control (FH2) with a Scanning Electron Microscope (Zeiss EVO 60) in secondary electrons mode. For each sample three sections were observed: external cross section, internal cross section and internal radial section. The internal sections were obtained splitting the block by hand, without the use of blades.

3.1.5.1. Retention of impregnation products and anti-shrink efficiency

The retention of consolidants (Y) was calculated following the procedure reported by Giachi et al. (2010).

In order to evaluate the dimensional variations of treated wood, all the six sides of treated blocks, freeze- and air-dried controls were scanned on high definition scanner (HP scanjet G3010), together with a measuring ruler, before the consolidation treatment and after the drying procedure (wood equilibrated at 20 °C, 50% RH). Using an image processing software (ImageJ 1.52n) the cross section surface and the average height of each block were measured. The obtained values were used to calculate the blocks' volume (V_W = volume of waterlogged blocks, V_D = volume of treated blocks and controls after drying).

For each block, the percentage shrinkage, S , was calculated following the equation:

$$S(\%) = \frac{V_W - V_D}{V_W} * 100$$

The S values obtained for each treated sample were used to determine the anti-shrink efficiency (ASE) with respect to freeze-dried (ASE_F) and air-dried (ASE_A) controls following the equations:

$$ASE_F(\%) = \frac{S_F - S_T}{S_F} * 100 \quad \text{and}$$

$$ASE_A(\%) = \frac{S_A - S_T}{S_A} * 100$$

Where S_F and S_A are the shrinkages of freeze- and air-dried controls, respectively, and S_T is the shrinkage of treated blocks.

The ASE_C , expressed as the percentage of shrinkage that has been suppressed by freeze-drying treatment as compared to the shrinkage of air-dried controls, was calculated as follows:

$$ASE_C(\%) = \frac{S_A - S_F}{S_A} * 100$$

3.1.5.2. Equilibrium moisture content

To evaluate the effect of the different consolidation treatments with respect to moisture absorption the equilibrium moisture content (EMC) was determined. The test was performed on the treated wood blocks and freeze-dried controls not used for SEM observations. The obtained results were compared to sound wood EMC from literature (Giordano, 1981) and to values obtained for sound wood controls. The latter, four blocks of silver fir and six blocks of holm oak, 2.0 x 2.0 x 0.5 cm in dimensions, were marked with a progressive number.

The samples were put in a climatic chamber at 20 °C and the RH was varied according to the sequence 10, 35, 45, 55, 65, 85% reaching in each step the EMC (Giordano, 1981). The EMC for the RH value of 100% was obtained by putting the wood blocks in a closed container in presence of liquid water. At the end of the RH cycle, the samples were completely dried in a oven at 103 °C $\pm$ 2 °C up to a constant weight.

3.2. Waterlogged wood preservation

3.2.1. Essential oils

Three essential oils (EOs) from organic farming, provided by Flora srl, were tested during the experimentation: *Cinnamomum zeylanicum* Blume (cinnamon bark, C), *Thymus serpyllum* L. (wild thyme, WT) and *Thymus vulgaris* L. (common thyme, CT).

Flora srl characterized the EOs composition through gas chromatography mass spectrometry (GC-MS): instrument, Perkin Elmer Clarus 500GC/FID/MS; column, 5% diphenyl + 95% dimethylpolysiloxano. The results are reported in Tab. 4.

Tab. 4 Composition of the EOs selected for the experimentation

Cinnamon (C)		Wild thyme (WT)		Common thyme (CT)	
Component	%	Component	%	Component	%
α -pinene	0.67	α -thujene	1.02	canfene	1.09
canfene	0.25	α -pinene	0.76	α -pinene	0.66
β -pinene	0.26	sabinene	0.28	α -thujene	1.21
α -phellandrene	1.09	β -myrcene	1.42	β -pinene	0.15
α -terpinene	0.06	α -terpinene	1.19	β -myrcene	1.76
p-cymene	1.54	p-cymene	5.24	α -phellandrene	0.16
limonene	0.55	limonene	0.25	α -terpinene	1.24
β - phellandrene	1.88	β -phellandrene	0.23	limonene	0.49
linalool	3.61	1.8 cineol	0.33	1.8 cineol + β -phellandrene	0.27

hydrocinnamaldehyde	0.40	y-terpinene	4.02	y-terpinene	9.85
c-cinnamaldehyde	0.36	c-sabinene hydrate	0.46	p-cymene	17.69
o-anisaldehyde	0.07	linalool	8.22	terpinolene	0.11
t-cinnamaldehyde	69.81	borneol	0.42	t-thujanol	0.25
cinnamic alcohol	0.01	terpinen-4-ol	0.62	camphora	0.81
eugenol	3.05	α -terpineol	0.61	linalool	4.71
hydrocinnamil acetate	0.07	thymol	2.59	β -caryophyllene	2.29
α -copaene	0.63	carvacrol	68.49	neral	0.08
β -caryophyllene	5.58	β -caryophyllene	0.94	α -terpineol	0.12
cinnamil acetate	2.04			borneol	1.79
α -humulene	0.97			verbenone	0.04
benzyl benzoate	1.43			geranial	0.02
caryophyllene oxide	0.64			δ -cadinene	0.08
				γ -cadinene	0.04
				geraniol	0.06
				p-cymene-8-ol	0.06
				thymol	47.58
				iso-carvacrol	0.11
				carvacrol	2.79

3.2.2. Fungal strains

In order to test the EOs biocidal activity against fungi that could cause damage to archaeological wood, four cellulolytic fungal strains were selected: *Chaetomium* sp., *Fusarium* sp., *Aspergillus japonicus*, and *Stachybotrys chartarum*. The fungi were isolated from paper artifacts and identified to the genus or species level through appropriate identification keys (among the others Wang et al., 2016; Watanabe, 2002). The isolates were maintained on Malt Extract Agar (MEA) (Sigma-Aldrich), stored at 4 °C. Spore suspensions adjusted to the concentration $44\text{--}56 \times 10^4$ spores/ml were obtained for each strain by mixing the spores with sterile saline solution containing 0.01% (v/v) Tween 20 to prevent spore aggregation. Spores concentration in the suspensions was quantified in a Thoma counting chamber.

3.2.3. Biocidal activity assays

The biocidal activity of the selected EOs was evaluated both *in vitro* and on the microbiota present in archaeological wood samples.

For *in vitro* tests, the oils were diluted in a solution 50/50 v/v of deionised water and ethanol. While, for the experiments carried out on waterlogged wood the oils were dissolved in an equal volume of ethanol and then added to the storage water or consolidation bath (for further details see next paragraphs).

During the experimentation the biocidal activity of the EOs was evaluated through cultural analyses, the quantification of ATP bioluminescence and/or by characterizing the microbiota present inside waterlogged archaeological wood through Next-Generation Sequencing. Preliminary tests were carried out before the beginning of the analyses to set the protocols for the two last methodologies.

3.2.3.1. Methods development

3.2.3.1.1. ATP bioluminescence

To find an efficient and cost-effective method to extract ATP from fungal spores and cells three different extraction procedures were compared.

Spore suspensions

Spore suspensions of *Chaetomium* sp., *Fusarium* sp. and *Aspergillus japonicus* were adjusted to three concentrations (A: $44\text{--}56 \times 10^4$ spores/ml, B: $15\text{--}19 \times 10^4$ spores/ml and C: $5\text{--}6 \times 10^4$ spores/ml). For each strain, the tests were performed on all the suspensions and on an aliquot of A sterilised by autoclave at 121 °C for 15 minutes, named S.

Extraction reagents

The following extraction reagents were tested:

- Lysis Reagent A: (Promega - CS193106) from the Water-Glo™ System, preserved at +4 °C and kept at room temperature for half an hour before use to equilibrate the temperature;
- Lysis Reagent B: containing 25 mM tris hcl, pH 7.5, 0.2% triton x-100, and 0.2% benzalkonium chloride, preserved at -20 °C and kept at room temperature for two hours before use to equilibrate the temperature;
- Lysis reagent C: Dimethyl sulphoxide (DMSO) at a concentration of 90% in TAE buffer (a buffer solution containing a mixture of tris(hydroxymethyl)-aminomethane base, acetic acid and ethylene diamine tetra-acetic acid), at pH 7.75.

Detection reagent

The ATP Water-Glo® detection reagent was used for the tests. The reagent (Promega – CS193119) is provided as a powder, which was reconstituted by adding ATP Water-Glo® Reconstitution Buffer (Promega – CS 193109). The reconstituted Detection Buffer was stored at +4 °C and kept at room temperature for half an hour before use.

Standard ATP

The standard ATP (Promega – REF: F203A), preserved at -20 °C until use, was used for calibration.

Six concentrations (50,000 pg/ml; 10,000 pg/ml; 2,000 pg/ml; 400 pg/ml; 80 pg/ml and 20 pg/ml) were used to create a calibration curve.

For each concentration 100 µl of standard ATP were added to 100 µl of detection buffer and then immediately vortexed for approximately 5 s. The bioluminescence was read with a luminometer GloMax® 20/20 (Promega) combined with GloMax® Spreadsheet Interface Software (GLOMAX SIS v1.10.0) using the default setting.

Extraction/Detection protocols

Three protocols were compared during the experimentation, details are listed in Tab. 5. The ATP was extracted in a reaction volume of 100 µl containing 20 µl of spore suspension and 80 µl of extraction reagent. The ATP bioluminescence was evaluated by adding 100 µl of detection reagent to the lysate.

Free ATP was quantified replacing the extraction reagent with the same volume of sterile saline solution. Blank tests were performed by substituting the sample with the same volume of extraction reagent for cellular ATP tests, and of saline sterile solution in the case of free ATP. The cellular ATP values reported in the results were obtained subtracting the free ATP and the blank values to the registered values of cellular ATP.

The bioluminescence was read through a luminometer GloMax® 20/20 combined with GloMax® Spreadsheet Interface Software (GLOMAX SIS v1.10.0) using the default setting. The values of bioluminescence registered as relative light unit (RLU) were converted in ATP content (ng/ml) by using the standard calibration curve.

All the tests were performed on three aliquots of the sample and the results are reported as the average value of the three readings.

Tab. 5 Protocol procedures used to extract and detect cellular ATP.

Protocol name	Extraction reagent	Temperature of extraction	Incubation time	Bioluminescence Reading
Protocol 1	Lysis Reagent A	Room temperature	5 min	After vortexing for 5 s
Protocol 2	Lysis Reagent B	Room temperature	10 min	After vortexing for 5 s
Protocol 3*	Lysis Reagent C (pre-heated at 100 °C for 2 minutes)	100 °C	1 min, then rapidly cooled in ice-bath	After vortexing for 5 s

* extraction settings from (Rakotonirainy et al., 2003a)

3.2.2.1.2. Next-generation sequencing (NGS)

To set a valuable protocol for the extraction of microbial DNA from wood a preliminary test was carried out on ten samples of waterlogged archaeological wood selected from the material available at the biology laboratory of the IsCR. The sample name, wood species and provenance are resumed in Tab. 6.

Tab. 6. List of waterlogged archaeological wood samples used to set a protocol for the extraction of microbial DNA from wood

Sample name	Wood species	Provenance
N1	Silver fir – <i>Abies alba</i> Mill.	Ancient port of Neapolis – archaeological site of Piazza Municipio, Naples, Italy
N4	Silver fir – <i>Abies alba</i> Mill.	
N9	Elm – <i>Ulmus</i> sp.	
N39	Elm – <i>Ulmus</i> sp.	
N88	Silver fir – <i>Abies alba</i> Mill.	
PF	Ash – <i>Fraxinus</i> sp.	Archaeological site of San Rossore, Pisa, Italy
PO	Elm – <i>Ulmus</i> sp.	
PQ	Oak – <i>Quercus</i> sp.	
PL1	Holm oak – <i>Quercus ilex</i> L.	
PL2	Holm oak – <i>Quercus ilex</i> L.	

The wood remains from Naples were recovered in 2015 and were stored in water at 4 °C, in the dark. They pertain the shipwreck named F and are dated to the end of the 2nd century AD (Di Donato et al., 2018). The samples from Pisa were excavated in 2000 and were stored in water at room temperature. The fragments were sampled from different wooden remains pertaining the urban harbor of Etruscan and Roman Pisa (VII cent. B.C. to V cent. A.D) (Giachi et al., 2003).

In collaboration with Dr Guerrieri, Responsible for the Genomics facility of the Center For Life- Nanoscience - Istituto Italiano di Tecnologia (IIT), and Dr Pozzana, Technical Service Scientist, Promega Italy, two protocols for the DNA extraction were developed (Fig. 2) basing on the extraction kits Maxwell® RSC Plant DNA Kit (AS1490) and Maxwell® RSC PureFood GMO and Authentication Kit (AS1600). Total genomic microbial DNA was extracted from the selected wood samples with Maxwell® RSC Instrument (Promega, Wisconsin, USA). In order to compare the extraction efficiency on the same microbial community, both protocols were applied to the same wood sample (Tab. 7). The only exception is represented by samples PL1 and PL2 for whom the wood material was not sufficient to perform the double extraction.

Tab. 7 List of samples used for the two DNA extraction protocols

Protocol 1 - Maxwell® RSC Plant DNA Kit	Protocol 2 - Maxwell® RSC PureFood GMO and Authentication Kit
1N1	2N1
1N4	2N4
1N9	2N9
1N39	2N39
1N88	2N88
1PF	2PF
1PL1	2PO
1PL2	2PQ
1PO	
1PQ	

The concentration and purity of the extracted DNA were evaluated through the Nanodrop microvolume sample retention system.

V3-V4 region of 16S rRNA gene (amplified using the primers described in Illumina 16S protocol: # 15044223 Rev. B) and ITS2 fungal region (amplified using the following primers: ITS3 PCR Forward Primer 5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-GCATCGATGAAGAACGCAGC-3' and ITS4 Reverse Primer 5' GTCTCGTGGGCTCGGAG-ATGTGTATAAGAGACAGTCCTCCGCTTATTGATATGC-3') were subject to amplicon library preparation (according to Illumina's instructions, 16S Metagenomic Sequencing Library Preparation, Part # 15044223 Rev. B).

Sequencing was performed by Miseq (2x300 paired-end, 600-cycle) Illumina platform. The marker data were analyzed using qiime2 (<https://qiime2.org>), according to the standard pipelines. Briefly, quality trimming and

OTU-picking was done using DADA2 (Callahan et al., 2016), representative sequences were aligned using mafft (Kato and Standley, 2014), uninformative positions were masked and a phylogenetic tree was built with fasttree (Price et al., 2009). The alpha diversity values and the beta diversity (i.e. UniFrac distance) were calculated on rarefied samples. Assessment of significant variation of alpha diversity between categories was determined using the Kruskal-Wallis test. Beta diversity significance (among categories) test was calculated with PERMANOVA and Mantel test, respectively. Taxonomic assignment was given to representative sequences using the most updated version of the SILVA database (release 132) (Quast et al., 2012), or (for fungal data) the UNITE database (Nilsson et al., 2018). The feature classifier was trained using the qiime2 classify-sklearn plugin on the database; the same plugin also classifies the reads in the real dataset.



DNA da legno antico con Maxwell®RSC

Materiali:

- Maxwell® RSC Instrument (AS4500)
- Maxwell® RSC Plant DNA Kit (AS1490)
- Maxwell® RSC PureFood GMO and Authentication Kit (AS1600)
- Termoblocco
- Centrifuga da banco
- Vortex
- Tubi 2ml
- Polyvinyl polypyrrolidone (PVPP): (Esempio: Sigma # 77627)
- Proteinase K (MC5005 o MC5008)



Procedura:

- Disgregare il campione di partenza con un opportuno tool di omogenizzazione (es: azoto liquido)
- **Metodo 1: Maxwell® RSC Plant DNA Kit (AS1490)**
 - Aggiungere 3% w/v di PVPP a quanto serve di Tail Lysis Buffer (A509C)
 - Pesare 100mg di campione ed aggiungerlo ad un tubo da 2ml.
 - Aggiungere al campione 1,2ml di Tail Lysis Buffer (con PVPP)
 - Vortexare per 10 secondi
 - Incubare il campione a 95°C per 5 minuti.
 - Aggiungere 40ul di Proteinase K (MC5005 o MC5008) e vortexare
 - Incubare per 25 minuti a 65°C.
 - Centrifugare alla massima velocità per 2 minuti.
 - Prelevare 300ul di surnatante e caricarlo nella well 1 della cartuccia
 - Aggiungere 300ul di Nuclease-free Water (P119C) nella well 1 della cartuccia
 - Aggiungere un plunger nella well 8 della cartuccia.
 - Aggiungere 50-100ul di Elution Buffer (A828D) nei tubi di eluizione.
 - Impostare ed avviare sulla strumentazione il metodo *Plant DNA*.
- **Metodo 2: Maxwell® RSC PureFood GMO and Authentication Kit (AS1600)**
 - Aggiungere 3% w/v di PVPP a quanto serve di CTAB Buffer (MC1411)
 - Pesare 100mg di campione ed aggiungerlo ad un tubo da 2ml.
 - Aggiungere al campione 1ml di CTAB Buffer (con PVPP)
 - Incubare per 5 minuti a 95°C.
 - Aggiungere 40ul di Proteinase K (MC500C) e vortexare
 - Incubare per 25 minuti a 65°C.
 - Centrifugare alla massima velocità per 10 minuti
 - Aggiungere un plunger nella well 8 della cartuccia.
 - Prelevare 300ul di surnatante e caricarlo nella well 1 della cartuccia
 - Aggiungere 300ul di Lysis Buffer (MC501C) nella well 1 della cartuccia
 - Aggiungere un plunger nella well 8 della cartuccia.
 - Aggiungere 50-100ul di Elution Buffer (A828D) nei tubi di eluizione.
 - Impostare ed avviare sulla strumentazione il metodo *PureFood GMO and Authentication kit*.

Figure 2. The two protocols for the DNA extraction based on the extraction kits Maxwell® RSC Plant DNA Kit (AS1490) and Maxwell® RSC PureFood GMO and Authentication Kit (AS1600)

3.2.3.2. Evaluation of the biocidal activity *in vitro*

The effectiveness values of four different concentrations (3%, 1%, 0.7% and 0.5%) of the selected EOs was estimated by Minimum Inhibitory Concentration (MIC).

For each oil, the four fungal strains spore suspensions (1 ml) were uniformly distributed on a set of 3 Petri dishes (9 cm Ø) containing Malt Extract Agar (MEA) (Sigma-Aldrich). After the inoculum, 500 µl of biocidal solution were sprayed on the medium surface and the plates were incubated at 27 ± 2 °C. Control tests were carried out under the same condition by substituting the biocide with a solution 50/50 v/v of deionised water and ethanol (WE). To evaluate the spore vitality one Petri dish was inoculated with the spore suspension and incubated at the same temperature.

Fungal growth was visually evaluated after 3, 6 and 10 days of incubation; the antifungal activity was assessed by the presence/absence of fungal growth.

The biocidal activity of the established MIC was evaluated on the mycelium of the same selected strains.

For each strain, a Petri dish containing MEA was inoculated with fungal spores and incubated at 27 ± 2 °C for three days. The grown not-sporogenous mycelia were sprayed with 1 ml of the solutions containing the MIC of the three oils. Control plates were treated with a solution of deionised water and ethanol (WE).

The biocide efficacy was evaluated through cultural and biochemical tests.

Seven days after the treatment, three small pieces of mycelium (about 10 mg each) were taken from each of the treated plates and transferred on new culture medium. The plates were then incubated at 27 ± 2 °C. The biocidal activity was assessed basing on the presence/absence of fungal growth.

Biochemical tests involved the quantification of the ATP bioluminescence before the oil application and after seven days from the treatment.

Basing on the results obtained during the preliminary experimentation (§ 3.2.3.1.1. and 4.2.1) the extraction and detection protocol was set as follow. To extract the ATP from fungal cells, 10 mg of mycelium were dispersed in 1 ml of deionised water containing sterile glass beads and vortexed for about 3 minutes. The fungal cells were then lysed with Dimethyl sulphoxide (DMSO) at a concentration of 90% in TAE buffer (a buffer solution containing a mixture of trishydroxymethyl-aminomethane base, acetic acid and ethylene diamine tetra-acetic acid), at pH 7.75. DMSO was preheated in a sterile 1.5 ml Eppendorf tube at 100 °C for two minutes, after adding the fungal suspension it was incubated for one minute at 100 °C and then rapidly cooled in an ice-bath, as described by (Rakotonirainy et al., 2003a). The ATP was extracted in a reaction volume of 100 µl containing 20 µl of mycelium suspension and 80 µl of DMSO. The cellular ATP bioluminescence was evaluated by adding to the lysate 100 µl of detection reagent obtained by mixing the ATP Water-Glo® Substrate (Promega – CS193119), provided as a powder, and ATP Water-Glo® Reconstitution Buffer (Promega – CS 193109).

Free ATP was quantified replacing the extraction reagent with the same volume of sterile saline solution. Blank tests were performed by substituting the sample with the same volume of extraction reagent (for cellular ATP tests) and of saline sterile solution (in the case of free ATP). The cellular ATP values reported in the results were obtained subtracting the free ATP and the blank values to the registered values of cellular ATP. All the tests were performed on three aliquots of the sample and the results are reported as the average value of the three readings. The bioluminescence was registered as relative light unit (RLU) through a luminometer GloMax® 20/20 combined with GloMax® Spreadsheet Interface Software (GLOMAX SIS v1.10.0) using the default setting.

The possible influence of the EOs on the ATP quantification was evaluated by adding 1 µl of the three EOs to 99 µl of standard ATP (Promega – REF: F203A) at concentration 10,000 pg/ml. The registered RLUs were compared to those obtained by substituting the oils with 1 µl of sterile deionised water.

3.2.3.3. Evaluation of the biocidal activity on the waterlogged archaeological wood microbiota

To evaluate the biocidal activity of the EOs MIC against the microbiota present inside the waterlogged archaeological wood two different tests were carried out.

3.2.3.3.1. Waterlogged archaeological wood samples

The first experimentation was carried out in the Biology laboratory of the IsCR. Basing on the results obtained during the preliminary tests with NGS (§3.2.3.1.2. and 4.2.2.), a sample of waterlogged archaeological wood (N9) characterized by the presence of a high number of cellulolytic fungi was selected for the experimentation. Five subsamples were obtained by cutting the wood with a sterile blade and were placed in sterile containers with 20 ml of the water in which the wood was stored until the test began. Three of the five subsamples were treated with cinnamon (C), wild thyme (WT) and common thyme (CT) oil: 1% of the EOs dissolved in ethanol was added to the storage water. The fourth fragment's water was treated with the same amount of ethanol used to dissolve the oils (E). The last subsample was kept as an untreated control (NT). The biocidal activity was evaluated through the quantification of ATP bioluminescence and by examining the total genomic DNA extracted from the wood.

The biochemical tests were carried out both on the storage water and the wood, before the application of the EOs and after one month from the treatment. For the tests on water, the lysis and detection procedures described in § 3.2.3.2. were followed, the 20 µl of fungal suspension were substituted with the same amount of storage water.

For the tests carried out on wood, 100 mg of wood were added to 1 ml of sterile water containing sterile glass beads and vortexed for about 3 minutes. The microbial cells extracted from wood were lysate and the ATP was quantified as described in § 3.2.3.2.

In order to evaluate the possible influence of wood extractives on the ATP quantification, 20 µl of the suspension obtained by vortexing the wood were added to 80 µl of standard ATP (Promega – REF: F203A) at concentration 10,000 pg/ml. The registered RLUs were compared to those obtained by substituting the suspension with 20 µl of sterile deionised water. Considering a possible variability in the amount of extractives present in the suspension after wood vortexing, the tests were carried out on all the lysate suspensions, both before and after the biocidal treatment.

To evaluate the effect of the essential oils on the microbiota composition, NGS sequencing was performed on the treated and untreated wood and on the storage water. Basing on the results obtained in the preliminary test (§ 3.2.2.1.2. and 4.2.2.), the extraction protocol 1 (Maxwell® RSC Plant DNA Kit) was selected. V3-V4 region of 16S rRNA gene and ITS2 fungal region were amplified and sequenced and the data were analyzed as described in § 3.2.2.1.2. The analyses on wood were performed in triplicate.

3.2.3.3.2. Waterlogged archaeological wood remains

The second experimentation was carried out in the frame of the thesis project of Cristian Pedone, graduand at ISCR restoration school. The project, entitled “Restauro del relitto E dagli scavi del porto antico di Napoli. Due metodi di consolidamento a confronto”, was aimed to carry on the restoration of the remains of the shipwreck named E (from the ancient port of Neapolis) and to test the efficacy of a mixture of lactitol and trehalose in the consolidation of waterlogged archaeological wood. The tests were performed on wooden poles recovered from the excavation area of Piazza Municipio in Naples. The poles were treated into four tanks containing the lactitol-trehalose impregnation baths. Two of the tanks were transparent, the others opaque. After one month from the beginning of the consolidation treatment a conspicuous microbial growth was observed in all the containers so it was decided to treat the baths with a biocide. Two of the tanks were treated with cinnamon essential oil (1%) dissolved in an equal amount of ethanol. The choice of cinnamon oil was made based on the results obtained in the previous tests. The effect of the EO was compared to that of a usually used biocide, Preventol RI80, added to the two remaining tanks, 1% in concentration. The experimentation details are resumed in Tab. 8.

Tab. 8 Details of the experimentation carried out during the consolidation of wooden poles

Tank name	Tank feature	Biocide
V2	Transparent	Preventol RI80
V3	Opaque	Preventol RI80
V4	Opaque	Cinnamon essential oil
V5	Transparent	Cinnamon essential oil

The biocidal effect of cinnamon EO and of Preventol RI80 was evaluated through biochemical tests and cultural analyses before the application of the biocides and after one week.

The ATP bioluminescence was quantified on an aliquot of the impregnation baths of the four tanks. The lysis and detection procedures described in § 3.2.3.2. were followed, the 20 µl of fungal suspension were substituted with the same amount of impregnation bath.

Two tests were carried out to evaluate the possible influence of lactitol-trehalose and of Preventol RI80 on the ATP quantification. First of all, 20 µl of lactitol-trehalose solution (concentration 25%, as for the impregnation bath) were added to 80 µl of standard ATP (Promega – REF: F203A) at concentration 10,000 pg/ml. Secondly, 20 µl of the impregnation bath treated with 1% of Preventol RI80 were added to 80 µl of standard ATP at the same concentration. The registered RLUs were compared to those obtained by substituting the solution and bath aliquots with 20 µl of sterile deionised water.

Cultural analyses were carried out in the same days of the biochemical tests. A stock solution was prepared by adding 1 ml of impregnation bath to 9 ml of sterile saline solution. For the pre-biocide test, 1 ml of the dilutions 10^{-4} - 10^{-6} were inoculated on Malt Extract Agar in duplicate. For the tests performed after the application of the biocides, basing on the results of ATP quantification it was decided to plate the stock solution and the 10^{-1} dilution for the Preventol treated tanks and the 10^{-2} and 10^{-3} dilutions for cinnamon treated baths. The plates were incubated at 27 ± 2 °C and the colonies were counted after 3, 7 and 14 days. The results are reported as CFU/ml.

4. Results and Discussion

4.1. Waterlogged wood consolidation

4.1.1. Bacterial cellulose nanoparticles

SEM observations of bacterial nanocellulose allowed for characterizing the consolidant. Most part of the observed nanoparticles was more or less spherical in shape (Fig. 3), in some cases small, irregularly shaped particles were observed. The size distribution (Fig. 4) showed that the nanoparticles could be divided into two dimensional classes with diameter ranges of 0.04-0.50 µm and 3-50 µm. Most part of the BC particles had a diameter of ca 10 µm.

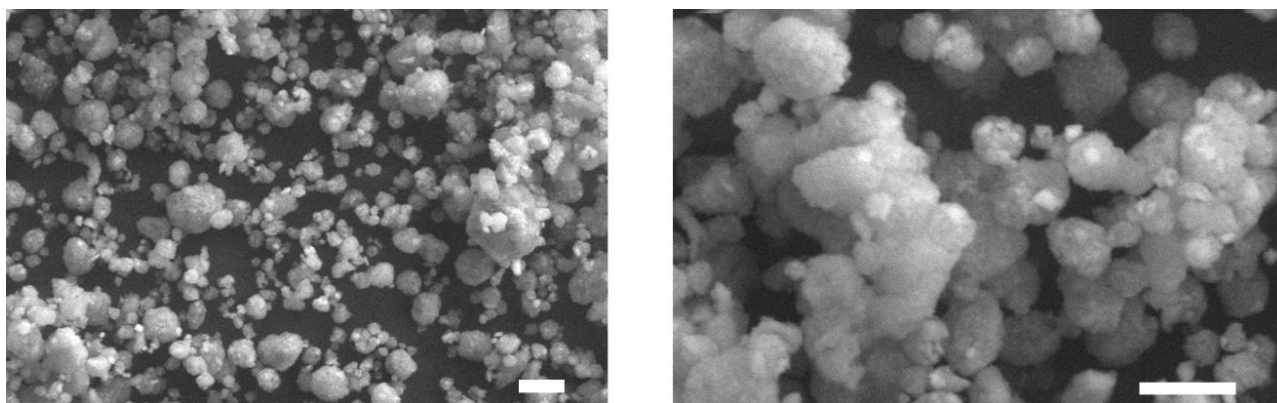


Figure 3. SEM images of the spray dried bacterial cellulose. Scale bars 10 µm

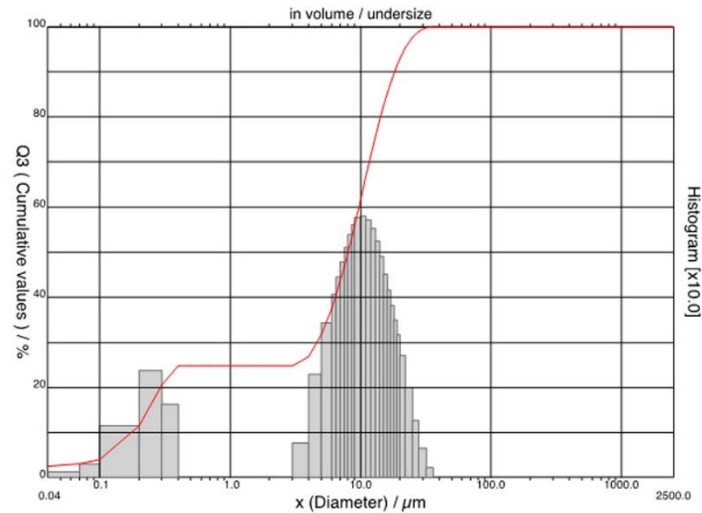


Figure 4. Size distribution of the spray dried bacterial cellulose

4.1.2. Wood characterization

Results regarding wood species identification and physical analyses are reported in Tab. 9. The softwood samples were referred to stone pine (*Pinus pinea* L.), silver fir (*Abies alba* Mill.) and cypress (*Cupressus sempervirens* L.). Regarding the hardwood samples, the sample FTdis 9 was identified as elm (*Ulmus* sp.) while the blocks obtained from the wooden pier belonged to the evergreen oak group and can be probably attributed to holm oak (*Quercus ilex* L.).

Tab. 9 Results of physical characterization of wood samples

Archaeological sample	Wooden species	$D_{bd} (g \times cm^{-3})$	MWC (%)	RD_b (%)
P2550	<i>Pinus pinea</i>	0.31	254	61
FTdis 4	<i>Abies alba</i>	0.41	193	108
FTdis 9	<i>Ulmus</i> sp.	0.34	250	61
FTdis 48	<i>Cupressus</i> sp.	0.25	331	41
2514.28	<i>Quercus</i> cfr. <i>ilex</i>	0.15	616	20
2514.30	<i>Quercus</i> cfr. <i>ilex</i>	0.12	741	17
2514.34	<i>Quercus</i> cfr. <i>ilex</i>	0.14	648	19

Micro-morphological analyses of wood thin sections allowed for observing the microbial decay of the tissue which was mainly ascribed to erosion bacteria (Fig. 5A), even though fungal hyphae and spores were observed in several sections (Fig. 5B). The observations carried out in polarized light showed a severe loss of the birefringence typical of cellulose (Fig. 5C, D), a sign that the crystalline structure of the polymer was almost completely lost due to microbial attack.

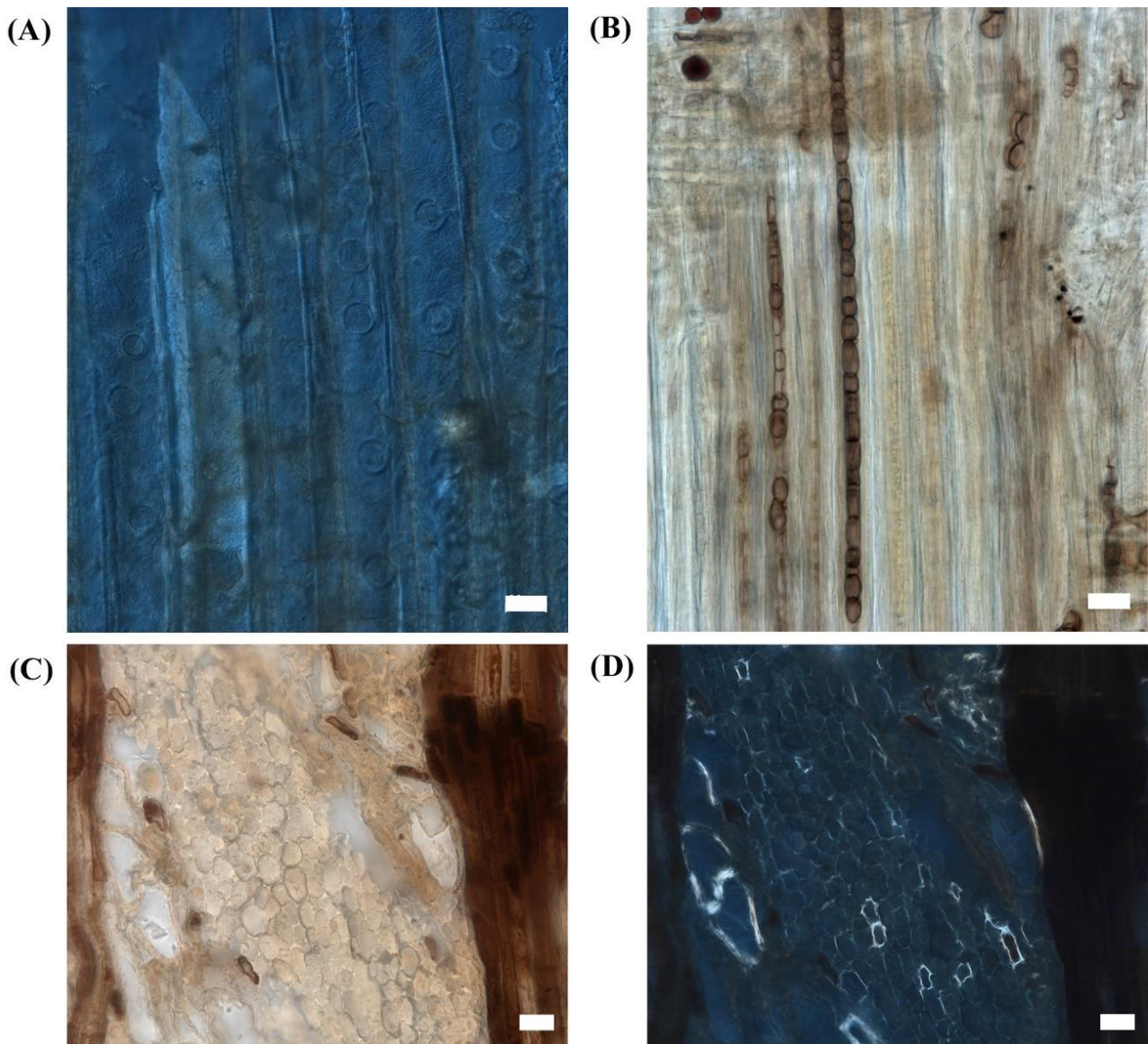


Figure 5. Micro-morphological characterization of microbial decay. (A) Sample FTdis4 – silver fir, radial section stained with methylene blue: signs of degradation produced by erosion bacteria are well visible on the cell walls; (B) Sample FTdis4 – silver fir, radial section: fungal spores present inside the wood cells; (C-D) Sample FTdis9 – elm, cross section observed in bright-field (C) and polarized light (D): a severe loss of cellulose birefringence highlights that the crystalline structure of the polymer was almost completely lost due to the microbial attack. Scale bars 20 μm.

The physical parameters used to assess the decay showed different levels of degradation for the selected woods. The highest MWC values were recorded for the holm oak samples (616, 741 and 648% respectively). For the three poles the residual density ranged between 17 and 20%. According to the degradation classes established by De Jong (Grattan and Clarke, 1987) and by McConnachie et al. (2008) these samples can be considered as highly degraded.

The stone pine samples were the best preserved of the analyzed woods with MWC value of 254% and RD_b of 61%. Cypress showed an intermediate level of degradation with an MWC of 331% and an RD_b of 41%. According to De Jong these woods belong to the intermediate class of degradation.

Silver fir and elm samples deserve a special mention. Based on the MWC values (193% and 250%) these samples resulted as the best preserved of all analyzed wood. However, the fir RD_b value (108%) and the fact that elm was much more degraded (spongy tissue, not resisting to the cut) with respect to wood with similar MWC indicated that the results could not be considered as reliable. The wood was characterized by the presence of several shipworm tunnels filled with sediments. During the cleaning operations carried out before the tests it was not possible to remove all the sediments from the samples, especially from the innermost part. It is known that the presence of sediments and/or mineral depositions inside archaeological waterlogged wood

influences the RD_b values (Schniewind, 1990), so the unreliable results obtained for the physical analyses must be attributed to this cause.

4.1.3. Evaluation of efficacy of consolidation treatments

Macroscopic observation of treated samples and comparison between consolidated and un-consolidated wood allowed for a first evaluation of the effect of the respective consolidation treatments.

In contrast to air-dried controls, all treated samples and freeze-dried controls apparently maintained the original shape and no dimensional differences were perceivable by naked eye between differently consolidated blocks and freeze-dried controls (Figs. 6-8). In few cases, of both consolidated and freeze-dried blocks, small pieces of wood had detached during consolidation or drying procedure and small openings were present on the samples' surfaces.

Wood color and aspect were widely affected by consolidation treatments. LNPs treated samples resulted in dark brown color and a fine brown powder was present on the sample surface, even after freeze-drying. BC consolidated blocks were whitish or yellowish in color. When broken for SEM observation, it was evident that the color change had occurred throughout the block thickness. Finally, CNCs treated samples showed a color similar to the controls, but a thin, colorless film was present on almost all the surface of the blocks. That film was easily removable by touching the wood.

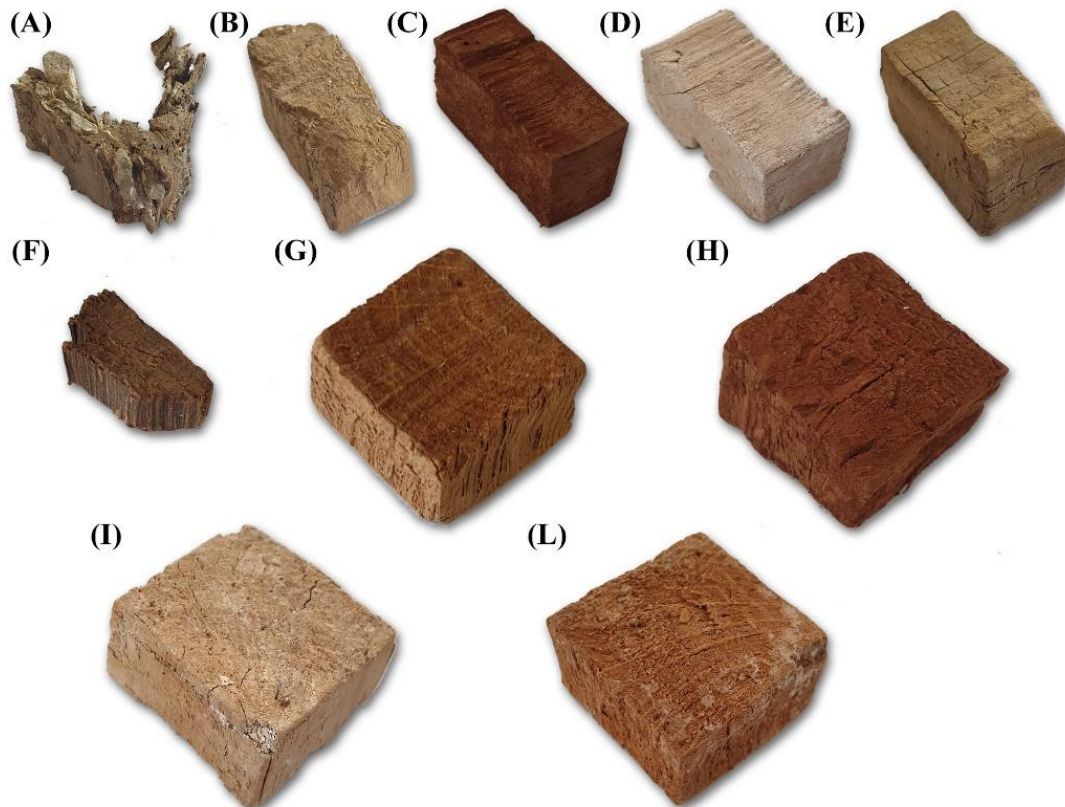


Figure 6. Aspect of selected treated blocks and controls. (A) AS3 – silver fir; (B) FS3 – cypress; (C) LS1 - stone pine; (D) BCS1 - stone pine; (E) CNCS3 – cypress; (F) AH3 - elm; (G) FH4 - holm oak; (H) LH4 - holm oak; (I) BCH6 - holm oak; (L) CNCH7 - holm oak

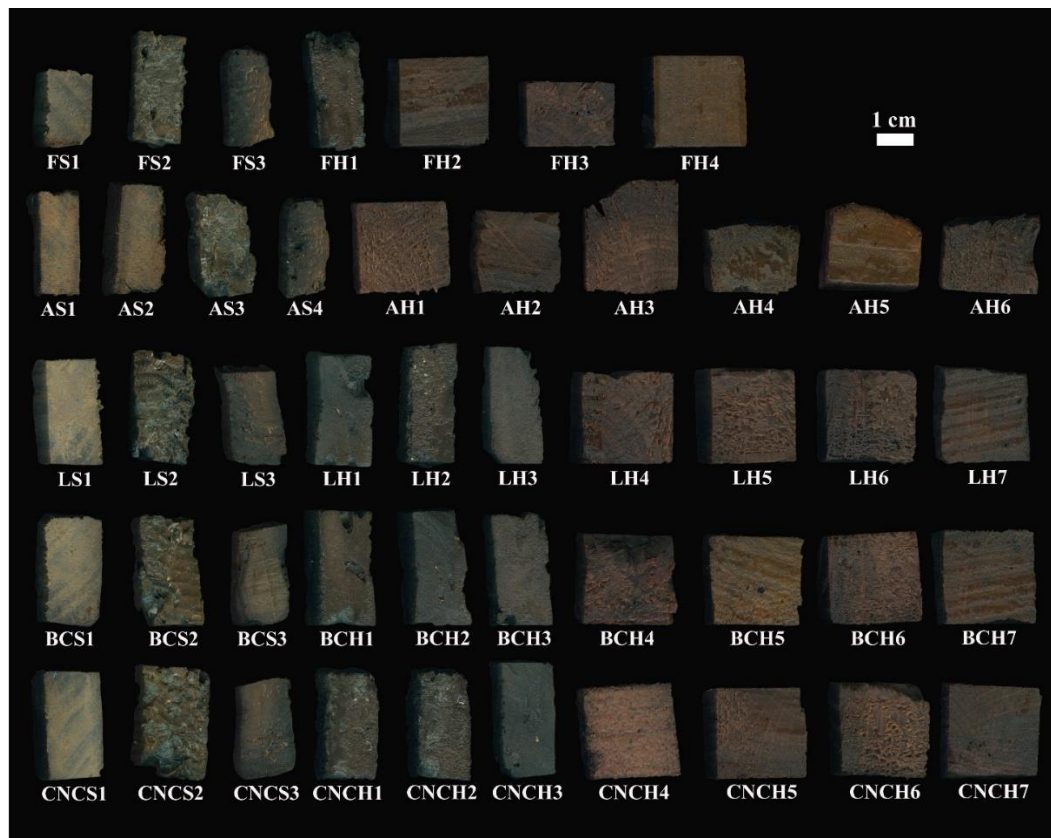


Figure 7. Aspect of the waterlogged blocks before the beginning of the consolidation treatment.

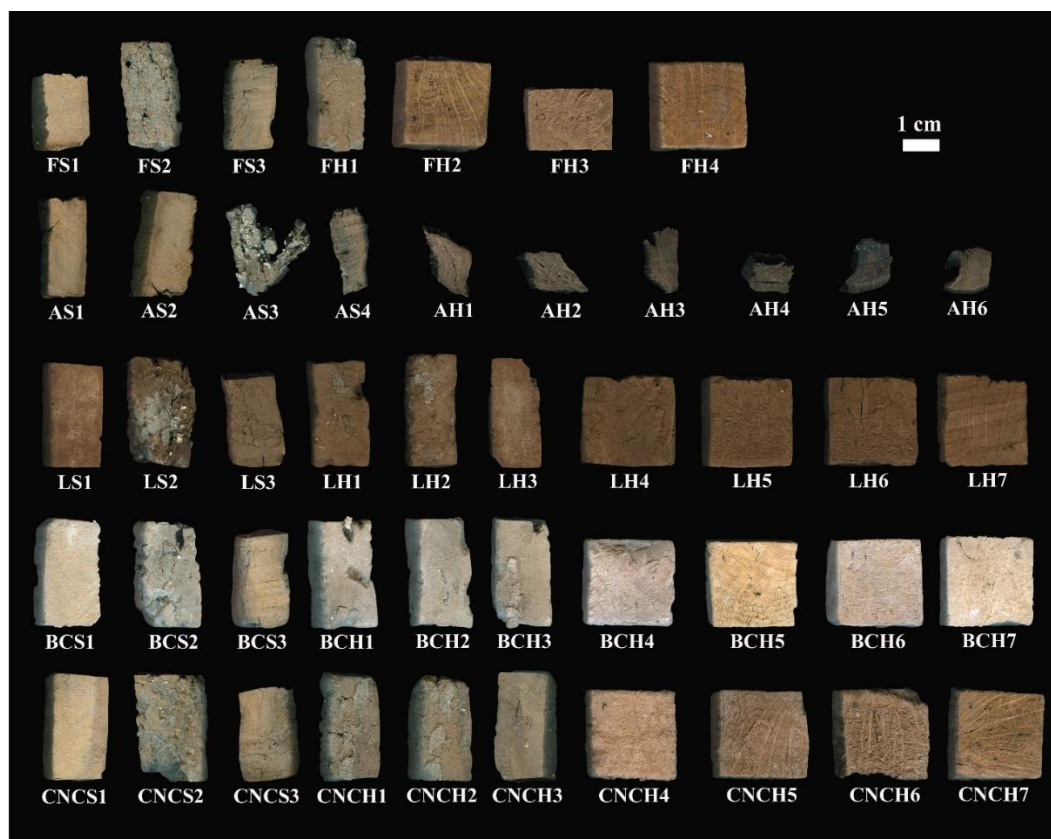


Figure 8. Aspect of the freeze-dried controls, air-dried controls and treated blocks after the consolidation treatment and lyophilization. Wood equilibrated at 20 °C, 50% RH.

SEM observations of the treated wood samples allowed for observing how the nanoparticles had penetrated inside the wood tissues. The analysis of a control un-consolidated block showed that in the cross sections, both external and internal (Fig. 9A,B), cell lumens were empty and in several cases the secondary cell walls appeared mostly detached from the middle lamella (ML) with only thin filaments connecting the cell wall to the ML (Fig. 9B). In radial section (Fig. 9C) the vessel and fiber walls appeared smooth and pits were well visible.

In the LNPs treated block a layer of nanoparticles was observed on the external cross section (Fig. 9D). Most of the cell lumens were covered with compact deposits of LNPs. In other cases, a coating of nanoparticles was well visible covering the whole cell lumen wall. Observing the radial section, it resulted that LNPs penetrated inside some vessels up to a depth of ca 1.2 mm and formed a more or less compact and irregular layer covering the cell walls for the first 400 μm (Fig. 9E). Only few sporadic particles penetrated deeper into the wood tissue and a part of wood vessels and fibers appeared completely empty (Fig. 9F).

On the external cross section of the BC treated block, only few sporadic deposits of nanoparticles were observed. Most of the cell lumens were filled with consolidant, the rest seemed empty and the secondary cell walls detached from the ML were well visible. Observing the internal cross section of the block, in most cases the degraded secondary cell walls appeared as “tubes” coming out of the section (Fig. 9G). In other cases, only the ML was present and the cell lumen was empty. In the radial section, a homogeneous and compact layer of BC was observed on the cell walls of both vessels and fibers throughout the thickness of the block, covering most part of the pits (Fig. 9H, I).

In the external cross section of the CNCs treated block, the cell lumens were empty and only in some cases a thin layer of CNCs was observed on the surface of the cell walls (Fig. 9L). Nanoparticles had penetrated up to a depth of ca 1.3 mm forming a compact and homogeneous coating that covered almost completely the pits, as visible in radial section (Fig. 9M). At greater depths, the CNCs layer was not visible anymore thus cell walls appeared smooth like observed for the control sample and all pits were well visible (Fig. 9N).

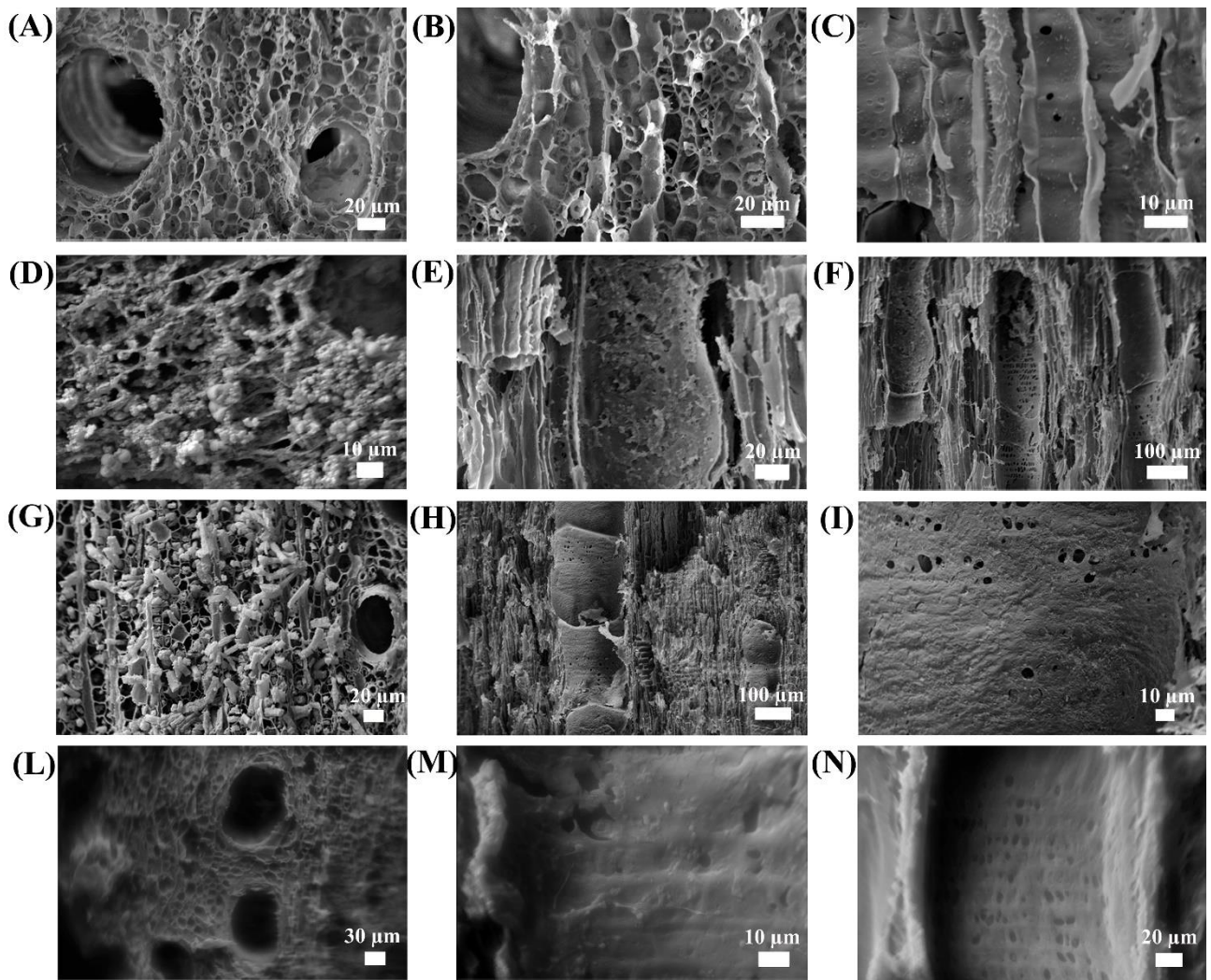


Figure 9. SEM images of freeze-dried control and treated blocks. (A-C) FH2; (D-F) LH5; (G-I) BCH5; (L-N) CNCH5. (A-B) FH2 external and internal cross sections: cell lumens are empty, secondary cell walls appear almost completely detached or linked by thin filaments to the middle lamella; (C) FH2 radial section: vessel and fiber walls appear smooth and the pits are well visible; (D) LH5 external cross section: a layer of nanoparticles is present on the surface, sometimes a coat of nanoparticles is visible inside the cells, all around the lumen; (E-F) LH5 radial section: LNPs are present inside some of the vessels forming a layer on the cell walls for the first 400 μm , the other vessels and the wood fibers are empty; (G) BCH5 internal cross section: the degraded secondary cell walls appear as “tubes” coming out of the section; (H-I) BCH5 radial section: a homogeneous and compact layer of BC is visible on the cell walls of both vessels and fibers, covering most part of the pits; (L) CNCH5 external cross section: the cell lumens are empty and only in some cases a thin layer of CNCs is visible on the surface of cell walls; (M) CNCH5 radial section: nanoparticles formed a compact and homogeneous coat almost completely covering the pits; (N) CNCH5 radial section: at depths greater than 1.3 mm the CNC layer is no more visible, the cell walls appear smooth and the pits are well visible

As shown, the results obtained with the three nano-scale consolidants were quite different. Shape and dimensions of the nanoparticles did not affect the treatment. In fact, CNCs showed almost the same penetration behavior as LNPs despite being much smaller and having a needle-like shape that initially suggested a greater ease of penetration inside the cells. In contrast, the BC nanoparticles penetrated evenly inside the material despite a more irregular shape and larger particle size.

The scarce LNPs penetration may be mainly linked to the nanoparticles hydrophobicity that lead to their aggregation and precipitation during the consolidation procedure and could present an obstacle for the penetration into the water-soaked samples. Further, substances present in the sediments inside the wood could have influenced the suspension's pH accelerating this phenomenon.

As mentioned above, the CNCs impregnation bath turned into a gel during the treatment, indicating that nanoparticles interacted with each other forming the gel matrix and thus preventing successful penetration inside the sample material. BC nanoparticles showed no interaction with each other and penetrated inside the

wood not filling up the voids of the structure but creating a compact layer on cell walls. This observation suggested that the consolidant interacted with OH groups available from cellulose and/or degraded lignin in the cell walls. The presence of “tubes” coming out the internal cross section, not observed in the control block, suggested that in the block treated by BC the nanoparticles had impregnated the detached cell walls and thus prevented breaking of the cell walls during splitting of the block. Nevertheless, the consolidation was not successful enough to allow the secondary walls to re-join the ML. Indeed, while in the control block in some cases subtle connections were observed between ML and secondary wall (Fig. 9B), in the BC treated wood these connections got lost. Probably, the BC present in the walls suffered from stress during freeze-drying that broke these links. The absence of this desired consolidant effect explains the fragile appearance of the treated wood, material loss and creation of openings in the blocks during treatment.

The nanocellulose shape affects how it interacts with the consolidated material. BC and CNCs penetrate inside the wood and precipitate on the cell walls’ surface creating hydrogen bonds with the wall components. Christensen et al. (2012) report of an attempt of consolidation of waterlogged archaeological wood by using cellulose whiskers, rod-like nanoparticles 15 nm in diameter and 200-300 nm in length. The authors observed also in their case problems with flocculation and an altered wood aspect caused by the superficial adhesion of nanoparticles. However, a consolidation effect was observed, the whiskers acted as gap fillers interacting between each other and creating an open net-structure inside wood tissues.

4.1.4. Retention of impregnation products and anti-shrink efficiency

Table 10 shows the retention of consolidant (Y) and the m_{WNT} used to calculate Y for the treated blocks. Y was lower than 20% for all the samples, except for BCS2, and in 13 of the 27 blocks negative values for Y were registered. For LNPs and BC, the lowest values were obtained for blocks S3, H1, H2 and H3 corresponding to the cypress and elm samples from Naples. For CNCs the lowest values were registered for the blocks S1, S3, H3 and H4, with S1 corresponding to the sample from Isola Sacra. The highest Y (47.93%) corresponded to the block BCS2. As reported in Giachi et al. (2010) the parameter Y is calculated as the increase of weight of treated wood with respect to the weight of the same untreated sample. The latter is an estimation obtained by attributing to each sample the same MWC of the nearby sample used for the diagnostic evaluations. Obviously, the use of this estimated weight cause an error in the formula of the retention of consolidants. The low and negative values obtained in the present work for LNPs and CNCs treated samples can be explained assuming that the change in weight due to the nanoparticles penetrated in the outer part of wood blocks was lower than the method’s intrinsic error. In spite of its good penetration observed through SEM, the Y values obtained for BC were very similar to those of LNPs and CNCs. Considering the lightweight of nanocellulose materials, it can be stated that also in this case the weight increase due to the consolidant is negligible.

Tab.10 Values of MWC used in the calculation of m_{WNT} and of the retention of consolidants (Y)

Sample	MWC (%)	m_{WNT} (g)	Y (%)
LS1	254	6.45	0.2
LS2	193	9.34	0.2
LS3	331	5.18	-1.6
LH1	250	7.02	-22.4
LH2	250	7.32	-5.0
LH3	250	6.18	-29.6
LH4	616	11.19	0.1
LH6	741	10.75	8.3
LH7	648	10.61	12.1
BCS1	254	7.90	-0.6
BCS2	193	4.86	47.9
BCS3	331	7.27	-35.0
BCH1	250	7.29	-24.6
BCH2	250	7.28	- 21.2
BCH3	250	8.28	-31.5
BCH4	616	9.83	6.5
BCH6	741	9.77	9.2
BCH7	648	10.79	6.5
CNCS1	254	8.50	-2.3
CNCS2	193	9.89	16.7
CNCS3	331	5.42	-1.5
CNCH1	250	7.04	14.1
CNCH2	250	7.32	10.0
CNCH3	250	7.18	-32.6
CNCH4	616	10.54	-4.4
CNCH6	741	9.09	6.3
CNCH7	648	10.40	0.8

The values of ASE_F , ASE_A and ASE_C are shown in Table 11. The percentage of shrinkage suppressed by the consolidation and freeze-drying process with respect to air-dried controls, ASE_A , was higher than 50% for all samples with the only exception of block CNCS1. More specifically, for all the consolidation treatments ASE_A obtained for hardwood ranged between 80% and 88%, while the values obtained for softwood showed a greater variability between the different consolidants as well as among the blocks treated with the same nanoparticles. In fact, for LNPs treated blocks the registered values ranged between 51% and 69%, for BC treated blocks between 55% and 94%, and for CNCs treated samples between 37% and 66%.

ASE_F compares the volumetric shrinkages of consolidated/freeze-dried blocks to those of unconsolidated/freeze-dried controls representing the contribution of the consolidants to the shrinkage suppression. The results obtained for this parameter were generally lower than those calculated for ASE_A , and for six of the treated blocks negative values were obtained. The highest value was registered for the block BCS2 (75%), the other positive results ranged between a minimum of 2% and a maximum of 46%. The low and negative values obtained for LNPs and CNCs were obviously linked to the fact that the consolidants had penetrated only into a thin layer inside the sample material and thus did not counteract sample shrinkage. The negative value of BCS3 and the low positive values obtained for the others BC treated samples may be linked to the fact that the bacterial cellulose was not able to recreate the bonds between middle lamella and cell wall upon successful sample penetration, as mentioned above.

Regarding the high ASE_A values calculated comparing treated blocks and air-dried controls the effect of freeze-drying must be considered. The values of ASE_C , representing the percentage of shrinkage suppressed by freeze-drying treatment compared to the shrinkage of air-dried controls, confirm the predominant anti-shrink efficiency of freeze-drying. In fact, for hardwood samples ASE_C values ranged between 78% and 80% and for

softwood samples between 51% and 75%. It is known from literature (e.g. Pearson, 2014) that freeze-drying of waterlogged archaeological wood allows for avoiding collapses and reduces shrinkage conserving object shape and dimension. However, the use of lyophilization without a previous consolidation procedure leads to stress-caused cracking in the fragile material. Aspect and consistency of the nanoparticles treated blocks reflected the just mentioned problems, confirming that an effective consolidation was not achieved. As shown in Figure 10A and B no relations were found between Y and MWC or ASE.

Tab. 11 Anti-shrink efficiency of the consolidation treatments with respect to freeze-dried (ASE_F) and air-dried (ASE_A) controls and anti-shrink efficiency of freeze-drying procedure with respect to air-drying (ASE_C)

Sample	V _w (mm ³)	V _D (mm ³)	S (%)	ASE _F (%)	ASE _A (%)	ASE _C (%)
LS1	5861.81	5449.78	7.02	17	60	-
LS2	5403.67	4196.31	22.34	-96	51	-
LS3	4121.53	3567.99	13.43	2	69	-
LH1	5608.21	5001.67	10.81	40	- *	-
LH2	6646.23	5704.47	14.17	21	-	-
LH3	5702.48	4981.53	12.64	30	-	-
LH4	11070.66	9921.53	10.38	43	88	-
LH5	9264.25	7679.67	17.10	7	80	-
LH6	9917.58	8714.65	12.13	28	86	-
LH7	10308.65	9062.89	12.08	36	86	-
BCS1	6320.24	5918.37	6.36	25	55	-
BCS2	5317.96	5164.92	2.88	75	94	-
BCS3	3314.78	2851.31	13.98	-2	68	-
BCH1	6625.52	5748.63	13.23	26	-	-
BCH2	6390.79	5620.76	12.05	33	-	-
BCH3	6342.45	5479.22	13.61	24	-	-
BCH4	9015.00	7552.07	16.23	12	81	-
BCH5	10739.92	9173.65	14.58	21	83	-
BCH6	9509.61	8041.09	15.44	9	82	-
BCH7	9736.55	8260.79	15.16	20	82	-
CNCS1	7580.56	6754.18	10.90	-28	37	-
CNCS2	6322.14	5337.42	15.58	-37	66	-
CNCS3	5700.13	4781.69	16.11	-18	63	-
CNCH1	5377.48	4854.08	9.73	46	-	-
CNCH2	5165.71	4516.12	12.57	30	-	-
CNCH3	6111.08	4970.19	18.67	-4	-	-
CNCH4	9557.40	8067.11	15.59	15	82	-
CNCH5	11624.52	10036.73	13.66	26	84	-
CNCH6	8864.71	7521.15	15.16	10	82	-
CNCH7	12059.45	10306.49	14.54	23	83	-
FS1	4482.83	4101.29	8.51	-	-	51
FS2	4402.86	3902.14	11.37	-	-	75
FS3	3178.20	2742.74	13.70	-	-	69
FH2	11569.56	9446.78	18.35	-	-	78
FH3	5413.94	4500.20	16.88	-	-	80
FH4	8646.58	7014.09	18.88	-	-	78

* these ASE values are missing because the archaeological material was not enough to obtain an air-dried control

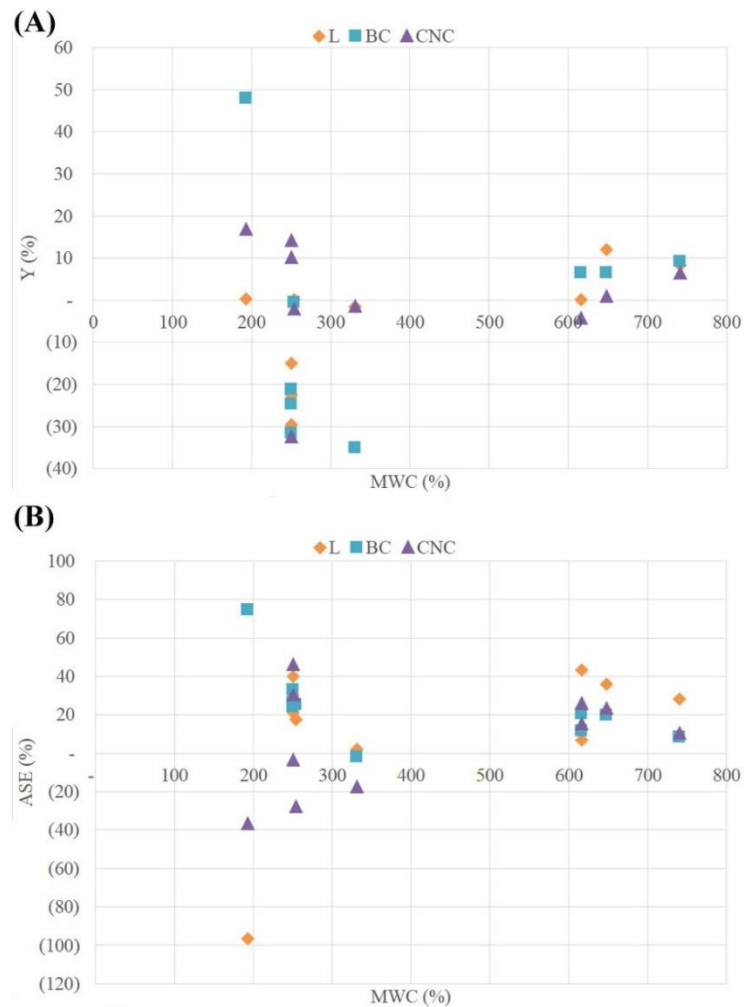


Figure 10. (A) Graph of the retention of consolidant (Y) vs Maximum Water Content (MWC); (B) graph of anti-shrink efficiency (ASE) vs MWC

4.1.5. Equilibrium moisture content

Table 12 and Figure 11 show the values and trends of the EMC of treated blocks compared to freeze-dried controls, sound wood controls (oak and fir) and sound wood values reported in literature. The trends, obtained by averaging the values in Table 12, showed that the EMC of sound wood controls used during the experimentation were lower with respect to literature values. This can be linked to the natural variability of wood. The values obtained for freeze-dried wood were comparable to those of sound controls for all tested RH.

In general, the EMC values obtained for blocks belonging to the same archaeological source but treated with different consolidants varied slightly and not significantly. At 100% RH, the CNCs treated blocks had EMC values slightly lower than wood treated with the other two consolidants and comparable with freeze-dried controls. At the same RH, the LNPs and BC treated blocks showed an EMC in average higher with respect to lyophilized controls (2-3% and 3-8% respectively). However, the observed differences were too low to be considered significant. Based on the obtained results it could be concluded that the moisture equilibrium content of treated samples was not influenced by the hygroscopic features of nano-scale consolidants. Obvious deviations from data uniformity were observed for the blocks S2, both those treated with nanoparticles and the freeze-dried control, CNCH1 and CNCH2 whose EMC values were on average 2-15% lower with respect to the others. This discrepancy can be explained taking into account what already mentioned above (§ 4.1.2.) with respect to fir and elm samples, the presence of sediments inside the wood must have influenced its relation with moisture.

It is interesting to note that in the RH range used for conservative purposes (35-85%), the average EMC values for all blocks never exceeded the 18% considered as the threshold for the risk of biological attacks.

Tab. 12 Equilibrium moisture content (EMC) of treated samples, freeze-dried controls, sound wood controls and sound wood from the literature at every RH stage

Sample	EMC						
	10%	35%	45%	55%	65%	85%	100%
LS1	4.43	8.05	11.24	13.24	13.35	19.67	31.83
LS2	2.84	4.08	4.55	4.85	5.38	8.69	15.87
LS3	4.52	6.59	8.92	9.97	11.55	19.90	32.72
LH1	4.71	7.76	10.84	12.45	13.03	18.04	26.65
LH2	3.29	7.03	9.53	11.13	14.24	17.61	23.74
LH3	5.26	8.44	12.12	14.17	14.53	19.91	32.49
LH4	3.55	6.81	9.95	11.74	11.76	17.30	27.25
LH5	4.29	7.20	10.46	12.14	12.29	17.72	27.57
LH7	3.18	6.43	9.61	11.18	11.07	16.52	25.92
BCS1	4.30	7.53	10.56	12.31	13.06	18.84	31.83
BCS2	2.79	3.77	4.75	5.25	5.74	9.90	17.56
BCS3	4.21	6.14	8.44	9.68	11.35	18.53	33.60
BCH1	4.72	7.48	10.38	11.97	13.03	18.03	27.55
BCH2	4.66	6.91	9.66	11.25	12.15	16.67	28.66
BCH3	4.40	6.84	9.77	11.41	12.52	17.03	25.62
BCH4	4.18	6.39	9.11	10.51	11.53	17.42	40.32
BCH6	4.16	7.01	9.64	11.16	12.79	18.62	36.22
BCH7	4.27	6.75	9.55	10.93	12.26	17.98	35.81
CNCS1	4.36	8.14	11.56	13.46	13.30	19.40	27.04
CNCS2	2.83	3.50	4.26	4.28	4.72	7.10	13.24
CNCS3	4.13	6.25	8.59	9.68	11.26	18.74	32.85
CNCH1	2.69	4.61	6.49	7.60	8.34	10.93	15.99
CNCH2	2.95	4.53	6.40	7.42	8.65	11.28	15.34
CNCH3	4.83	8.08	11.27	12.91	13.15	18.61	28.81
CNCH4	3.35	6.47	9.57	11.36	11.27	16.64	24.69
CNCH6	3.30	6.25	9.46	10.99	10.68	16.09	28.19
CNCH7	3.17	6.50	9.65	11.20	10.83	16.42	26.52
FS1	4.67	8.32	11.59	13.39	13.16	19.02	27.05
FS2	2.76	3.77	4.00	4.25	4.54	7.21	12.72
FS3	4.68	6.60	8.95	10.06	11.55	20.22	33.53
FH1	3.31	6.29	9.67	12.12	14.09	17.05	22.87
FH3	4.08	6.65	9.83	11.08	10.85	15.96	22.93
FH4	3.29	6.45	9.67	11.31	10.77	16.36	26.34
Oak1	3.79	6.67	9.32	10.83	10.45	16.66	27.22
Oak2	4.31	6.55	9.20	10.71	10.17	16.26	23.87
Oak3	4.22	7.24	9.88	11.51	10.69	16.05	25.07
Oak4	4.60	7.23	9.85	11.48	10.26	14.37	24.08
Oak5	4.28	6.97	9.53	11.15	10.33	15.17	23.91
Oak6	3.94	6.75	9.29	10.94	10.23	15.58	24.43
Fir1	3.41	6.46	9.34	10.68	11.21	15.62	21.90
Fir2	3.56	6.58	9.39	10.68	11.09	15.75	21.92
Fir3	3.77	6.71	9.67	10.88	11.52	16.05	22.42
Fir4	3.78	6.66	9.57	10.79	11.52	15.91	22.14
Sound wood	3	7	9	10	12	18	30

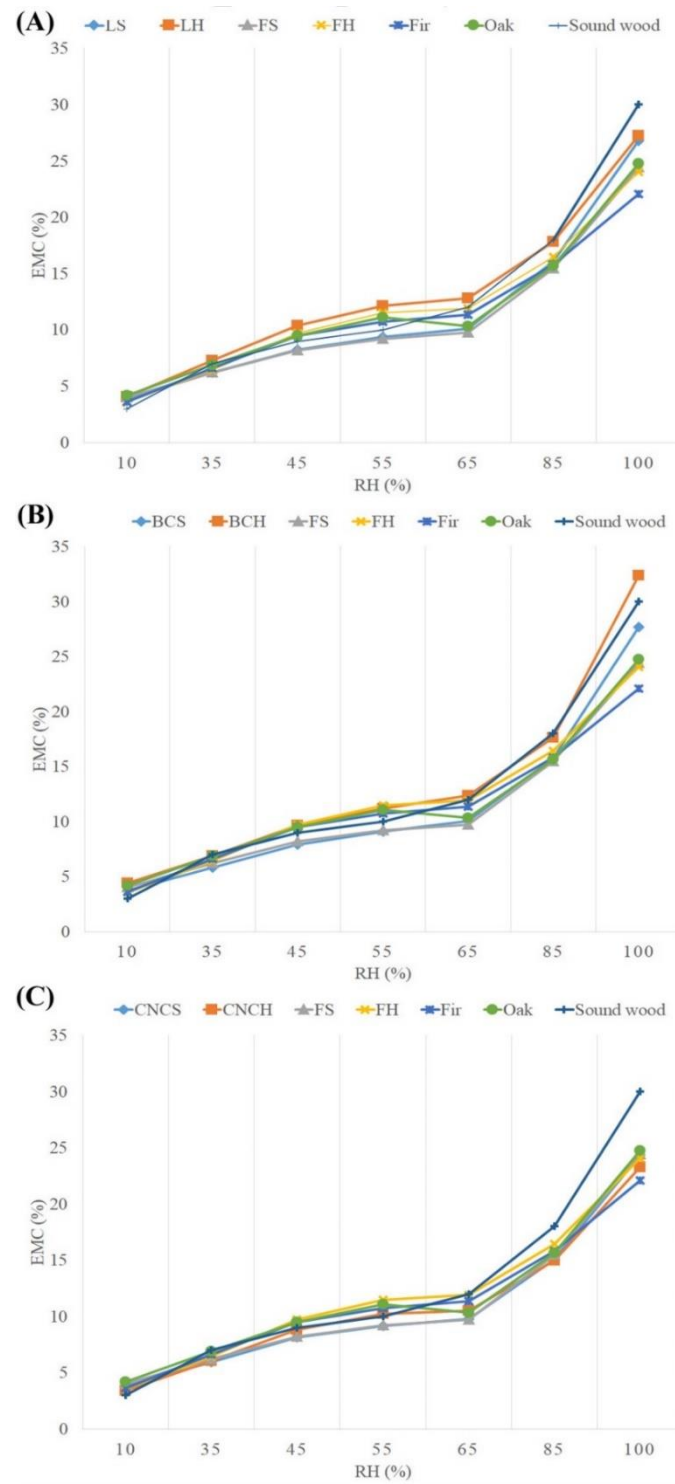


Figure 11. Equilibrium moisture content (EMC) trends of treated blocks compared to freeze-dried controls, sound wood controls and sound wood values obtained from the literature. (A) LNP; (B) BC; (C) CNC

4.2. Waterlogged wood preservation

4.2.1. ATP bioluminescence

The quantification of ATP through bioluminescence is currently widely used and a variety of technical options exist for the extraction and detection of ATP from bacterial cells (Hoffner et al., 1999; Janaszek et al., 1987; Lundin and Thore, 1975; Stanley et al., 1989). Instead, protocols for ATP extraction and quantification from

fungus cells and spores are limited. The comparison of the three extraction/detection protocols was aimed to find a robust and cost-effective analytical tool for routine biomonitoring fungi.

Table 13 reports the values of mean RLU and ATP concentration obtained for each fungal strain with the three protocols. Fig. 12 compares the ATP concentrations values and Fig. 13 shows the percentage efficacy ratios of protocols 1 and 2 compared with protocol 3 that allowed obtaining the best results (set as 100%).

Tab. 13 Mean RLUs, standard deviation and ATP concentration obtained for *Aspergillus japonicus*, *Fusarium* sp. and *Chaetomium* sp. with the three tested protocols

<i>Aspergillus japonicus</i>				
	Suspension	Cellular ATP (Mean RLU)	Standard deviation	ATP concentration (ng/ml)
Protocol 1	A	6,543,417	493,159	47.6
	B	1,801,194	117,168	13.1
	C	620,384	78,219	4.4
	S	1,994	721	0
Protocol 2	A	7,244,445	1,151,998	53.3
	B	5,687,961	515,578	41.7
	C	2,606,873	334,001	19
	S	5,417	1,332	0
Protocol 3	A	39,926,420	1,106,106	294.7
	B	15,114,552	650,721	111.4
	C	5,798,030	172,802	42.7
	S	2,522	810	0
<i>Fusarium</i> sp.				
	Suspension	Cellular ATP (Mean RLU)	Standard deviation	ATP concentration (ng/ml)
Protocol 1	A	7,586,927	225,646	55.8
	B	2,560,273	243,469	18.7
	C	951,713	125,057	6.9
	S	401	169	0
Protocol 2	A	11,360,793	955,166	83.7
	B	3,845,934	709,902	28.2
	C	1,384,797	87,741	10.1
	S	1,190	659	0
Protocol 3	A	15,168,799	985,948	111.8
	B	4,675,032	233,061	34.4
	C	2,393,679	32,314	17.6
	S	4,196	4,203	0
<i>Chaetomium</i> sp.				
	Suspension	Cellular ATP (Mean RLU)	Standard deviation	ATP concentration (ng/ml)
Protocol 1	A	482,371	368,451	3
	B	172,481	15,676	1.1
	C	54,833	3,280	0.27
	S	3,899	787	0
Protocol 2	A	442,246	65,010	3.1
	B	476,511	42,434	3.2
	C	119,401	14,474	0.7
	S	630	453	0
Protocol 3	A	1,333,803	78,001	9.5
	B	1,104,589	46,719	7.8
	C	205,163	774	1.2
	S	1,691	1,483	0

For *Aspergillus japonicus* (Fig. 12A), Protocol 3 allowed to register the highest ATP concentration for A, B and C suspensions, so the Lysis reagent C can be considered as the best extraction reagent. The Lysis reagent A seemed to be the less effective against the spores of this fungal strain, with ATP values corresponding to

10-16% of Protocol 3, while the Protocol 2 (Lysis Reagent B) allowed to obtain intermediate concentration values (45-19% of Protocol 3 value) (Fig. 13A).

Also for *Fusarium* sp. (Fig. 12B), Protocol 3 resulted the best one allowing to obtain the highest values of ATP concentration. Fig. 13B clearly shows that Protocol 2 accounted for 57-82% of Protocol 3 values while Protocol 1 registered lower concentrations corresponding to 39-54% of the highest ones.

Protocol 3 gave the best results also for *Chaetomium* sp. (Fig. 12C) but in this case the ATP concentrations were much lower with respect to the other fungi. For this strain, Protocol 2 allowed obtaining results accounting for 32-54% of Protocol 3 values (Fig. 13C), while Protocol 1 gave the lowest concentration levels equal to 22% and 13% of Protocol 3 values. It is necessary to underline that for this strain Protocol 1 and 2 seem to lack in sensibility for low ATP concentrations. In fact, for the less concentrated spore suspension (C), Protocol 1 and 2 allowed obtaining values close to zero (0.27 and 0.7 ng/ml, respectively). Furthermore, the graphic reported in Fig. 12C clearly highlights some incongruences in the trend of the curves (e.g. for Protocol 2 the concentration value obtained for B suspension is higher with respect to A). These results can be explained by hypothesizing that, for *Chaetomium* sp., the amount of Tween 20 added to the saline solution of the spore suspension was not enough to prevent spore aggregation.

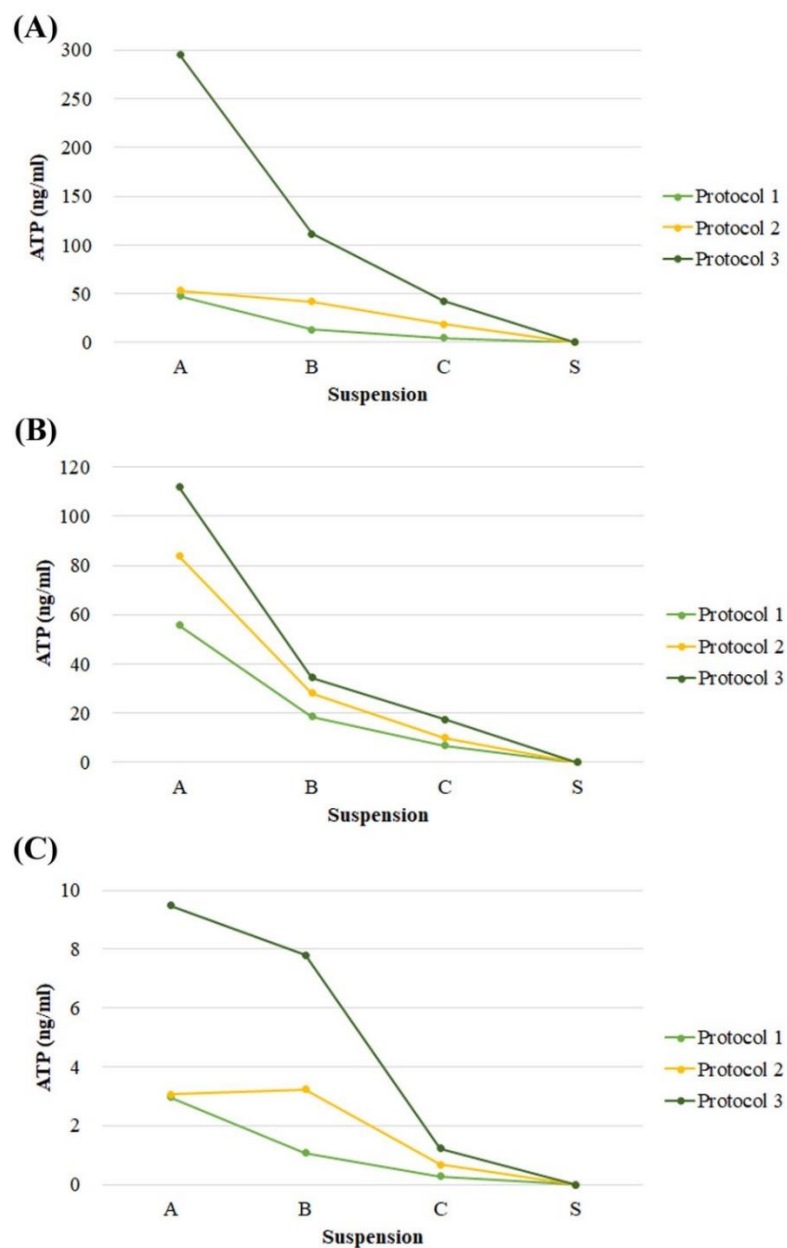


Figure 12. Comparison of the ATP concentrations (ng/ml) obtained with the three protocols for the spore suspensions of *Aspergillus japonicus* (A), *Fusarium* sp. (B) and *Chaetomium* sp. (C)

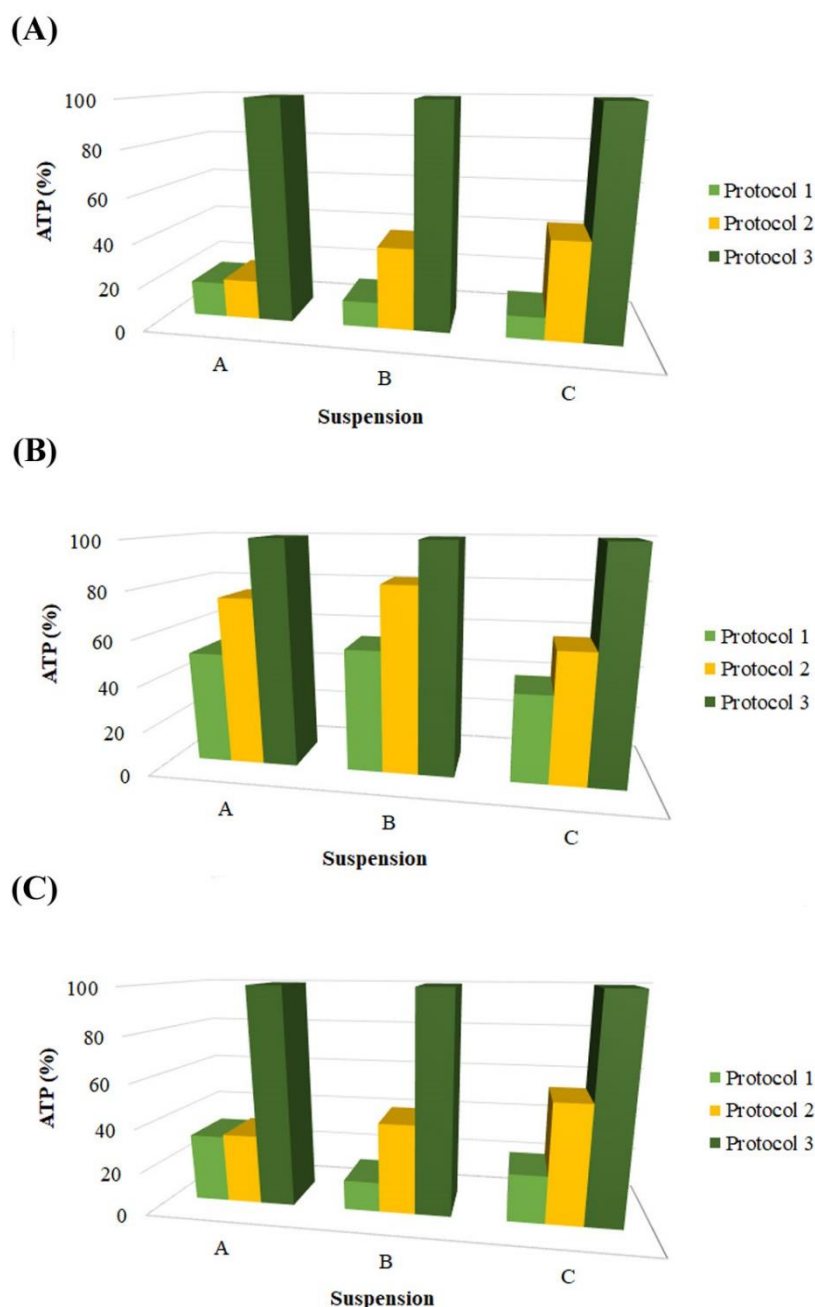


Figure 13. Percentage efficacy ratios of the three protocols. Protocol 3, which had given the best results, was set as 100%. *Aspergillus japonicus* (A), *Fusarium* sp. (B) and *Chaetomium* sp. (C)

All the graphics reported in Fig. 12 show that the curves obtained with the protocol using Lysis Reagent C have the same trend for all fungal strains and that this reagent, used at 100 °C, acted as the best extraction reagent in all the performed tests. Considering these results Protocol 3 was selected for the tests on the EOs biocidal activity.

Of note, comparing the ATP concentration values obtained for all the analyzed fungal strains with this method (Fig. 14) it is evident that suspensions with similar spore concentration gave different results depending on the fungus. In particular, *A. japonicus* had the highest ATP concentration values, reaching almost 300 ng/ml for A suspension, *Fusarium* sp. gave intermediate results with an ATP concentration for A slightly higher than 111 ng/ml, while *Chaetomium* sp. had the lowest values with ca. 9.5 ng/ml for the most concentrated spore suspension. Two different explanations can be hypothesized to clarify these results. It is well known that the fungal cell wall has an extremely complex chemical composition that varies depending on several factors, such as the growth phase, the age of the culture, the environmental conditions of growth, the carbon and nitrogen

sources, ion concentration, temperature, pH, illumination, and so forth (Ruiz-Herrera, 2016). Furthermore, several studies demonstrated that the cell wall composition can be different in several taxonomic groups, not only among phyla or classes but also at genus or species level (Bartnicki-Garcia, 1968; Crook and Johnston, 1962; Mitchell and Taylor, 1969; Ruiz-Herrera, 2016; Schoffemeer et al., 1999). The analyses performed during the present work involved, as said, spore suspended in the same medium, sterile saline solution containing 0.01% (v/v) Tween 20, and stored at the +4 °C for the same time frame. Furthermore, the spores were obtained from mycelia grown for the same amount of time on Malt Extract Agar. Therefore, the only variable to which the composition of the wall can be linked is the different taxonomic groups. As already highlighted by Rakotonirainy et al., 2003, DMSO have a different interaction, and so a different lysis effect, with differently composed cell wall, and so the differences in ATP concentration observed among the analyzed strains could be attributed to a difference in the lysis efficiency.

The other possible explanation to the data reported in Fig. 14 is that the difference in ATP levels is related to the species: different species are characterized by different cellular ATP content. The works carried out by Rakotonirainy and colleagues (Rakotonirainy et al., 2003a, 2003b; Rakotonirainy and Arnold, 2008) on spore suspensions of ten fungal strains confirm this statement.

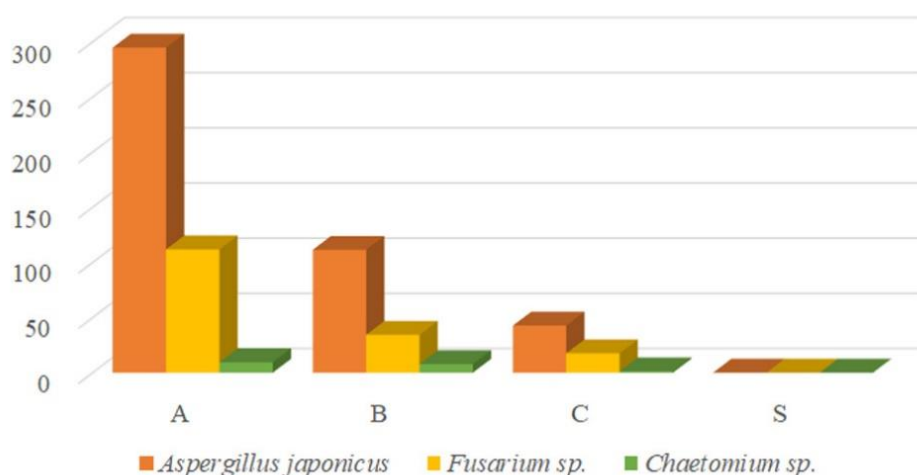


Figure 14. Comparison of ATP concentrations (ng/ml) in the three analyzed strains evaluated through Protocol 3.

4.2.2. Next-generation sequencing (NGS)

NGS has been rarely applied to the study of the microbiota present in waterlogged archaeological wood. Due to the novelty of the application, the preliminary test aimed to set a correct procedure for the extraction of microbial DNA from wood was necessary.

Table 14 reports the values of DNA concentration and purity obtained for the two protocols. It is evident that for all the double extracted samples, the DNA concentration obtained with Protocol 1 is higher with respect to that of Protocol 2. The purity of the extracted DNA was evaluated in term of the ratio of absorbance at 260 nm and 280 nm. A ratio of ~1.8 can be considered as “pure”, if the ratio is appreciably lower or higher it may indicate the presence contaminants like proteins or phenols. Also in this case, the Protocol 1 gave us best results. The high impurity of some of the samples treated with Protocol 2 may probably indicate that the kit’s reagents acted also on wood extractives. This hypothesis is confirmed by the dark color of some of the DNA suspensions.

Table 14. Values of DNA concentration and purity obtained for the two extraction protocols.

Protocol 1 - Maxwell® RSC Plant DNA Kit			Protocol 2 - Maxwell® RSC PureFood GMO and Authentication Kit		
Sample	DNA concentration (ng/μl)	260/280 Ratio	Sample	DNA concentration (ng/μl)	260/280 Ratio
1N1	33.5	1.73	2N1	13.2	1.38
1N4	49.8	1.29	2N4	8.5	1.63
1N9	19.5	1.36	2N9	7.3	2.56
1N39	45.7	1.20	2N39	5	3.34
1N88	-	-	2N88	4	22.78
1PF	233.6	0.98	2PF	123.3	1.05
1PO	67.3	1.27	2PO	40.1	40.1
1PQ	780.4	0.91	2PQ	35.9	35.9
1PL1	170.8	1.11			
1PL2	551.7	0.96			

The dendrogram showed in Fig. 15 depicts UPGMA clustering of the samples treated with both protocols based on weighted community similarity. It is interesting to observe that the community composition obtained with the two extraction method for the same wood sample is always very similar. This let hypothesizing that the results obtained are not biased by an extraction limit and that can be considered as representative of the actual microbial community.

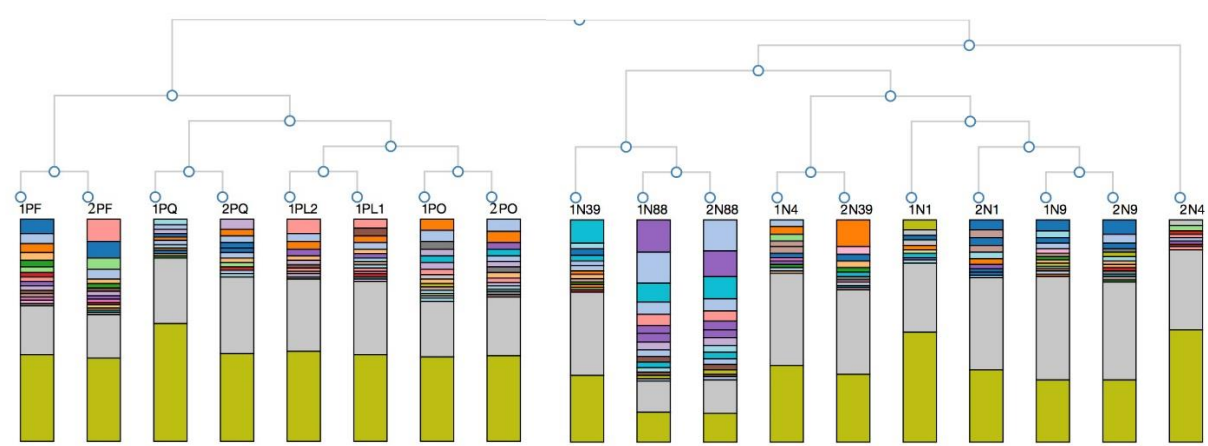


Figure 15. UPGMA clustering of the samples treated with both protocols based on weighted community similarity

The Principal Component Analysis (PCA) with respect to order, as expected, well separates N woods from P ones (Fig. 16).

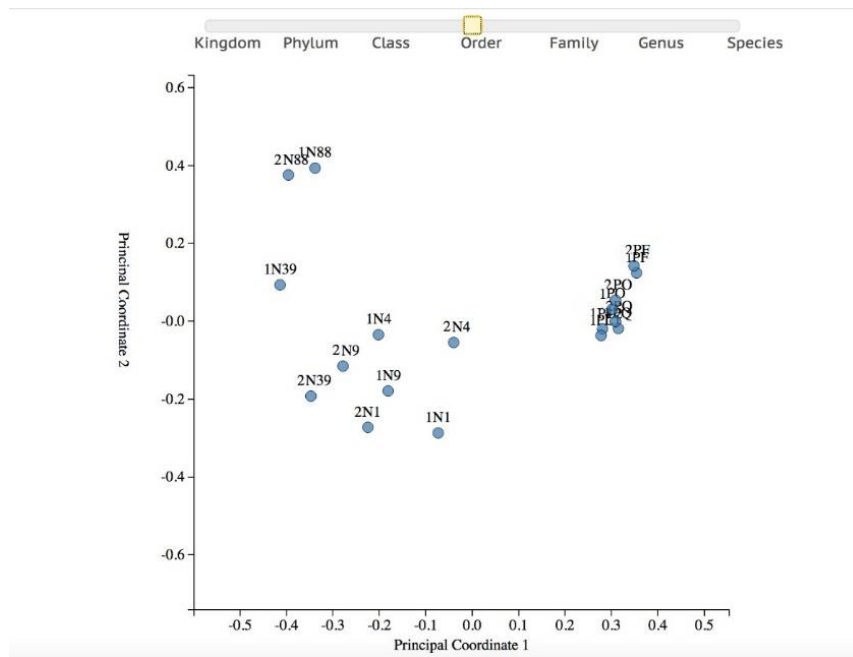


Figure 16. Principal Component Analysis with respect to order

Considering the obtained results, Protocol 1 was chosen as principal protocol for the DNA extractions step and performed for the rest of the experimentation.

Taken together, these results allowed the characterization of the bacterial and fungal communities present in the analyzed samples and a comparison of the microbiome composition between woods coming from the two archaeological sites.

Bacterial community

The Beta diversity reported in Fig. 17A shows the generic distribution of microbial community considering the difference in the bacterial communities present in Naples and Pisa samples. The graphic clearly highlight that the samples cluster in two well-defined groups, each representing an archaeological site. Alpha diversity, based on observed OTUs and on Chao1/ACE/Shannon/Simpson indices, indicates the richness and the biodiversity of each sample community. Fig 17B shows the boxplot of Shannon index in the two sites. The median Shannon values for the two communities are similar but the index varies in a wider range for Naples samples.

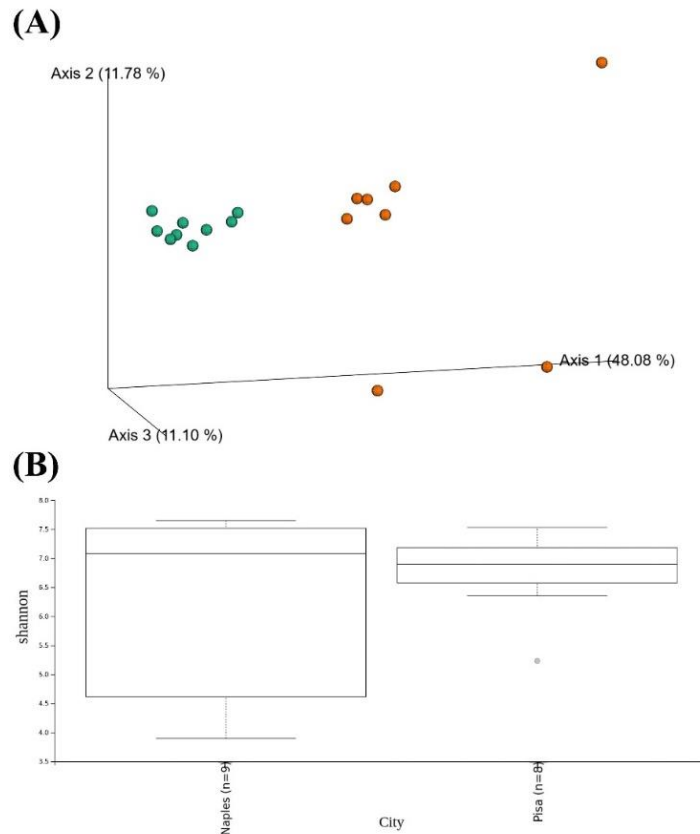


Figure 17. Ecological parameters of 16S rRNA gene sequencing data. (A) Beta diversity represented by Principle Coordinate Analysis Emperor plot on a Bray-Curtis distance matrix. Green dots: Naples, orange dots: Pisa. (B) Boxplots of Alpha-diversity Index (calculated as Shannon index) in the two archaeological sites

The sequencing results showed that the bacterial communities of almost all the analyzed samples were dominated by taxa belonging to the phyla Proteobacteria, Acidobacteria, and Planctomycetes. In particular, Proteobacteria accounted for more than 50% of the total reads in all the Naples samples, reaching 96% for N88, and for more than 30% for Pisa woods. The phylum Bacteroidetes was well represented in Naples communities, in some cases reaching relative frequencies of 10-20%, while it accounted for less than 5% in Pisa samples. Opposite results were registered for the phylum Chloroflexi.

At class level, the communities of N samples were mainly composed by Gammaproteobacteria, Alphaproteobacteria, Planctomycetacia, and Bacteroidia with a neat prevalence of the first taxon. Instead, Alphaproteobacteria dominated in P samples and together with Gammaproteobacteria, Planctomycetacia, and Acidobacteriia accounted for more than 60% of the whole communities (Fig. 18A).

The communities of Naples samples were mainly composed of microorganisms belonging to the families Pseudomonadaceae (g. *Pseudomonas*), Burkholderiaceae (g. *Janthinobacterium*), Methylophilaceae, Pirellulaceae, and Xanthobacteraceae. Instead, the genera of the families Xanthobacteraceae (g. *Pseudolabrys*), Caulobacteraceae, Solibacteraceae (g. *Bryobacter*), and Hyphomicrobiaceae (g. *Hyphomicrobium*) were enriched in P samples (Fig. 18B and 19).

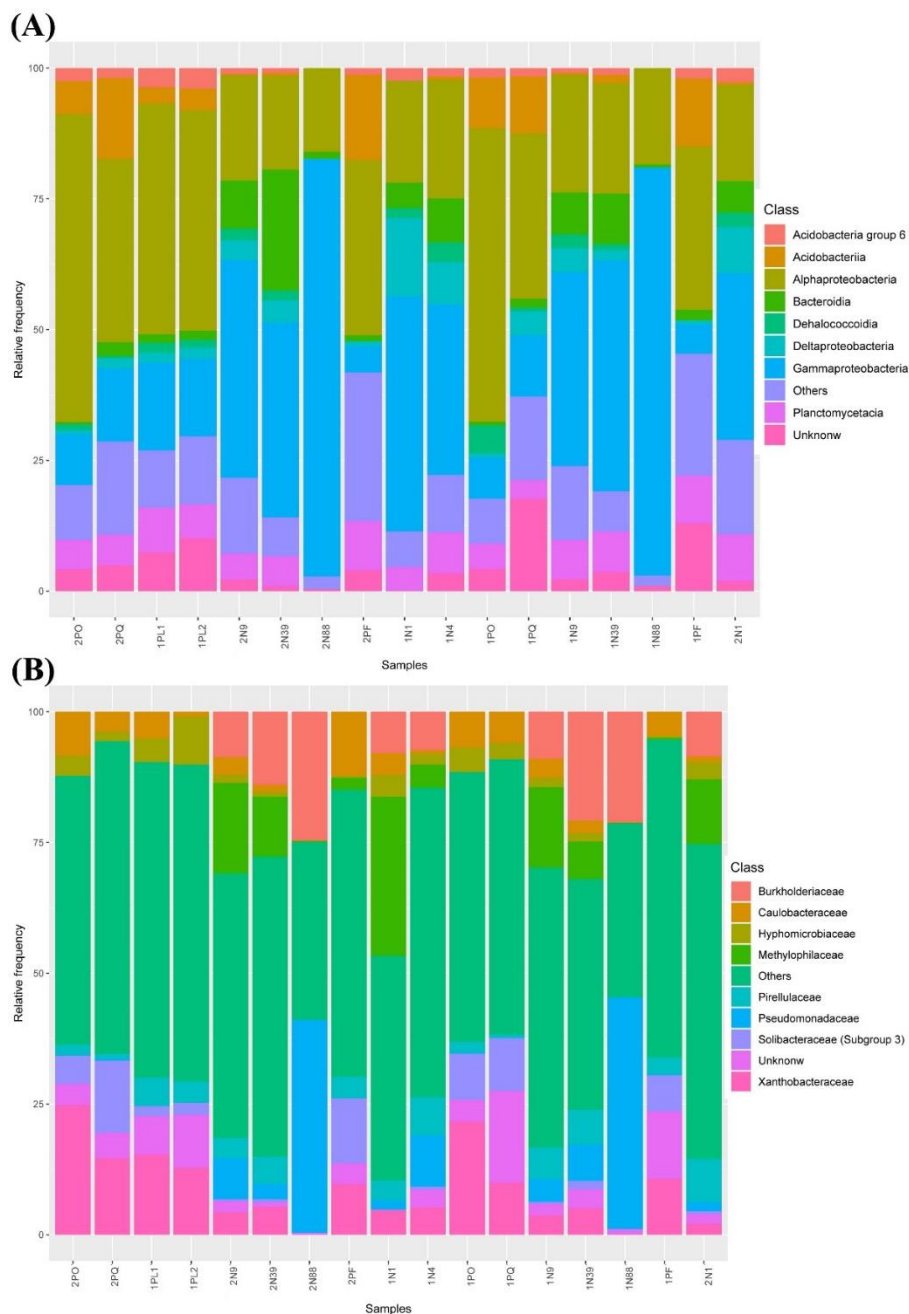


Figure 18. 16S. Stacked barcharts of the taxonomic profile at the class (A) and family (B) level, low abundance taxa are lumped together in the “Others” category

Even if the role of bacteria in the biodegradation of waterlogged archaeological wood is well-known and several studies investigated the degradation patterns produced by these microorganisms (Björðal, 2012; Björðal et al., 2000; Blanchette, 2000; Blanchette and Jurgens, 2005; Kim et al., 1996) very few is known about the bacterial genera involved in this phenomenon. Fig. 19 shows the stacked barcharts of the 16S taxonomic profile at the genus level sorted by city (A) and sample name (B). Some of the genera identified during the present work (e.g. *Pseudomonas*, *Janthinobacterium*, *Flavobacterium*, *Brevundimonas*, *Sphingomonas*, and *Spirochaeta*) are known as members of the microbial community present in waterlogged wood and as active degraders of cellulose and/or lignin (Helms et al., 2004; Landy et al., 2008; Palla et al., 2013, 2014; Wagner et al., 2018; Yin et al., 2019). More in detail, *Pseudomonas* sp. characterized the community of all the samples from Naples, reaching more than 40% of the relative frequency in N88, while the taxon was present only in four of the analyzed Pisa samples, always with frequencies lower than 0.5%. Microorganisms belonging to the genus *Pseudomonas* are strict aerobes or facultative anaerobes. Some species are involved in denitrification or are implicated in sulfur and iron metabolism (Landy et al., 2008). A study

carried out on chips of *Eucalyptus grandis* x *Eucalyptus urophylla*, *Populus canadensis*, and *Larix olgensis* demonstrated that the strain *Pseudomonas* sp. PKE117 is able to produce a wood weight loss ranging from 8% to 27% in 60 days. The characterization of the degraded wood showed that the lignin structure was degraded more than the cellulose (Shui Yang et al., 2007). Other studies demonstrated that several species belonging to the genus *Pseudomonas* are able to degrade lignin and lignin model compounds, to oxidize carbohydrates and to degrade cellulose via different metabolic pathways (Bugg et al., 2011; Chen et al., 2011; Dumova and Kruglov, 2009; Palleroni and Doudoroff, 1957; Stern et al., 1960; Wood and Schwerdt, 1953; Zimmermann, 1990).

The species belonging to this genus are considered as cosmopolites and no specific relations have been reported with one or more wood species. In the present study *Pseudomonas* sp. was identified in silver fir, elm, ash and holm oak samples. Landy et al. (2008) reported *Pseudomonas* spp. from archaeological waterlogged wood pilings from different sites across the Europe. The species were associated with spruce, fir, scot's pine, oak and poplar. Palla et al. (2013) identified *Pseudomonas* spp. from pine samples belonging to the wood recovered inside a rostrum in the site of Aqualadroni (Messina, Sicily). Wagner et al. (2018) identified three *Pseudomonas* strains from *Quercus robur/petraea* remains recovered from different sites across the Europe.

In waterlogged archaeological wood, the genus *Janthinobacterium* is reported in association with *Pseudomonas*, in samples coming from sites characterized by limy soil (Landy et al., 2008; Wagner et al., 2018). Some species of the genus are reported from lake sediments, as part of the bacterioplankton of maritime Antarctic lake and from Antarctic snow (Lopatina et al., 2013; Pearce et al., 2003; Shivaji et al., 2011). In our samples it was identified only in N88 and N39, respectively silver fir and elm, in association with *Pseudomonas*. Ravindran and Yang (2016) demonstrated that the strain *Janthinobacterium* sp. AR-129 has cellulolytic activity and is able to produce high thermal stable cellulase.

Flavobacterium was identified in all the Naples samples but not in Pisa. The genus is reported as part of the bacterial community of waterlogged archeological wood piles coming from two archaeological sites from the Netherlands (Landy et al., 2008) and it has also been identified in the storage water of lacquerware from the Nanhai No. 1 shipwreck (China) (Yin et al., 2019). Members of the genus *Flavobacterium* have a wide distribution, they mostly occur in aquatic ecosystems ranging in salinity from freshwater to seawater, but have also been isolated from soil and sediments (Bernardet and Bowman, 2006). The species *F. akiainvivens* was isolated from decaying wood of the Hawaiian shrub *Wikstroemia oahuensis* (Kuo et al., 2013). The known species belonging to this genus are able to degrade cellulose derivative but not crystalline cellulose, some strains have proved to be able to lysate algae (Bernardet and Bowman, 2006; Dong et al., 2013).

Sphingomonas was found in samples N4, N9 and N39. It was isolated from spruce, Scot's pine and oak pile dwellings and from the Aqualadroni rostrum (Landy et al., 2008; Palla et al., 2013). The species belonging to this genus are usually isolated from soil but can also be animal and human pathogens (Baraniecki et al., 2002; Nishiyama et al., 1992; White et al., 1996; Yang et al., 2006). *Sphingomonas* strains are able to degrade cellulose, lignin and lignin derivatives (Bugg et al., 2011; Chen et al., 2011; Dumova and Kruglov, 2009; Masai et al., 1999).

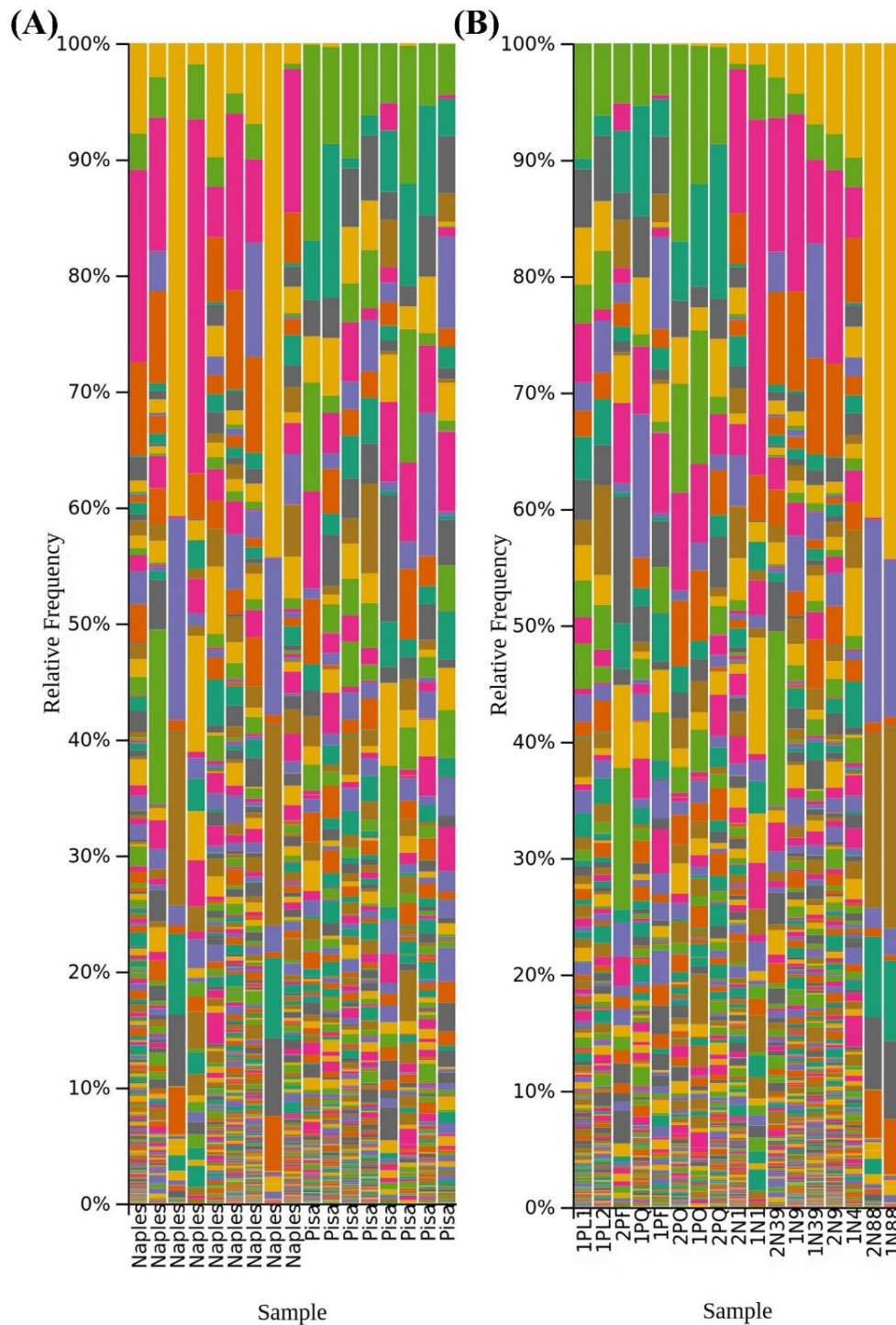
Some of the identified genera never reported before in waterlogged archaeological wood deserve a special mention. The genus *Methylovirgula* was identified only in two samples N88 (silver fir) and PF (ash). It is part of the bacterial community present in decaying wood of the species *Fagus sylvatica*, *Picea abies* and *Pinus sylvestris* (Hoppe et al., 2015; Kielak et al., 2016). The species *Methylovirgula ligni* has been found on beechwood blocks attacked by white-rot fungi (Vorob'ev et al., 2009).

Methylovirgula bacteria are obligated methylotrophs, and can use methanol as the sole carbon source. Methanol is produced during the decomposition of woody materials and this could explain why these microorganisms are found in wood colonized by fungi and in advanced stages of decay (Hoppe et al., 2015; Kielak et al., 2016).

The genus *Bryobacter* was identified in both Naples and Pisa samples. In the latter, it reached a relative frequency of 13% while in N wood the frequency was always equal to or lower than 1%. The genus comprises acidotolerant, strictly aerobic, slow-growing chemoorganotrophic bacteria, which inhabit acidic wetlands and soils and are capable of hydrolyzing several heteropolysaccharides (Dedysh, 2019). Currently, only the species *Bryobacter aggregatus* is described for this genus, it was isolated from boreal Sphagnum peat bogs. This species is able to hydrolyze several substrates, among which pectin and starch, but not cellulose (Kulichevskaya et al., 2010).

Most of the other identified genera are usually isolated from soil and sediments or from water (freshwater or seawater) (e.g. *Pseudolabrys*, *Hyphomicrobium*, *Desulfosporosinus*, *Rhodanobacter*, *Aquicella*, *Reyranella*, *Devosia*, *Dongia*, and *Curvibacter*) (Baik et al., 2013; Ding and Yokota, 2010; Garcia-Sanchez et al., 2013;

Kampfer et al., 2006; Kim et al., 2016; Kostka et al., 2012; Lee et al., 2017; Leifson, 1964; Pagnier et al., 2011; Ramamoorthy et al., 2006; Robertson et al., 2001; Yoon et al., 2007). Species belonging to some of the identified genera are reported as sulfur and iron-oxidising (e.g. *Acidithiobacillus*, *Thiomonas*, *Pseudolabrys*, and *Hyphomicrobium*) (Chen et al., 2004; De Bont et al., 1981; Hallberg et al., 2010; Ramírez et al., 2004; Zecchin et al., 2019) or denitrifying bacteria (e.g. *Rhodanobacter*) (Green et al., 2012; Van Den Heuvel et al., 2010). It is interesting to note that the genera *Acidithiobacillus*, *Thiomonas* and *Rhodanobacter* were identified only in sample N88, indicating a concentration of sulfur, iron and nitrogen in the wood. SEM-EDS analyses carried out on the sample during an experimentation external to the PhD project confirmed the presence of sulfur and iron (iron aluminosilicate) in the wood (data not published).



D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Pseudomonadales;D_4_Pseudomonadaceae;D_5_Pseudomonas
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Xanthobacteraceae;_
D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Methylophilaceae;_
D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Burkholderiaceae;D_5_Janthinobacterium
D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Burkholderiaceae;_
D_0_Bacteria;D_1_Acidobacteria;D_2_Acidobacteriia;D_3_Solibacterales;D_4_Solibacteraceae (Subgroup 3);D_5_Bryobacter
D_0_Bacteria;_:_:_:_
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Beijerinckiaceae;D_5_Methylovirgula
D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;_:_:_
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;_:_
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Caulobacteriales;D_4_Caulobacteraceae;D_5_uncultured
Unassigned;_:_:_:_
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D_0_Bacteria;D_1_Acidobacteria;D_2_Subgroup 6;_:_:_
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D_0_Bacteria;D_1_Planctomycetes;D_2_Planctomycetacia;D_3_Pirellulales;D_4_Pirellulaceae;D_5_uncultured
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D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Acidithiobacillales;D_4_Acidithiobacillaceae;D_5_Acidithiobacillus
D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Burkholderiaceae;D_5_Thiomonas
D_0_Bacteria;D_1_Planctomycetes;D_2_Planctomycetacia;D_3_Pirellulales;D_4_Pirellulaceae;_
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D_0_Bacteria;D_1_Verrucomicrobia;D_2_Verrucomicrobiae;D_3_Pedosphaerales;D_4_Pedosphaeraceae;_
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D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Amb-165-1323;D_5_uncultured bacterium
D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Oceanospirillales;D_4_Pseudohongiellaceae;D_5_Blyi10
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D_0_Bacteria;D_1_Planctomycetes;D_2_Planctomycetacia;D_3_Planctomycetales;D_4_uncultured;_
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D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Diploricetisiales;D_4_Diploricetisaceae;D_5_Aquicella
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Caulobacteriales;D_4_Caulobacteraceae;_
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D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Cytophagales;D_4_Microscillaceae;D_5_uncultured
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D_0_Bacteria;D_1_Chloroflexi;D_2_Ktedonobacteria;D_3_Ktedonobacteriales;D_4_Ktedonobacteraceae;_
D_0_Bacteria;D_1_Acidobacteria;D_2_Aminicenantia;D_3_Aminicenantales;_:_
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Devosiaceae;D_5_Devosia
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Chitinophagales;D_4_Chitinophagaceae;_
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Micropepsales;D_4_Micropepsaceae;D_5_uncultured
D_0_Bacteria;D_1_Spirochaetes;D_2_Spirochaetia;D_3_Spirochaetales;D_4_Spirochaetaceae;D_5_Spirochaeta 2
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Dongiiales;D_4_Dongiaceae;D_5_Dongia
D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Burkholderiaceae;D_5_Curvibacter
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D_0_Bacteria;D_1_Chloroflexi;D_2_Dehalococcoidia;D_3_S085;_:_
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D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Xanthobacteraceae;D_5_Bradyrhizobium
D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Gammaproteobacteria Incertae Sedis;D_4_Unknown Family;D_5_Acidibacter
D_0_Bacteria;D_1_Chlamydiae;D_2_Chlamydiae;D_3_Chlamydiales;_:_
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D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_A0839;D_5_uncultured bacterium
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Sphingomonadales;D_4_Sphingomonadaceae;D_5_Sphingobium
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Micropepsales;D_4_Micropepsaceae;_
D_0_Bacteria;D_1_Patescibacteria;D_2_Parcubacteria;D_3_Candidatus Kaiserbacteria;_:_
D_0_Bacteria;D_1_Gemmatimonadetes;D_2_Gemmatimonadetes;D_3_Gemmatimonadales;D_4_Gemmatimonadaceae;D_5_Gemmatimonas
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Cytophagales;_:_
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D_0_Bacteria;D_1_Chlamydiae;D_2_Chlamydiae;D_3_Chlamydiales;D_4_Simkaniaceae;D_5_uncultured
D_0_Bacteria;D_1_Planctomycetes;D_2_Phycisphaerae;D_3_Tepidisphaerales;D_4_WD2101 soil group;D_5_uncultured bacterium
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Rhizobiales Incertae Sedis;D_5_Bauldia
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Rhizobiaceae;D_5_Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium
D_0_Bacteria;D_1_Patescibacteria;D_2_Parcubacteria;D_3_Candidatus Moranbacteria;D_4_uncultured bacterium;D_5_uncultured bacterium
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Caulobacteriales;D_4_Hyphomonadaceae;D_5_SWB02
D_0_Bacteria;D_1_Chlamydiae;D_2_Chlamydiae;D_3_Chlamydiales;D_4_cvE6;D_5_uncultured bacterium
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Sphingomonadales;D_4_Sphingomonadaceae;D_5_Sphingopyxis
D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Nitrosomonadaceae;D_5_MND1
D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Rhodocyclaceae;_
D_0_Bacteria;D_1_Bacteroidetes;D_2_Ignavibacteria;D_3_Kryptoniales;D_4_Kryptoniaceae;D_5_uncultured bacterium
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Chitinophagales;D_4_Chitinophagaceae;D_5_uncultured
D_0_Bacteria;D_1_Acidobacteria;D_2_Acidobacteriia;D_3_Subgroup 2;_:_
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;_:_:_
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Elsteriales;D_4_uncultured;D_5_uncultured bacterium
D_0_Bacteria;D_1_Planctomycetes;D_2_Phycisphaerae;D_3_Phycisphaerales;D_4_Phycisphaeraceae;D_5_SM1A02

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;_:;:_

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Hyphomicrobiaceae;D_5_Hyphomicrobium

D_0_Bacteria;D_1_Planctomycetes;D_2_Planctomycetacia;D_3_Pirellulales;D_4_Pirellulaceae;D_5_uncultured

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Rhizobiales;D_4_Xanthobacteraceae;D_5_uncultured

D_0_Bacteria;D_1_Planctomycetes;D_2_Planctomycetacia;D_3_Pirellulales;D_4_Pirellulaceae;D_5_Pir4 lineage

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Peptococcaceae;D_5_Desulfosporosinus

D_0_Bacteria;D_1_Proteobacteria;D_2_Bacteroidia;D_3_Flavobacteriales;D_4_Flavobacteriaceae;D_5_Flavobacterium

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Acidithiobacillales;D_4_Acidithiobacillaceae;D_5_Acidithiobacillus

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Burkholderiaceae;D_5_Thiomonas

D_0_Bacteria;D_1_Planctomycetes;D_2_Planctomycetacia;D_3_Pirellulales;D_4_Pirellulaceae;_

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Sva0485;_:;:_

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Sphingomonadales;D_4_Sphingomonadaceae;_

D_0_Bacteria;D_1_Verrucomicrobia;D_2_Verrucomicrobiae;D_3_Pedosphaerales;D_4_Pedosphaeraceae;_

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Hyphomicrobiaceae;_

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_CCD24;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Amb-16S-1323;D_5_uncultured bacterium

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Oceanospirillales;D_4_Pseudohongiellaceae;D_5_Blyi10

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_R7C24;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_uncultured;_:;:_

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Cytophagales;D_4_Microscillaceae;_

D_0_Bacteria;D_1_Gemmatimonadetes;D_2_Gemmatimonadetes;D_3_Gemmatimonadales;D_4_Gemmatimonadaceae;D_5_uncultured

D_0_Bacteria;D_1_Planctomycetes;D_2_Planctomycetacia;D_3_Planctomycetales;D_4_uncultured;_

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Xanthomonadales;D_4_Rhodanobacteraceae;D_5_Rhodanobacter

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Diploricetttiales;D_4_Diploricetttiaceae;D_5_Aquicella

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Caulobacteriales;D_4_Caulobacteraceae;_

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Reyranellales;D_4_Reyranellaceae;D_5_Reyranella

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Caulobacteriales;D_4_Caulobacteraceae;D_5_Brevundimonas

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Elsterales;D_4_uncultured;_

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Cytophagales;D_4_Microscillaceae;D_5_uncultured

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Sphingomonadales;D_4_Sphingomonadaceae;D_5_Sphingomonas

D_0_Bacteria;D_1_Planctomycetes;D_2_Planctomycetacia;D_3_Gemmatales;D_4_Gemmataceae;D_5_Gemmata

D_0_Bacteria;D_1_Planctomycetes;D_2_Planctomycetacia;D_3_Gemmatales;D_4_Gemmataceae;D_5_uncultured

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;_:;:_

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_CCD24;_:;:_

D_0_Bacteria;D_1_Acidobacteria;D_2_Acidobacteriia;D_3_Solibacteriales;D_4_Solibacteraceae (Subgroup 3);D_5_Candidatus Solibacter

D_0_Bacteria;D_1_Chloroflexi;D_2_Ktedonobacteria;D_3_Ktedonobacteriales;D_4_Ktedonobacteraceae;_

D_0_Bacteria;D_1_Acidobacteria;D_2_Aminicenantia;D_3_Aminicenantales;_:;:_

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Devosiaceae;D_5_Devosia

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Chitinophagales;D_4_Chitinophagaceae;_

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Micropepsales;D_4_Micropepsaceae;D_5_uncultured

D_0_Bacteria;D_1_Spirochaetes;D_2_Spirochaetia;D_3_Spirochaetales;D_4_Spirochaetaceae;D_5_Spirochaeta 2

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Dongiales;D_4_Dongiaceae;D_5_Dongia

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Burkholderiaceae;D_5_Curvibacter

D_0_Bacteria;D_1_Patescibacteria;D_2_Parcubacteria;D_3_Candidatus Adlerbacteria;_:;:_

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;_:;:_

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Acetobacteriales;D_4_Acetobacteraceae;_

D_0_Bacteria;D_1_Chloroflexi;D_2_Dehalococcoidia;_:;:_

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Nitrosomonadaceae;_

D_0_Bacteria;D_1_Chloroflexi;D_2_KD4-96;_:;:_

D_0_Bacteria;D_1_Chloroflexi;D_2_Dehalococcoidia;D_3_S085;_:;:_

D_0_Bacteria;D_1_Chloroflexi;D_2_Dehalococcoidia;D_3_S085;D_4_metagenome;D_5_metagenome

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_TRA3-20;_

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Xanthobacteraceae;D_5_Bradyrhizobium

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Gammaproteobacteria Incertae Sedis;D_4_Unknown Family;D_5_Acidibacter

D_0_Bacteria;D_1_Chlamydiae;D_2_Chlamydiae;D_3_Chlamydiales;_:;:_

D_0_Bacteria;D_1_Acidobacteria;D_2_Holophagae;D_3_Subgroup 7;_:;:_

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Rhizobiaceae;_

D_0_Bacteria;D_1_Chloroflexi;D_2_G30 KF-CM66;D_3_uncultured bacterium;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Azospirillales;D_4_Azospirillaceae;D_5_Azospirillum

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Caulobacteriales;D_4_Hyphomonadaceae;D_5_Hirschia

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_A0839;D_5_uncultured bacterium

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Sphingomonadales;D_4_Sphingomonadaceae;D_5_Sphingobium

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Micropepsales;D_4_Micropepsaceae;_

D_0_Bacteria;D_1_Patescibacteria;D_2_Parcubacteria;D_3_Candidatus Kaiserbacteria;_:;:_

D_0_Bacteria;D_1_Gemmatimonadetes;D_2_Gemmatimonadetes;D_3_Gemmatimonadales;D_4_Gemmatimonadaceae;D_5_Gemmatimonas

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Cytophagales;_:;:_

D_0_Bacteria;D_1_Verrucomicrobia;D_2_Verrucomicrobiae;D_3_Opitutales;D_4_Opitutaceae;D_5_Lacunisphaera

D_0_Bacteria;D_1_Chlamydiae;D_2_Chlamydiae;D_3_Chlamydiales;D_4_Simkaniaceae;D_5_uncultured

D_0_Bacteria;D_1_Planctomycetes;D_2_Phycisphaerae;D_3_Tepidisphaerales;D_4_WD2101 soil group;D_5_uncultured bacterium

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Rhizobiales Incertae Sedis;D_5_Bauldia

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Rhizobiaceae;D_5_Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium

D_0_Bacteria;D_1_Patescibacteria;D_2_Parcubacteria;D_3_Candidatus Moranbacteria;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Caulobacteriales;D_4_Hyphomonadaceae;D_5_SWB02

D_0_Bacteria;D_1_Chlamydiae;D_2_Chlamydiae;D_3_Chlamydiales;D_4_cvE6;D_5_uncultured bacterium

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Sphingomonadales;D_4_Sphingomonadaceae;D_5_Sphingopyxis

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Nitrosomonadaceae;D_5_MND1

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Rhodocyclaceae;_

D_0_Bacteria;D_1_Bacteroidetes;D_2_Ignavibacteria;D_3_Kryptoniales;D_4_Kryptoniaceae;D_5_uncultured bacterium

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Chitinophagales;D_4_Chitinophagaceae;D_5_uncultured

D_0_Bacteria;D_1_Acidobacteria;D_2_Acidobacteriia;D_3_Subgroup 2;_:;:_

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;_:;:_

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Elsterales;D_4_uncultured;D_5_uncultured bacterium

D_0_Bacteria;D_1_Planctomycetes;D_2_Phycisphaerae;D_3_Phycisphaerales;D_4_Phycisphaeraceae;D_5_SM1A02

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_R7C24;_:;:_

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Methyloiligellaceae;D_5_uncultured

D_0_Bacteria;D_1_Chloroflexi;D_2_Dehalococcoidia;D_3_t0.6.f;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Hyphomicrobiaceae;D_5_Pedomicrobium

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Chitinophagales;D_4_Saprosiraceae;_

D_0_Bacteria;D_1_Acidobacteria;D_2_Acidobacteriia;D_3_Acidobacteriales;D_4_Acidobacteriaceae (Subgroup 1);_

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Burkholderiaceae;D_5_Hydrogenophaga

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Legionellales;D_4_Legionellaceae;D_5_Legionella

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Caulobacteriales;D_4_Caulobacteraceae;D_5_Phenylobacterium

D_0_Bacteria;D_1_Actinobacteria;D_2_Acidimicrobia;D_3_IMCC26256;_:;:_

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Desulfuromonadales;D_4_Geobacteraceae;D_5_Geobacter

D_0_Bacteria;D_1_Acidobacteria;D_2_Acidobacteriia;D_3_Acidobacteriales;D_4_Acidobacteriaceae (Subgroup 1);D_5_Occallatibacter

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Sulfuricellaceae;D_5_Ferrotrophicum
D_0_Bacteria;D_1_Acidobacteria;D_2_Acidobacteriia;D_3_Acidobacteriales;D_4_Koribacteraceae;D_5_Candidatus Koribacter
D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Burkholderiaceae;D_5_Massilia
D_0_Bacteria;D_1_Acidobacteria;D_2_Acidobacteriia;D_3_Acidobacteriales;D_4_uncultured;_
D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Bdellovibrionales;D_4_Bdellovibrionaceae;D_5_Bdellovibrio
D_0_Bacteria;D_1_Acidobacteria;D_2_Thermoanaerobaculia;D_3_Thermoanaerobaculales;D_4_Thermoanaerobaculaceae;D_5_Subgroup 10
D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Alteromonadales;D_4_Alteromonadaceae;D_5_Rheinheimeria
D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Xanthomonadales;D_4_Rhodanobacteraceae;D_5_uncultured
D_0_Bacteria;D_1_Nitrospirae;D_2_Thermodesulfobivibronia;D_3_uncultured;D_4_uncultured Nitrospirae bacterium;D_5_uncultured Nitrospirae bacterium
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Rhizobiales Incertae Sedis;D_5_uncultured
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Chitinophagales;D_4_Saprospiraceae;D_5_uncultured
D_0_Bacteria;D_1_Verrucomicrobia;D_2_Verrucomicrobiae;D_3_Opitutales;D_4_Opitutaceae;D_5_Opitutus
D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Syntrophobacteriales;D_4_Syntrophaceae;D_5_Desulfobacca
D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Gammaproteobacteria Incertae Sedis;D_4_Unknown Family;_
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Holospirales;D_4_Holospiraceae;D_5_uncultured
D_0_Bacteria;D_1_Planctomycetes;D_2_Planctomycetacia;D_3_Pirellulales;D_4_Pirellulaceae;D_5_Pirellula
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Cytophagales;D_4_Microscillaceae;D_5_OLB12
D_0_Bacteria;D_1_Spirochaetes;D_2_Spirochaetia;D_3_Spirochaetales;D_4_Spirochaetaceae;D_5_uncultured
D_0_Bacteria;D_1_Chloroflexi;D_2_Dehalococcoidia;D_3_GIF9;D_4_uncultured bacterium;D_5_uncultured bacterium
D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Bdellovibrionales;D_4_Bacteriovoraceae;D_5_Peredibacter
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_KFJG30-B3;D_5_uncultured bacterium
D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Steroidobacteriales;D_4_Steroidobacteraceae;D_5_Steroidobacter
D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Diploricetksiales;D_4_Diploricetksiaceae;D_5_uncultured
D_0_Bacteria;D_1_Hydrogenedentes;D_2_Hydrogenedentia;D_3_Hydrogenedentiales;D_4_Hydrogenedensaceae;D_5_metagenome
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Sphingobacteriales;_
D_0_Bacteria;D_1_Dependentiae;D_2_Babeliae;D_3_Babeliales;_
D_0_Bacteria;D_1_Acidobacteria;D_2_Acidobacteriia;D_3_Solibacteriales;D_4_Solibacteraceae (Subgroup 3);_
D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Myxococcales;D_4_Haliangiaceae;D_5_Haliangium
D_0_Bacteria;D_1_Actinobacteria;D_2_Thermoleophilia;D_3_Gaiellales;_
D_0_Bacteria;D_1_Verrucomicrobia;D_2_Verrucomicrobiae;D_3_Opitutales;D_4_Opitutaceae;_
D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Sulfuricellaceae;_
D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Myxococcales;D_4_Archangiaceae;D_5_Anaeromyxobacter
D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Methylophilaceae;D_5_Methylotenera
D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_PLTA13;_
D_0_Bacteria;D_1_Chloroflexi;D_2_Dehalococcoidia;D_3_GIF3;D_4_uncultured bacterium;D_5_uncultured bacterium
D_0_Bacteria;D_1_Actinobacteria;D_2_Actinobacteria;D_3_Frankiales;D_4_Acidothermaceae;D_5_Acidothermus
D_0_Bacteria;D_1_Dependentiae;D_2_Babeliae;D_3_Babeliales;D_4_Vermiphilaceae;_
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Sporobacter
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Chitinophagales;D_4_Saprospiraceae;D_5_Haliscomenobacter
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Devosiaceae;_
D_0_Bacteria;D_1_Nitrospirae;D_2_Nitrospira;D_3_Nitrospirales;D_4_Nitrospiraceae;D_5_Nitrospira
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Caulobacteriales;D_4_Hyphomonadaceae;D_5_Hyphomonas
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Sphingomonadales;D_4_Sphingomonadaceae;D_5_Novosphingobium
D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Hydrogenophilaceae;D_5_Thiobacillus
D_0_Bacteria;D_1_Acidobacteria;D_2_Subgroup 6;D_3_metagenome;D_4_metagenome;D_5_metagenome
D_0_Bacteria;D_1_Chloroflexi;D_2_Dehalococcoidia;D_3_GIF9;D_4_AB-539-J10;D_5_SCGC-AB-539-J10
D_0_Bacteria;D_1_Spirochaetes;D_2_Spirochaetia;D_3_Spirochaetales;D_4_Spirochaetaceae;_
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Chitinophagales;D_4_Chitinophagaceae;D_5_Sediminibacterium
D_0_Bacteria;D_1_Acidobacteria;D_2_Acidobacteriia;D_3_Acidobacteriales;D_4_uncultured;D_5_uncultured Acidobacteriaceae bacterium
D_0_Bacteria;D_1_Actinobacteria;D_2_Acidimicrobia;D_3_Microtrichiales;D_4_Illumatobacteraceae;D_5_Illumatobacter
D_0_Bacteria;D_1_Patesimicrobia;D_2_Parcubacteria;D_3_Candidatus Nomurabacteria;D_4_uncultured bacterium;D_5_uncultured bacterium
D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Syntrophobacteriales;D_4_Syntrophaceae;D_5_uncultured
D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Myxococcales;D_4_Sandaracinaceae;D_5_uncultured
D_0_Bacteria;D_1_Bacteroidetes;D_2_Ignavibacteria;D_3_Kryptoniales;D_4_BSV26;_
D_0_Bacteria;D_1_Chloroflexi;D_2_Ktedonobacteria;D_3_Ktedonobacteriales;D_4_Ktedonobacteraceae;D_5_uncultured
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Sphingobacteriales;D_4_env.OPS 17;_
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Chitinophagales;D_4_Chitinophagaceae;D_5_Ferruginibacter
D_0_Bacteria;D_1_Chloroflexi;D_2_Anaerolineae;D_3_SBR1031;D_4_A4b;_
D_0_Bacteria;D_1_Planctomycetes;D_2_Phycisphaerae;D_3_Pla1 lineage;D_4_uncultured bacterium;D_5_uncultured bacterium
D_0_Bacteria;D_1_Chloroflexi;D_2_Dehalococcoidia;D_3_661239;D_4_uncultured bacterium;D_5_uncultured bacterium
D_0_Bacteria;D_1_Elusimicrobia;D_2_4-29;D_3_uncultured bacterium;D_4_uncultured bacterium;D_5_uncultured bacterium
D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Burkholderiaceae;D_5_Aquabacterium
D_0_Bacteria;D_1_Bacteroidetes;D_2_Ignavibacteria;D_3_SJA-28;D_4_uncultured bacterium;D_5_uncultured bacterium
D_0_Bacteria;D_1_Elusimicrobia;D_2_Elusimicrobia;D_3_Lineage IV;D_4_uncultured bacterium;D_5_uncultured bacterium
D_0_Bacteria;D_1_TA06;_
D_0_Bacteria;D_1_Acidobacteria;D_2_Subgroup 17;_
D_0_Bacteria;D_1_Proteobacteria;_
D_0_Bacteria;D_1_Chloroflexi;D_2_Dehalococcoidia;D_3_t0.6.f;_
D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Nitrosomonadaceae;D_5_Ellin6067
D_0_Bacteria;D_1_Actinobacteria;D_2_Thermoleophilia;D_3_Gaiellales;D_4_uncultured;_
D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Xanthomonadales;D_4_Xanthomonadaceae;_
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Caulobacteriales;D_4_Caulobacteraceae;D_5_Caulobacter
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Eubacteriaceae;_
D_0_Bacteria;D_1_Armatimonadetes;D_2_Fimbriimonadia;D_3_Fimbriimonadales;D_4_Fimbriimonadaceae;D_5_uncultured bacterium
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;_
D_0_Bacteria;D_1_Planctomycetes;D_2_Planctomycetacia;D_3_Isosphaerales;D_4_Isosphaeraceae;D_5_Aquisphaera
D_0_Bacteria;D_1_Chloroflexi;_
D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Myxococcales;_
D_0_Bacteria;D_1_BRC1;_
D_0_Bacteria;D_1_Patescibacteria;D_2_Parcubacteria;_
D_0_Bacteria;D_1_Actinobacteria;D_2_Acidimicrobia;D_3_Microtrichiales;D_4_Illumatobacteraceae;D_5_CL500-29 marine group
D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Sulfuricellaceae;D_5_Sulfuriferula
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Chitinophagales;D_4_Chitinophagaceae;D_5_Terrimonas
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Parvibaculales;D_4_Parvibaculaceae;D_5_Parvibaculum
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Paracaedibacteriales;D_4_Paracaedibacteraceae;D_5_uncultured
D_0_Bacteria;D_1_Planctomycetes;D_2_Planctomycetacia;D_3_Planctomycetales;D_4_uncultured;D_5_metagenome
D_0_Bacteria;D_1_Chloroflexi;D_2_TK10;_
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Acetobacteriales;D_4_Acetobacteraceae;D_5_uncultured
D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Desulfarculales;D_4_Desulfarculaceae;D_5_Desulfatiglanis
D_0_Bacteria;D_1_Verrucomicrobia;D_2_Verrucomicrobiae;D_3_Verrucomicrobiales;D_4_Verrucomicrobiaceae;_
D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Myxococcales;D_4_Nannocystaceae;D_5_uncultured
D_0_Bacteria;D_1_Actinobacteria;D_2_Actinobacteria;D_3_Micrococcales;D_4_Microbacteriaceae;_
D_0_Bacteria;D_1_Chloroflexi;D_2_Anaerolineae;D_3_Anaerolineales;D_4_Anaerolineaceae;D_5_uncultured

D_0_Bacteria;D_1_Planctomycetes;D_2_Planctomycetacia;D_3_Planctomycetales;D_4_Rubinisphaeraceae;D_5_SH-PL14
D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Immundisolibacterales;D_4_Immundisolibacteraceae;D_5_Immundisolibacter
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhodospirillales;D_4_Rhodospirillaceae;D_5_uncultured
D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Myxococcales;D_4_Phaselicystidaceae;D_5_Phaselicystis
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Caulobacteriales;D_4_Caulobacteraceae;D_5_Asticcacaulis
D_0_Bacteria;D_1_Planctomycetes;D_2_Planctomycetacia;D_3_Planctomycetales;D_4_uncultured;D_5_uncultured bacterium
D_0_Bacteria;D_1_Actinobacteria;D_2_WCHB1-81;D_3_uncultured bacterium;D_4_uncultured bacterium;D_5_uncultured bacterium
D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Syntrophobacteriales;D_4_Syntrophaceae;D_5_Desulfomonile
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Cytophagales;D_4_Spirosomaceae;D_5_Lacihabitans
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Beijerinckiacae;_
D_0_Bacteria;D_1_Actinobacteria;D_2_Thermoleophilina;D_3_Solirubrobacteriales;D_4_67-14;_
D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Myxococcales;D_4_Biri41;_
D_0_Bacteria;D_1_Hydrogenedentes;D_2_Hydrogenedentia;D_3_Hydrogenedentiales;D_4_Hydrogenedensaceae;_
D_0_Bacteria;D_1_Planctomycetes;D_2_Phycisphaerae;D_3_mle1-8;D_4_uncultured bacterium;D_5_uncultured bacterium
D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Desulfarculales;D_4_Desulfarculaceae;D_5_uncultured
D_0_Bacteria;D_1_Chloroflexi;D_2_Anaerolineae;D_3_Caldilineales;D_4_Caldilineaceae;D_5_uncultured
D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Sva0485;D_4_uncultured delta proteobacterium;D_5_uncultured delta proteobacterium
D_0_Bacteria;D_1_Dadabacteria;D_2_Dadabacteria;D_3_Dadabacteriales;_
D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Acidiferrobacteriales;D_4_Acidiferrobacteraceae;D_5_Sulfurifustis
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Xanthobacteraceae;D_5_Pseudorhodoplanes
D_0_Bacteria;D_1_Planctomycetes;D_2_Planctomycetacia;D_3_Planctomycetales;D_4_Schlesneriaceae;D_5_Planctopirus
D_0_Bacteria;D_1_Planctomycetes;D_2_Planctomycetacia;D_3_Isosphaerales;D_4_Isosphaeraceae;D_5_uncultured
D_0_Bacteria;D_1_Actinobacteria;D_2_Thermoleophilina;D_3_Solirubrobacteriales;D_4_Solirubrobacteraceae;_
D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Xanthomonadales;D_4_Rhodanobacteraceae;D_5_Dyella
D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Myxococcales;D_4_Blf119;_
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Sphingobacteriales;D_4_Sphingobacteriaceae;D_5_Pedobacter
D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Burkholderiaceae;D_5_Rhizobacter
D_0_Bacteria;D_1_Patescibacteria;D_2_Saccharimonadia;D_3_Saccharimonadales;_
D_0_Bacteria;D_1_Planctomycetes;D_2_Phycisphaerae;D_3_Phycisphaerales;D_4_Phycisphaeraceae;D_5_CL500-3
D_0_Bacteria;D_1_Atribacteria;D_2_J51;D_3_uncultured bacterium;D_4_uncultured bacterium;D_5_uncultured bacterium
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_NRL2;_
D_0_Bacteria;D_1_Chloroflexi;D_2_OLB14;D_3_uncultured bacterium;D_4_uncultured bacterium;D_5_uncultured bacterium
D_0_Bacteria;D_1_Calditrichaeta;D_2_Calditrichia;D_3_Calditrichales;D_4_Calditrichaceae;_
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhodospirillales;D_4_Magnetospiraceae;D_5_uncultured
D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Cellvibrionales;D_4_Cellvibrionaceae;D_5_Cellvibrio
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_uncultured;D_4_uncultured Rhodospirillaceae bacterium;D_5_uncultured Rhodospirillaceae bacterium
D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Deltaproteobacteria Incertae Sedis;D_4_Syntrophorhabdaceae;D_5_Syntrophorhabdus
D_0_Bacteria;D_1_Patescibacteria;D_2_Gammaproteobacteria;D_3_Steroidobacteriales;D_4_Steroidobacteraceae;_
D_0_Bacteria;D_1_Patescibacteria;D_2_Parcubacteria;D_3_Candidatus Adlerbacteria;D_4_uncultured bacterium;D_5_uncultured bacterium
D_0_Bacteria;D_1_Latescibacteria;D_2_Latescibacteria;D_3_Latescibacteriales;D_4_Latescibacteraceae;D_5_uncultured bacterium
D_0_Bacteria;D_1_Armatimonadetes;D_2_uncultured;_
D_0_Bacteria;D_1_Planctomycetes;D_2_Planctomycetacia;D_3_Isosphaerales;D_4_Isosphaeraceae;_
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Cytophagales;D_4_Spirosomaceae;D_5_Dyadobacter
D_0_Bacteria;D_1_Acidobacteria;D_2_Holophagae;D_3_Holophagales;D_4_Holophagaceae;D_5_Geothrix
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Sphingobacteriales;D_4_Sphingobacteriaceae;_
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_KF-JG30-B3;_
D_0_Bacteria;D_1_Planctomycetes;D_2_Phycisphaerae;D_3_Tepidisphaerales;D_4_Tepidisphaeraceae;D_5_Tepidisphaera
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhodobacteriales;D_4_Rhodobacteraceae;D_5_Pseudorhodobacter
D_0_Bacteria;D_1_Actinobacteria;D_2_WCHB1-81;_
D_0_Bacteria;D_1_Actinobacteria;D_2_Acidimicrobia;_
D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Myxococcales;D_4_P3OB-42;D_5_metagenome
D_0_Bacteria;D_1_Planctomycetes;D_2_Planctomycetacia;D_3_Isosphaerales;D_4_Isosphaeraceae;D_5_Singulisphaera
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;_
D_0_Bacteria;D_1_Patescibacteria;D_2_Parcubacteria;D_3_Candidatus Nomurabacteria;_
D_0_Bacteria;D_1_Nitrospirae;D_2_Thermodesulfobacteriales;D_3_uncultured;D_4_uncultured bacterium;D_5_uncultured bacterium
D_0_Bacteria;D_1_Nitrospirae;D_2_4-29-1;D_3_uncultured bacterium;D_4_uncultured bacterium;D_5_uncultured bacterium
D_0_Bacteria;D_1_Acidobacteria;D_2_Acidobacteria;D_3_Solibacterales;D_4_Solibacteraceae (Subgroup 3);D_5_PAUC26f
D_0_Bacteria;D_1_Gemmatimonadetes;D_2_Gemmatimonadetes;D_3_Gemmatimonadales;D_4_Gemmatimonadaceae;_
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Cytophagales;D_4_MWH-CFBK5;D_5_uncultured bacterium
D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Myxococcales;D_4_Polyangiaceae;_
D_0_Bacteria;D_1_Planctomycetes;D_2_Planctomycetacia;D_3_Planctomycetales;D_4_Rubinisphaeraceae;_
D_0_Bacteria;D_1_Planctomycetes;D_2_Planctomycetacia;D_3_Planctomycetales;_
D_0_Bacteria;D_1_Chloroflexi;D_2_OLB14;_
D_0_Bacteria;D_1_Planctomycetes;D_2_Planctomycetacia;_
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Cytophagales;D_4_Cyclobacteriaceae;D_5_Ekhidna
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Thalassobaculales;D_4_Thalassobaculaceae;D_5_Thalassobaculum
D_0_Bacteria;D_1_Chloroflexi;D_2_KD4-96;D_3_uncultured bacterium;D_4_uncultured bacterium;D_5_uncultured bacterium
D_0_Bacteria;D_1_Bacteroidetes;D_2_Ignavibacteria;_
D_0_Bacteria;D_1_Actinobacteria;D_2_Acidimicrobia;D_3_Microtrichiales;D_4_Illumatobacteraceae;_
D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Desulfobacteriales;D_4_Desulfobacteraceae;_
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Flavobacteriales;D_4_Crocinitomicaceae;D_5_Crocinitomix
D_0_Bacteria;D_1_Actinobacteria;D_2_Actinobacteria;D_3_Micromonosporales;D_4_Micromonosporaceae;_
D_0_Bacteria;D_1_Planctomycetes;D_2_Planctomycetacia;D_3_Planctomycetales;D_4_Rubinisphaeraceae;D_5_uncultured
D_0_Bacteria;D_1_Bacteroidetes;D_2_Ignavibacteria;D_3_Ignavibacteriales;D_4_PHOS-HE36;D_5_uncultured bacterium
D_0_Bacteria;D_1_Actinobacteria;D_2_Rubrobacteria;D_3_Rubrobacteriales;D_4_Rubrobacteriaceae;D_5_Rubrobacter
D_0_Bacteria;D_1_Acidobacteria;D_2_Acidobacteria;D_3_Solibacterales;D_4_Solibacteraceae (Subgroup 3);D_5_Paludibaculum
D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Coxiellales;D_4_Coxiellaceae;D_5_Coxiella
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Micavibrionales;_
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Flavobacteriales;_
D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Syntrophobacteriales;D_4_Syntrophaceae;D_5_Syntrophus
D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_MBNT15;D_4_uncultured bacterium;D_5_uncultured bacterium
D_0_Bacteria;D_1_Latescibacteria;D_2_Latescibacteria;D_3_Latescibacteriales;D_4_Latescibacteraceae;_
D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Syntrophobacteriales;D_4_Syntrophobacteraceae;_
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhodobacteriales;D_4_Rhodobacteraceae;_
D_0_Bacteria;D_1_Chloroflexi;D_2_JG30-KF-CM66;_
D_0_Bacteria;D_1_Bacteroidetes;D_2_Ignavibacteria;D_3_Ignavibacteriales;_
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Sphingomonadales;D_4_Sphingomonadaceae;D_5_Sphingouranticius
D_0_Bacteria;D_1_Actinobacteria;D_2_Actinobacteria;D_3_PeM15;_
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Flavobacteriales;D_4_Flavobacteriaceae;_
D_0_Bacteria;D_1_Patescibacteria;D_2_Parcubacteria;D_3_Candidatus Kaiserbacteria;D_4_uncultured bacterium;D_5_uncultured bacterium
D_0_Bacteria;D_1_Patescibacteria;D_2_Parcubacteria;D_3_Candidatus Kaiserbacteria;D_4_metagenome;D_5_metagenome
D_0_Bacteria;D_1_Chlamydiae;D_2_Chlamydiae;D_3_Chlamydiales;D_4_Parachlamydiaceae;_
D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Nitrosomonadaceae;D_5_GOUTA6
D_0_Bacteria;D_1_Spirochaetes;D_2_Leptospirae;D_3_Leptospirales;D_4_Leptospiraceae;D_5_Turneriella
D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Gallionellaceae;_
D_0_Bacteria;D_1_Acidobacteria;D_2_Subgroup 6;D_3_Unknown Order;D_4_Unknown Family;D_5_Luteitalea
D_0_Bacteria;D_1_Actinobacteria;D_2_RBG-16-55-12;D_3_uncultured bacterium;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Zixibacteria;D_2_uncultured bacterium;D_3_uncultured bacterium;D_4_uncultured bacterium;D_5_uncultured bacterium
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminiclostridium 1
D_0_Bacteria;D_1_Planctomycetes;D_2_Phycisphaerae;D_3_CCM11a;_:_
D_0_Bacteria;D_1_Patescibacteria;D_2_Saccharimonadia;D_3_Saccharimonadales;D_4_Saccharimonadaceae;D_5_uncultured bacterium
D_0_Bacteria;D_1_Chloroflexi;D_2_Dehalococcidia;D_3_Dehalococcoidales;D_4_uncultured;D_5_uncultured bacterium
D_0_Bacteria;D_1_Acidobacteria;D_2_Blastocatellia (Subgroup 4);D_3_Blastocatellales;D_4_Blastocatellaceae;_:_
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Peptococcaceae;D_5_Thermincola
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Azospirillales;D_4_Azospirillaceae;D_5_uncultured
D_0_Bacteria;D_1_Bacteroidetes;D_2_Ignavibacteria;D_3_Ignavibacteriales;D_4_SR-FBR-L83;D_5_uncultured bacterium
D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Myxococcales;D_4_Polyangiaceae;D_5_Polyangium
D_0_Bacteria;D_1_Planctomycetes;D_2_Phycisphaerae;D_3_Phycisphaerales;D_4_Phycisphaeraeaceae;D_5_uncultured
D_0_Bacteria;D_1_Chloroflexi;D_2_Anaerolineae;D_3_MSB-582;D_4_uncultured bacterium;D_5_uncultured bacterium
D_0_Archaea;D_1_Crenarchaeota;D_2_Bathyarchaea;_:_
D_0_Bacteria;D_1_Actinobacteria;D_2_Actinobacteria;D_3_Micrococcales;D_4_Micrococccaceae;_:_
D_0_Bacteria;D_1_Actinobacteria;D_2_Acidimicrobia;D_3_Microtrichales;_:_
D_0_Bacteria;D_1_Bacteroidetes;D_2_Ignavibacteria;D_3_SJA-28;_:_
D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Myxococcales;D_4_Sandaracinaceae;D_5_Sandaracinus
D_0_Bacteria;D_1_Chloroflexi;D_2_Anaerolineae;D_3_Ardenticatenales;D_4_uncultured;_:_
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Sphingobacteriales;D_4_env.OPS 17;D_5_uncultured bacterium
D_0_Bacteria;D_1_Actinobacteria;D_2_Thermoleophilia;D_3_Solirubrobacteriales;D_4_Solirubrobacteraceae;D_5_Conexibacter
D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Burkholderiaceae;D_5_Acidovorax
D_0_Archaea;D_1_Crenarchaeota;D_2_Bathyarchaea;D_3_uncultured methanogenic archaeon;D_4_uncultured methanogenic archaeon;D_5_uncultured methanogenic archaeon
D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Myxococcales;D_4_27F-1492R;D_5_uncultured bacterium
D_0_Bacteria;D_1_Verrucomicrobia;D_2_Verrucomicrobiae;D_3_Verrucomicrobiales;D_4_DEV007;_:_
D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Nitrosomonadaceae;D_5_IS-44
D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_NB1-j;D_4_uncultured organism;D_5_uncultured organism
D_0_Bacteria;D_1_TA06;D_2_uncultured bacterium;D_3_uncultured bacterium;D_4_uncultured bacterium;D_5_uncultured bacterium
D_0_Bacteria;D_1_Acidobacteria;D_2_Subgroup 18;_:_
D_0_Bacteria;D_1_Elusimicrobia;D_2_Elusimicrobia;D_3_Lineage IV;_:_
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Cytophagales;D_4_Spirosomaceae;D_5_Pseudarcicella
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Micavibrionales;D_4_Micavibrionaceae;D_5_uncultured
D_0_Bacteria;D_1_Planctomycetes;D_2_Planctomycetacia;D_3_Planctomycetales;D_4_Gimesiaceae;D_5_uncultured
D_0_Bacteria;D_1_Acidobacteria;D_2_Subgroup 21;D_3_uncultured bacterium;D_4_uncultured bacterium;D_5_uncultured bacterium
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Acetobacterales;D_4_Acetobacterales Incertae Sedis;D_5_uncultured
D_0_Bacteria;D_1_Armatimonadetes;D_2_Fimbrimonadia;D_3_Fimbrimonadales;D_4_Fimbrimonadaceae;_:_
D_0_Bacteria;D_1_Spirochaetes;D_2_Leptospirae;D_3_Leptospirales;D_4_Leptospiraceae;D_5_uncultured
D_0_Bacteria;D_1_Acidobacteria;D_2_Thermoanaerobaculia;D_3_Thermoanaerobaculales;D_4_Thermoanaerobaculaceae;D_5_TPD-58
D_0_Bacteria;D_1_Acidobacteria;D_2_Acidobacteria;D_3_Acidobacteriales;_:_
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-010
D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_MBNT15;_:_
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Chitinophagales;_:_
D_0_Bacteria;D_1_Acidobacteria;D_2_Subgroup 5;_:_
D_0_Bacteria;D_1_Modulibacteria;D_2_Moduliflexia;D_3_Moduliflexales;D_4_Moduliflexaceae;D_5_uncultured bacterium
D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Desulfovibrionales;D_4_Desulfovibrionaceae;D_5_Desulfovibrio
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Chitinophagales;D_4_37-13;_:_
D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Syntrophobacteriales;D_4_Syntrophaceae;_:_
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Acetobacterales;D_4_Acetobacteraceae;D_5_Roseomonas
D_0_Bacteria;D_1_Actinobacteria;D_2_Actinobacteria;D_3_Propionibacteriales;D_4_Propionibacteriaceae;D_5_Cutibacterium
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Cytophagales;D_4_Cyclobacteriaceae;_:_
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;_:_
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_uncultured;_:_
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Caulobacterales;D_4_Parvularculaceae;D_5_Amphipicatus
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Heliobacteriaceae;D_5_Hydrogenispora
D_0_Bacteria;D_1_Actinobacteria;D_2_MB-A2-108;D_3_uncultured actinobacterium;D_4_uncultured actinobacterium;D_5_uncultured actinobacterium
D_0_Bacteria;D_1_Nitrospirae;D_2_Thermodesulfuvibrionia;D_3_uncultured;_:_
D_0_Bacteria;D_1_Verrucomicrobia;D_2_Verrucomicrobiae;D_3_Opitutales;D_4_Opitutaceae;D_5_Alterococcus
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Prevotellaceae;D_5_Prevotella 9
D_0_Bacteria;D_1_Chloroflexi;D_2_Anaerolineae;D_3_uncultured;_:_
D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_TRA3-20;D_5_metagenome
D_0_Bacteria;D_1_Actinobacteria;D_2_Actinobacteria;D_3_Micrococcales;_:_
D_0_Bacteria;D_1_Chloroflexi;D_2_TK10;D_3_metagenome;D_4_metagenome;D_5_metagenome
D_0_Bacteria;D_1_Actinobacteria;D_2_Actinobacteria;D_3_Micrococcales;D_4_Microbacteriaceae;D_5_Microbacterium
D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Desulfarculales;D_4_Desulfarculaceae;D_5_Desulfocarbo
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rickettsiales;D_4_SM2D12;_:_
D_0_Bacteria;D_1_Acidobacteria;D_2_Blastocatellia (Subgroup 4);D_3_Blastocatellales;D_4_Blastocatellaceae;D_5_Aridibacter
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Sphingomonadales;D_4_Sphingomonadaceae;D_5_Altererythrobacter
D_0_Bacteria;D_1_Planctomycetes;D_2_Planctomycetacia;D_3_Gemmatales;D_4_Gemmataceae;D_5_Fimbrioglobus
D_0_Bacteria;D_1_Planctomycetes;D_2_Phycisphaerae;D_3_mle1-8;D_4_uncultured organism;D_5_uncultured organism
D_0_Bacteria;D_1_Planctomycetes;D_2_OM190;_:_
D_0_Bacteria;D_1_Acidobacteria;D_2_Subgroup 11;D_3_uncultured bacterium;D_4_uncultured bacterium;D_5_uncultured bacterium
D_0_Bacteria;D_1_Patescibacteria;D_2_Parcubacteria;D_3_uncultured bacterium;D_4_uncultured bacterium;D_5_uncultured bacterium
D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Syntrophobacteriales;D_4_Syntrophobacteraceae;D_5_Syntrophobacter
D_0_Bacteria;D_1_Chlamydiae;D_2_Chlamydiae;D_3_Chlamydiales;D_4_Parachlamydiaceae;D_5_Neochlamydia
D_0_Bacteria;D_1_BRC1;D_2_uncultured candidate division BRC1 bacterium;D_3_uncultured candidate division BRC1 bacterium;D_4_uncultured candidate division BRC1 bacterium;D_5_uncultured candidate division BRC1 bacterium
D_0_Bacteria;D_1_Acidobacteria;D_2_Subgroup 5;D_3_uncultured Acidobacteria bacterium;D_4_uncultured Acidobacteria bacterium;D_5_uncultured Acidobacteria bacterium
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_NRL2;D_4_uncultured alpha proteobacterium;D_5_uncultured alpha proteobacterium
D_0_Bacteria;D_1_Planctomycetes;D_2_Planctomycetacia;D_3_Gemmatales;D_4_Gemmataceae;_:_
D_0_Bacteria;D_1_Patescibacteria;D_2_Parcubacteria;D_3_Candidatus Adlerbacteria;D_4_metagenome;D_5_metagenome
D_0_Bacteria;D_1_Chloroflexi;D_2_Anaerolineae;D_3_Ardenticatenales;D_4_uncultured;D_5_uncultured organism
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Chitinophagales;D_4_Chitinophagaceae;D_5_Dinghuibacter
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Paracaedibacteriales;D_4_Paracaedibacteraceae;D_5_Candidatus Captivus
D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Bacillales;D_4_uncultured;D_5_uncultured bacterium
D_0_Bacteria;D_1_Chloroflexi;D_2_Anaerolineae;D_3_Anaerolineales;D_4_Anaerolineaceae;_:_
D_0_Bacteria;D_1_Actinobacteria;D_2_Acidimicrobia;D_3_Microtrichales;D_4_uncultured;_:_
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Bacteroidetes vadinHA17;_:_
D_0_Bacteria;D_1_Verrucomicrobia;D_2_Verrucomicrobiae;D_3_Verrucomicrobiales;D_4_Verrucomicrobiaceae;D_5_uncultured
D_0_Bacteria;D_1_Planctomycetes;D_2_Phycisphaerae;_:_
D_0_Bacteria;D_1_Patescibacteria;D_2_Parcubacteria;D_3_wastewater metagenome;D_4_wastewater metagenome;D_5_wastewater metagenome
D_0_Bacteria;D_1_Fibrobacteres;D_2_Fibrobacteria;D_3_Fibrobacteriales;D_4_Fibrobacteraceae;D_5_possible genus 04
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Bacteroidetes vadinHA17;D_5_uncultured bacterium
D_0_Bacteria;D_1_Actinobacteria;_:_
D_0_Bacteria;D_1_Actinobacteria;D_2_Actinobacteria;D_3_Frankiales;D_4_Sporichthyaceae;_:_
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Cytophagales;D_4_Hymenobacteraceae;D_5_Adhaeribacter
D_0_Bacteria;D_1_Acidobacteria;D_2_Thermoanaerobaculia;D_3_Thermoanaerobaculales;D_4_Thermoanaerobaculaceae;D_5_Thermoanaerobaculum
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Sphingobacteriales;D_4_KD3-93;D_5_metagenome
D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Gammaproteobacteria Incertae Sedis;D_4_Unknown Family;D_5_uncultured
D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Bdellovibrionales;D_4_Bacterivoracaceae;D_5_Bacterivorax

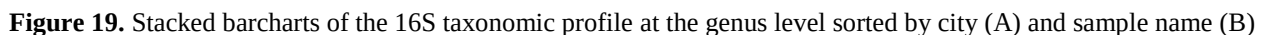


Figure 19. Stacked barcharts of the 16S taxonomic profile at the genus level sorted by city (A) and sample name (B)

Fungal community

Fig. 20 reports the Beta and Alpha diversities for ITS2 data. As for the 16S data, the Beta diversity index (Fig. 20A) shows a neat difference between the fungal communities of Naples and Pisa samples. The median Shannon values, indicating the Alpha diversity, are similar for the two communities (Fig. 20B).

The sequencing results showed that the fungal communities of the wood samples coming from Naples were dominated by the phylum Ascomycota, accounting on average for more than 50% and reaching in some cases more than 90% of the total reads. In Pisa samples a neat prevalence of the phylum Basidiomycota was registered. Sequences attributed to this phylum represented more than 65% of the identified taxa in almost all the analyzed samples. It is interesting to note the presence of sequences belonging to the fungal phyla Chytridiomycota and Rozellomycota in some of the P samples and of the phylum Cercozoa (supergroup Rhizaria) in both N and P samples (reaching more than 67% in sample PO).

At class level, the communities of N samples were mainly composed by Sordariomycetes (on average 55% of the total reads), Leotiomycetes (on average 39%) and Dacrymycetes (5% of the N88 community). Agaricomycetes dominated the P samples, accounting for the more than 70% of total reads on average (Fig. 21A). The class Dothideomycetes was identified in almost all the analyzed samples and reached more than 5% of the relative frequency in most part of them. The class Eurotiomycetes was present only in sample PF accounting for 14% (Protocol 2) and 19% (Protocol 1) of the total reads.

The most represented families in N samples are Pleurotheciaceae (genus *Pleurotheciella*), Lasiosphaeriaceae (g. *Podospora*), Coniochaetaceae (g. *Coniochaeta*), Herpotrichiellaceae (g. *Coniosporium*, *Phialophora* and *Exophiala*), Dacrymycetaceae (g. *Cerinosterus*, *Calocera*) and Reticulascaceae (g. *Reticulascus*). The Pleurotheciaceae accounted for more than 50% of the total reads in all the analyzed samples with the exception of N88. The genera of the families Serendipitaceae (unidentified genus) and Herpotrichiellaceae (g. *Cladophialophora*), were enriched in P samples. In particular, the family Serendipitaceae accounted for a number of reads ranging from 2% to 88% in the different samples.

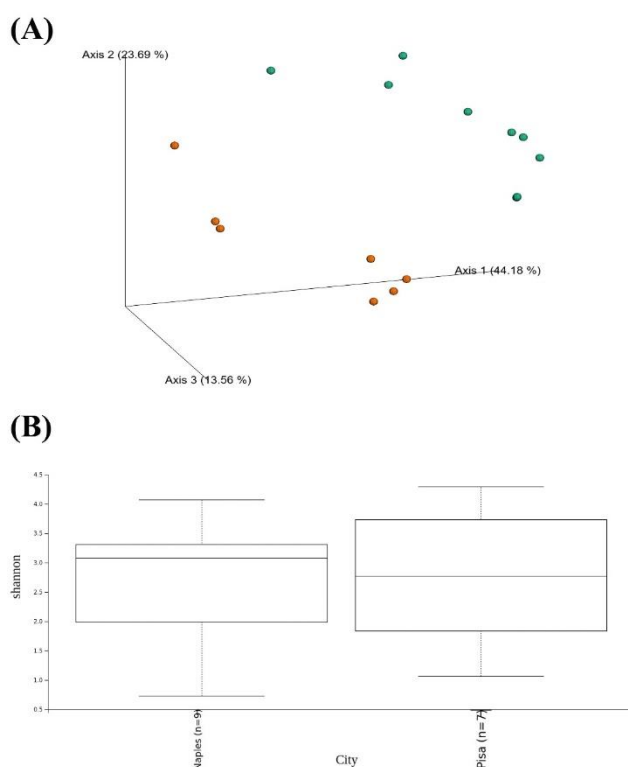


Figure 20. Ecological parameters of ITS2 sequencing data. (A) Beta diversity represented by Principle Coordinate Analysis Emperor plot on a Bray-Curtis distance matrix. Green dots: Naples, orange dots: Pisa. (B) Boxplots of Alpha-diversity Index (calculated as Shannon index) in the two archaeological sites

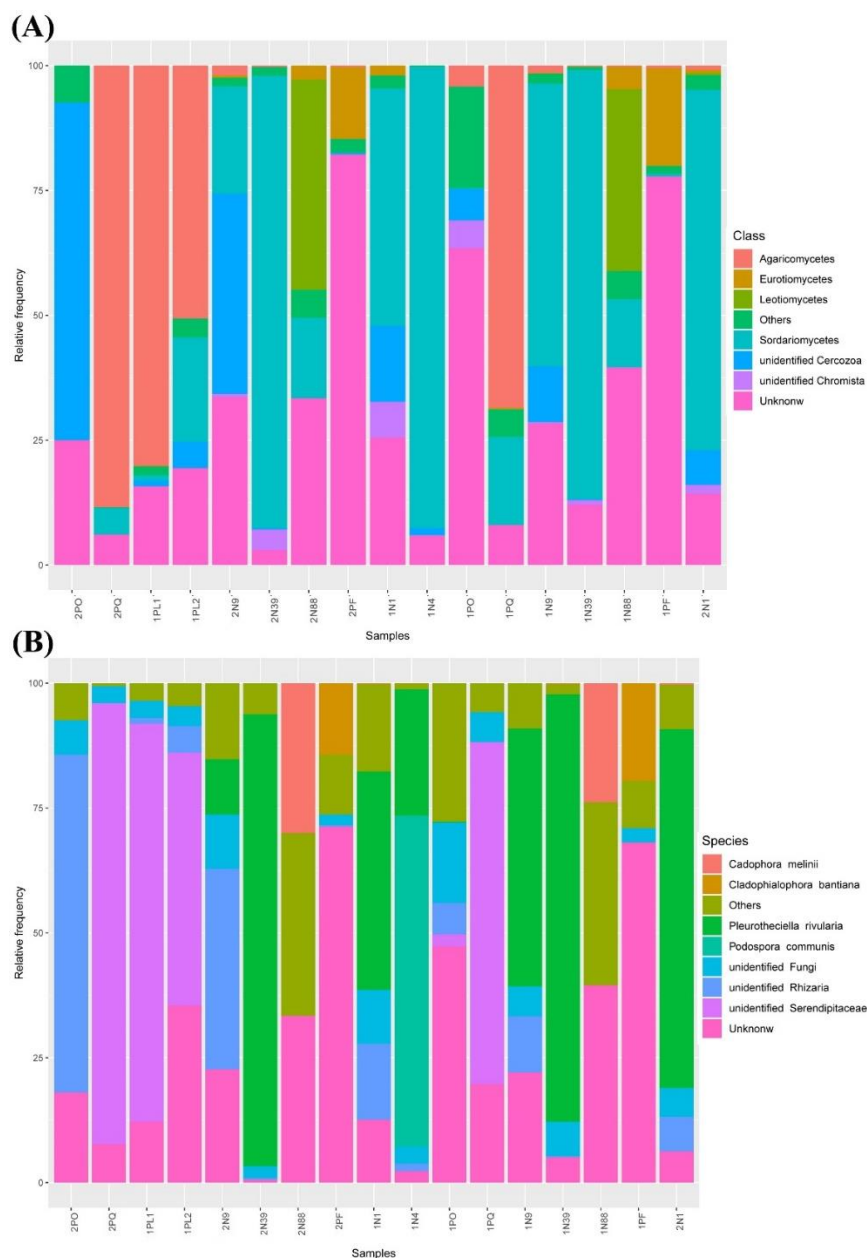


Figure 21. ITS2. Stacked barcharts of the taxonomic profile at the class (A) and species (B) level, low abundance taxa are lumped together in the “Others” category

Fig.22 shows the stacked barcharts of the ITS2 taxonomic profile at the species level sorted by city (A) and sample name (B). As Fig. 22A clearly shows, most part of the identified species are exclusive of one of the two archaeological sites. Differently from what observed in the bacterial communities, most part of fungal genera and species were exclusively identified in one or two of the analyzed samples. Usually the relative frequencies obtained for each taxon with the two extraction protocols do not coincide so, in the results discussion both values are reported. The genera and species identified in the Naples samples will be analyzed first.

The species *Pleurotheciella rivularia* is the most abundant in all the analyzed N samples except for N88 in which is totally absent. Species of the genus *Pleurotheciella* are usually isolated from freshwater habitats and the species *P. rivularia* was collected on decaying wood submerged in freshwater (Luo et al., 2018; Réblová et al., 2012). No literature is available about a possible ligninolytic or cellulolytic activity of this species.

Cadophora melinii was identified in samples N88 (relative frequencies 23% - Protocol 1 and 29.9% - Protocol 2), N39 (0.1% - Protocol 1, not detected with Protocol 2) and N1 (0.3% - Protocol 1, not detected with Protocol 2). It is reported as grapevine pathogen, causing the Petri disease (Travadon et al., 2015). Anyway, species belonging to the genus *Cadophora* are reported from decaying wood, soil and plants (Gramaje et al., 2011;

Harrington and McNew, 2003), from wooden artifacts from the Ross Sea region (Antarctica) and Deception Island (South Shetlands, Antarctica) and from decayed arctic driftwoods (Arenz et al., 2006; Blanchette et al., 2016, 2004; Held and Blanchette, 2017). *C. malorum*, *C. luteo-olivacea*, *C. fastigiata* and previously undescribed *Cadophora* species designated as *C. species H*, *C. species E* and *C. species NH* were proved to cause type 1 soft rot after 12 months of incubation on *Betula* and *Populus* sound wood (Blanchette et al., 2004). The species *Podospora communis* accounted for more than 66% of the total reads of the fungal community of sample N4. *Podospora* species are saprophytic, predominantly reported as coprophilous (Che et al., 2005; Lorenzo and Havrylenko, 2001; Miller and Huhndorf, 2005; Mirza and Cain, 1969). *Acidomelania panicicola* (5.9% - Protocol 1 and 7.6% - Protocol 2) and *Cerinosterus luteoalbus* (frequencies 5.3% - Protocol 1 and 5.2% - Protocol 2) were found only in sample N88. The first is closely related to endophyte species and is usually isolated from the roots of plants living in acid and nutrient-poor environments (Walsh et al., 2014). The latter was isolated from decayed historic wood on Deception Island (South Shetlands, Antarctica) and from arctic driftwood attacked by soft rot fungi (Blanchette et al., 2016; Held and Blanchette, 2017).

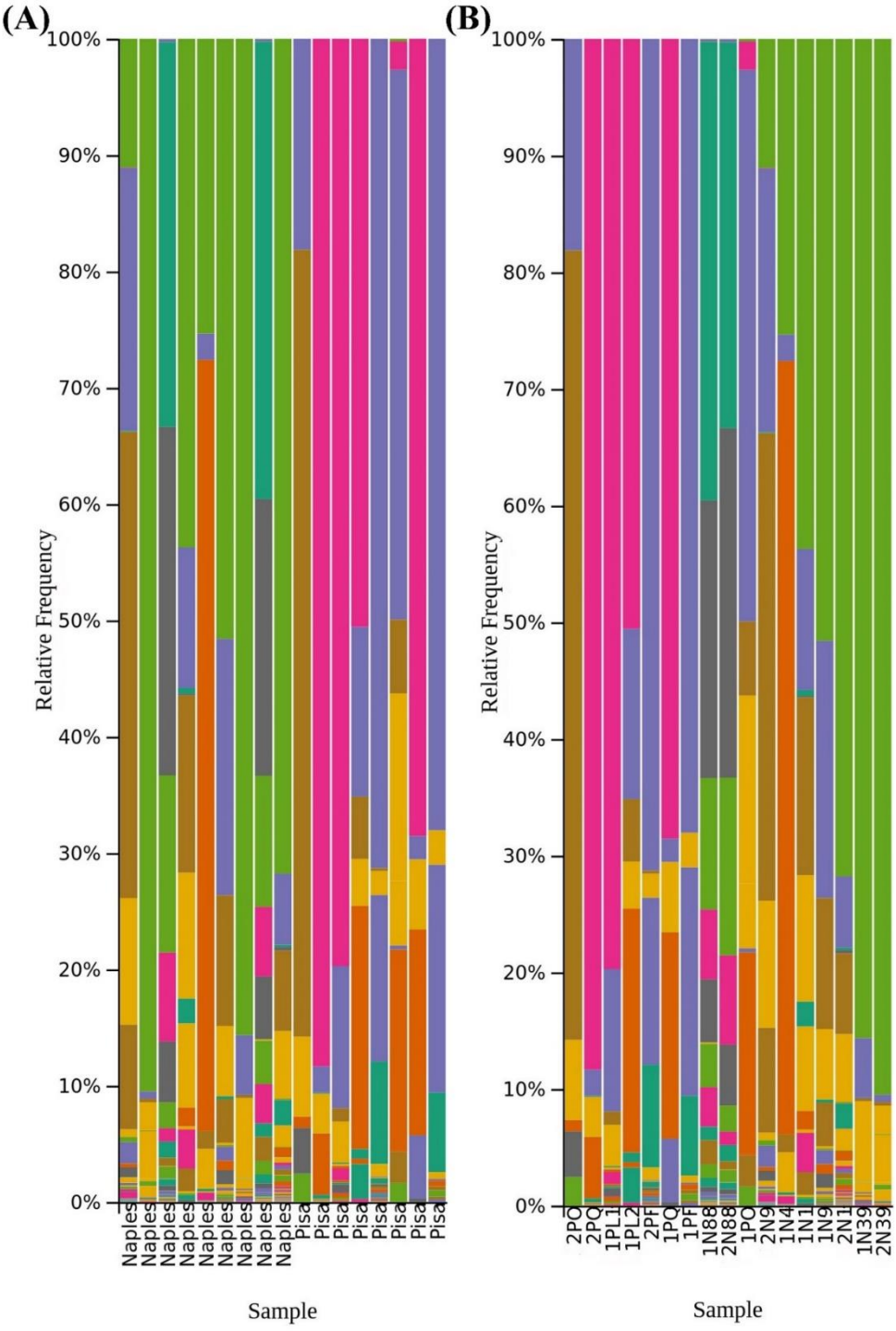
Some of the less abundant species deserve a special mention. *Reticulascus clavatus* was exclusively found in sample N9 (3.7% - Protocol 1 and 9% - Protocol 2). It is reported as the teleomorph form of *Cylindrotrichum clavatum*. The species was isolated from submerged wood of *Alnus glutinosa*, *Platanus* sp., *Fraxinus* sp. and from decayed wood of *Ulmus scabra* (Réblová et al., 2011). *Mollisia dextrinospora*, found in sample N88 (1.1% - Protocol 1 and 1.3% - Protocol 2) is reported as the teleomorph form of a *Cadophora*-like anamorph (Day et al., 2012). It was isolated from decaying wood (Crous et al., 2003). *Lecythophora (Coniochaeta) hoffmannii*, found only in sample N9 (1.2% - Protocol 1 and 0.8% - Protocol 2), is a facultative plant pathogen, it can colonize as saprotroph soils, leaf litter, and coarse wood debris or cause opportunistic mycosis in humans. But it is also reported as soft rot fungi, able to degrade the aromatic compounds (phenolics and aryl alcohols/aldehydes) that are produced during wood decomposition thanks to a pool of extracellular enzymes (Bugos et al., 1988; Leonhardt et al., 2018). *Penicillium simplicissimum*, identified only in sample N88 (0.7% - Protocol 1 and 0.5% - Protocol 2), is not considered a white-rot fungus but several works demonstrated its ability in degrade lignin and lignin-related compounds derivate from lignin degradation by wood rot fungi (Fraaije et al., 1997; Shen et al., 2013; Yu et al., 2005; Zeng et al., 2006). Other species of the genus *Penicillium* are reported as soil inhabitants able to degrade lignin and lignin related aromatic compounds, cellulose or as soft-rot agents (Hamed, 2013; Launen et al., 1995; Osman et al., 2014; Rodríguez et al., 1994; Tillett and Walker, 1990). *Exophiala xenobiotica* was part of the fungal communities of samples N9 (0.1% - Protocol 1 and 0.4% - Protocol 2) and N88 (3.7% - Protocol 1 and 2.2% - Protocol 2). The genus *Exophiala* includes agents of opportunistic infection in humans, potentially able to cause a wide diversity of mycoses, varying from cutaneous infections to disseminated syndromes (Aoyama et al., 2009; Morio et al., 2012; Zeng, 2007). Anyway, *E. xenobiotica* is reported as an opportunistic black yeast isolated from hydrocarbons-rich environments (De Hoog et al., 2006).

The fungal communities of Pisa samples are more difficult to analyze. Most of the sequences obtained remained unidentified, for some samples more than 60% of the total reads are generically reported as Fungi. However, in four of the analyzed woods more than 71% of the sequences are reported as belonging to the family Serendipitaceae so it can be said that the fungal communities of the Pisa samples are dominated by basidiomycetes. Several fungal species belonging to the phylum Basidiomycota are well known wood degraders. A large number of basidiomycetes have been typified as brown or white rot fungi, able to degrade cellulose and lignin (Cohen et al., 2005; D'Souza et al., 1999; Eaton and Hale, 1993; Gold et al., 1989; Yelle et al., 2008; Yoon and Kim, 2005). Among the few identified species, the most abundant basidiomycetes are *Angulomyces argentinensis* and *Schizophyllum commune*. *A. argentinensis* was identified in samples PO (17.2% - Protocol 1 and 0.9% - Protocol 2), PF (0.4% - Protocol 1, not detected with Protocol 2), PL1 (relative frequency 0.2%), and PL2 (relative frequency 0.4%). It has been isolated from water, pollen, soil and cellulosic material in tropical ponds (Letcher et al., 2008; Sutcliffe et al., 2018). *S. commune*, found only in sample PO (1.6% - Protocol 1, not detected with Protocol 2), is one of the most widely distributed white-rot fungi. Several studies proved its ability to degrade cellulose and lignin through different enzymatic ways (Baldrian and Valášková, 2008; Ghosh et al., 2005; Haltrich and Steiner, 1994; Irshad and Asgher, 2002; Ohm et al., 2010; Schmidt and Liese, 1980; Steiner et al., 1987).

Among the ascomycetes identified from Pisa samples, *Cladophialophora bantiana*, found in sample PF (19.5% - Protocol 1 and 14.3% - Protocol 2), deserve a mention. It is a thermotolerant multinucleated saprophytic black mold, isolated from decayed vegetation, wood and soil able to cause myelitis in humans (Ozgun et al., 2019; Shields and Castillo, 2002). Species belonging to the genus *Cladophialophora* have also been reported

as degrader of wooden artifacts from the Deception Island (South Shetlands, Antarctica) (Held and Blanchette, 2017).

For both 16S and ITS2 sequences, it was observed that the communities of the samples treated according the two extraction protocols were very similar, as it can be clearly observed in Figs. 19B and 22B. This let hypothesizing that the obtained results can be considered as representative of the actual communities present in the wood. The differences in the relative frequency registered for almost all the identified taxon could be attributed to the extraction method or to the anisotropic distribution of the microorgasnims inside the wood. As showed, most part of the identified bacterial and fungal taxa have cellulolytic or ligninolytic ability. These results underline that the waterlogged wood is exposed to a high biological risk during the storage pre-restoration phase.



k_Fungi;p_Ascomycota;c_Sordariomycetes;o_Pleurotheciales;f_Pleurotheciaceae;g_Pleurotheciella;s_Pleurotheciella_rivularia
k_Fungi;p_Basidiomycota;c_Agaricomycetes;o_Sebacinales;f_Serendipitaceae;g_unidentified;s_unidentified
k_Fungi; ; ; ; ;
k_Fungi;p_Ascomycota;c_Sordariomycetes;o_Sordariales;f_Lasiochaetaceae;g_Podospora;s_Podospora_communis
k_Fungi;p_Ascomycota; ; ; ; ;
k_Fungi;p_Ascomycota;c_Leotiomycetes;o_Helotiales;f_Helotiales_fam_Incertae_sedis;g_Cadophora;s_Cadophora_melinii
k_Rhizaria;p_Cercozoa;c_unidentified;o_unidentified;f_unidentified;g_unidentified;s_unidentified
k_Fungi;p_unidentified;c_unidentified;o_unidentified;f_unidentified;g_unidentified;s_unidentified
k_Fungi;p_Ascomycota;c_Sordariomycetes;o_Coniochaetales;f_Coniochaetaceae;g_Coniochaeta;_
k_Fungi;p_Ascomycota;c_Leotiomycetes;o_Helotiales;f_Helotiales_fam_Incertae_sedis;g_Acidomelania;s_Acidomelania_panicolica
k_Fungi;p_Ascomycota;c_Eurotiomycetes;o_Chaetothyriales;f_Herpotrichiellaceae;g_Cladophialophora;s_Cladophialophora_bantiana
k_Fungi;p_Ascomycota;c_Sordariomycetes; ; ; ; ;
Unassigned; ; ; ; ;
k_Fungi;p_Basidiomycota;c_Dacrymycetes;o_Dacrymycetales;f_Dacrymycetaceae;g_Cerinosterus;s_Cerinosterus_luteoalbus
k_Fungi;p_Ascomycota;c_Sordariomycetes;o_Glomerellales;f_Reticulascaceae;g_Reticulascus;s_Reticulascus_clavatus
k_Chromista;p_unidentified;c_unidentified;o_unidentified;f_unidentified;g_unidentified;s_unidentified
k_Fungi;p_Ascomycota;c_Eurotiomycetes;o_Chaetothyriales;f_Herpotrichiellaceae;g_Exophiala;s_Exophiala_xenobiotica
k_Fungi;p_Ascomycota;c_Leotiomycetes;o_Helotiales; ; ; ;
k_Fungi;p_Basidiomycota;c_Agaricomycetes; ; ; ; ;
k_Fungi;p_Basidiomycota;c_Pucciniomycetes;o_unidentified;f_unidentified;g_unidentified;s_unidentified
k_Fungi;p_Ascomycota;c_Leotiomycetes;o_Helotiales;f_Dermateaceae;g_Mollisia;s_Mollisia_dextrinospora
k_Fungi;p_Ascomycota;c_Sordariomycetes;o_Coniochaetales;f_Coniochaetaceae;g_Coniochaeta;s_Coniochaeta_hoffmannii
k_Fungi;p_Ascomycota;c_Sordariomycetes;o_Coniochaetales;f_Coniochaetaceae;g_Coniochaeta;s_unidentified
k_Fungi;p_Basidiomycota;c_Malasseziomycetes;o_Malasseziales;f_Malasseziaceae;g_Malassezia;s_Malassezia_restricta
k_Fungi;p_Ascomycota;c_Leotiomycetes;o_Helotiales;f_Helotiaceae;g_Colophora;s_unidentified
k_Fungi;p_Ascomycota;c_Sordariomycetes;o_Pleurotheciales;f_Pleurotheciaceae; ; ;
k_Fungi;p_Ascomycota;c_Dothideomycetes;o_Capnodiales; ; ; ;
k_Fungi;p_Chytridiomycota;c_Rhizophydiomycetes;o_Rhizophydiales;f_Angulomycetaceae;g_Angulomyces;s_Angulomyces_argentinensis
k_Fungi;p_Ascomycota;c_Eurotiomycetes;o_Eurotiales;f_Aspergillaceae;g_Penicillium;s_Penicillium_simplicissimum
k_Fungi;p_Ascomycota;c_Leotiomycetes;o_Helotiales;f_Vibrissaceae;g_Phialocephala;s_Phialocephala_dimorphospora
k_Fungi;p_Ascomycota;c_Eurotiomycetes;o_Chaetothyriales;f_Herpotrichiellaceae;g_Phialophora;s_Phialophora_cyclaminis
k_Fungi;p_Ascomycota;c_Dothideomycetes;o_Pleosporales;f_Pleosporaceae;g_Alternaria;s_Alternaria_alternata
k_Fungi;p_Ascomycota;c_Dothideomycetes;o_Capnodiales;f_Cladosporiaceae;g_Cladosporium;_
k_Fungi;p_Ascomycota;c_Sordariomycetes;o_Xylariales; ; ; ;
k_Fungi;p_Ascomycota;c_Leotiomycetes;o_Helotiales;f_Myxotrichaceae;g_Oidiodendron;s_Oidiodendron_griseum
k_Fungi;p_Basidiomycota;c_Agaricomycetes;o_Agaricales;f_Tricholomataceae;g_Clitocybe;s_Clitocybe_trulliformis
k_Fungi;p_Rozellomycota;c_unidentified;o_unidentified;f_unidentified;g_unidentified;s_unidentified
k_Fungi;p_Ascomycota;c_Dothideomycetes;o_Pleosporales;f_Pleosporaceae;g_Curvularia;_
k_Fungi;p_Ascomycota;c_Sordariomycetes;o_Hypocreales;f_Hypocreales_fam_Incertae_sedis;g_Sarocladium;s_Sarocladium_kiliense
k_Fungi;p_Basidiomycota; ; ; ; ;
k_Fungi;p_Ascomycota;c_Dothideomycetes;o_Pleosporales;f_Phaeosphaeriaceae;g_Phaeosphaeria;s_Phaeosphaeria_triglochonicola
k_Fungi;p_Basidiomycota;c_Malasseziomycetes;o_Malasseziales;f_Malasseziaceae;g_Malassezia;s_Malassezia_globosa
k_Fungi;p_Basidiomycota;c_Tremellomycetes; ; ; ; ;
k_Fungi;p_Ascomycota;c_Dothideomycetes;o_Capnodiales;f_Capnodiales_fam_Incertae_sedis;g_Arthrocatena;s_Arthrocatena_tenebrio
k_Fungi;p_Ascomycota;c_Sordariomycetes;o_Coniochaetales;f_Coniochaetaceae;g_Coniochaeta;s_Coniochaeta_fasciculata
k_Fungi;p_Ascomycota;c_Dothideomycetes;o_Pleosporales;f_Pleosporaceae;g_Alternaria;_
k_Fungi;p_Ascomycota;c_Dothideomycetes;o_Pleosporales;f_Pleosporaceae;g_Alternaria;s_Alternaria_hordeicola
k_Fungi;p_Ascomycota;c_Leotiomycetes;o_Helotiales;f_Myxotrichaceae;g_Oidiodendron;s_Oidiodendron_flavum
k_Fungi;p_Basidiomycota;c_Agaricomycetes;o_Hymenochaetales;f_Hymenochaetaceae;g_Fuscoporia;s_Fuscoporia_torulosa
k_Fungi;p_Ascomycota;c_Sordariomycetes;o_Microascales;f_Microascaceae;g_Microascus;_
k_Fungi;p_Basidiomycota;c_Dacrymycetes;o_Dacrymycetales;f_Dacrymycetaceae;g_Calocera;s_unidentified
k_Fungi;p_Basidiomycota;c_Agaricomycetes;o_Cantharellales;f_Cantharellales_fam_Incertae_sedis;g_Sistotrema;s_Sistotrema_coronilla
k_Fungi;p_Ascomycota;c_Dothideomycetes;o_Capnodiales;f_Mycosphaerellaceae;g_Ramularia;_
k_Fungi;p_Basidiomycota;c_Agaricomycetes;o_Russulales;f_Stereaceae;g_Stereum;s_Stereum_sanguinolentum
k_Fungi;p_Basidiomycota;c_Tremellomycetes;o_Tremellales;f_Bulleribasidiaceae;g_Vishniacozyma;s_unidentified
k_Fungi;p_Ascomycota;c_Eurotiomycetes;o_Eurotiales;f_Aspergillaceae;g_Aspergillus;s_unidentified
k_Fungi;p_Basidiomycota;c_Agaricomycetes;o_Cantharellales; ; ; ;
k_Fungi;p_Basidiomycota;c_Tremellomycetes;o_Filobasidiales;f_Filobasidiaceae;g_Filobasidium;s_Filobasidium_magnum
k_Fungi;p_Basidiomycota;c_Agaricomycetes;o_Russulales;f_Peniophoraceae; ; ; ;
k_Fungi;p_Basidiomycota;c_Malasseziomycetes;o_Malasseziales;f_Malasseziaceae;g_Malassezia;s_unidentified
k_Fungi;p_Basidiomycota;c_Agaricomycetes;o_Polyporales;f_Meruliaceae;g_Phaeophlebiopsis;s_Phaeophlebiopsis_caribbeana
k_Fungi;p_Ascomycota;c_Dothideomycetes;o_Pleosporales;f_Phaeosphaeriaceae; ; ;
k_Fungi;p_Basidiomycota;c_Microbotryomycetes;o_Microbotryomycetes_ord_Incertae_sedis;f_Microbotryomycetes_fam_Incertae_sedis;g_Colacogla
k_Fungi;p_Basidiomycota;c_Exobasidiomycetes;o_Geogheffichiales;f_Gjaerumiaceae;g_Gjaerumia;s_Gjaerumia_minor
k_Fungi;p_Basidiomycota;c_Agaricomycetes;o_Agaricales;f_Pluteaceae;g_Pluteus;_
k_Fungi;p_Ascomycota;c_Sordariomycetes;o_Hypocreales;f_Ophiocordycipitaceae;g_Purpureocillium;s_Purpureocillium_lilacinum
k_Fungi;p_Basidiomycota;c_Malasseziomycetes;o_Malasseziales;f_Malasseziaceae;g_Malassezia;s_Malassezia_sympodialis

k_Fungi;p_Basidiomycota;c_Agaricomycetes;o_Agaricales;f_Tricholomataceae;g_Mycena;_
 k_Fungi;p_Basidiomycota;c_Tremellomycetes;o_Filobasidiales;f_Filobasidiaceae;g_Filobasidium;s_Filobasidium_globisporum
 k_Fungi;p_Ascomycota;c_Eurotiomycetes;o_Chaetothyriales;f_Herpotrichiellaceae;g_Coniosporium;s_unidentified
 k_Fungi;p_Ascomycota;c_Sordariomycetes;o_Hypocreales;f_Cordycipitaceae;g_Lecanicillium;s_unidentified
 k_Fungi;p_Basidiomycota;c_Agaricomycetes;o_Russulales;f_Peniophoraceae;g_Peniophora;s_Peniophora_piceae
 k_Fungi;p_Basidiomycota;c_Agaricomycetes;o_Agaricales;f_Schizophyllaceae;g_Schizophyllum;s_Schizophyllum_commune
 k_Fungi;p_Ascomycota;c_Eurotiomycetes;o_Eurotiales;f_Aspergillaceae;g_Aspergillus;_
 k_Fungi;p_Basidiomycota;c_Tremellomycetes;o_Cystofilobasidiales;f_Mrakiaceae;g_Itersonilia;s_Itersonilia_annonica
 k_Fungi;p_Ascomycota;c_Sordariomycetes;o_Hypocreales;f_Hypocreaceae;g_Trichoderma;s_Trichoderma_viride
 k_Fungi;p_Ascomycota;c_Sordariomycetes;o_Hypocreales;f_Nectriaceae;g_Fusarium;s_Fusarium_solani
 k_Fungi;p_Ascomycota;c_Eurotiomycetes;o_Eurotiales;f_Thermoascaceae;g_Byssoscleromycetes;s_unidentified
 k_Fungi;p_Ascomycota;c_Dothideomycetes;o_Pleosporales;f_Didymellaceae;g_Didymella;_
 k_Fungi;p_Basidiomycota;c_Agaricomycetes;o_Agaricales;f_Agaricales;g_Agaricus;_
 k_Fungi;p_Basidiomycota;c_Cystobasidiomycetes;o_Cystobasidiomycetes_ord_Incertae_sedis;f_Symmetrosporaceae;g_Symmetrospora;s_Symmetr
 k_Fungi;p_Mucoromycota;c_Endogonomycetes;o_Endogonales;f_Endogonales_fam_Incertae_sedis;g_Densospora;s_unidentified
 k_Fungi;p_Basidiomycota;c_Agaricomycetes;o_Agaricales;f_Pleurotaceae;g_Pleurotus;s_Pleurotus_ostreatus
 k_Fungi;p_Ascomycota;c_Sordariomycetes;o_Hypocreales;f_Hypocreales_fam_Incertae_sedis;g_Acremonium;s_Acremonium_cavaeaeum
 k_Fungi;p_Basidiomycota;c_Tremellomycetes;o_Tremellales;f_Bulleribasidiaceae;g_Vishniacozyma;s_Vishniacozyma_carnescens
 k_Fungi;p_Ascomycota;c_Lecanoromycetes;o_Telochistales;f_Telochistaceae;g_Athallia;s_Athallia_cerinella
 k_Fungi;p_Basidiomycota;c_Microbotryomycetes;o_Sporidiobolales;f_Sporidiobolaceae;g_Sporidiobolus;s_unidentified
 k_Fungi;p_Ascomycota;c_Saccharomycetes;o_Saccharomycetales;f_Saccharomycetales_fam_Incertae_sedis;g_Candida;s_Candida_ethanolica
 k_Fungi;p_Ascomycota;c_Eurotiomycetes;o_Eurotiales;f_Aspergillaceae;g_Penicillium;_
 k_Fungi;p_Basidiomycota;c_Agaricomycetes;o_Polyporales;f_Meruliaceae;g_Merulius;_
 k_Fungi;p_Ascomycota;c_Sordariomycetes;o_Hypocreales;f_Cordycipitaceae;g_unidentified;s_unidentified
 k_Fungi;p_Ascomycota;c_Archaeorhizomycetes;o_Archaeorhizomycetales;f_Archaeorhizomycetales;g_Archaeorhizomyces;s_unidentified
 k_Fungi;p_Ascomycota;c_Leotiomycetes;o_Helotiales;f_Helotiales_fam_Incertae_sedis;g_Cadophora;s_Cadophora_luteo-olivacea
 k_Fungi;p_Ascomycota;c_Dothideomycetes;o_Pleosporales;f_Didymellaceae;g_Didymella;_
 k_Fungi;p_Basidiomycota;c_Agaricomycetes;o_Trechisporales;f_Trechisporales_fam_Incertae_sedis;g_Sistotremastrum;s_Sistotremastrum_guttulife
 k_Fungi;p_Ascomycota;c_Leotiomycetes;o_Helotiales;f_Vibrissaceae;g_Phialocephala;s_Phialocephala_scopiformis
 k_Fungi;p_Basidiomycota;c_Microbotryomycetes;o_Sporidiobolales;f_Sporidiobolaceae;g_Sporobolomyces;s_Sporobolomyces_ruberrimus
 k_Fungi;p_Basidiomycota;c_Agaricomycetes;o_Polyporales;f_Ganodermataceae;g_Ganoderma;s_Ganoderma_africana
 k_Fungi;p_Basidiomycota;c_Tremellomycetes;o_Tremellales;f_Bulleribasidiaceae;g_Vishniacozyma;s_Vishniacozyma_victoriae
 k_Fungi;p_Basidiomycota;c_Tremellomycetes;o_Holtermanniales;f_Holtermanniales_fam_Incertae_sedis;g_Holtermanniella;s_Holtermanniella_taka
 k_Fungi;p_Basidiomycota;c_Agaricomycetes;o_Polyporales;f_Meruliaceae;g_Bjerkandera;_
 k_Fungi;p_Ascomycota;c_Dothideomycetes;o_Capnodiales;f_Teratosphaeriaceae;g_Catenulostroma;_
 k_Fungi;p_Basidiomycota;c_Tremellomycetes;o_Tremellales;f_Bulleribasidiaceae;g_Dioszegia;s_Dioszegia_hungarica
 k_Fungi;p_Basidiomycota;c_Agaricomycetes;o_Agaricales;f_Psathyrellaceae;g_Coprinellus;s_Coprinellus_disseminatus
 k_Fungi;p_Ascomycota;c_Dothideomycetes;o_Capnodiales;f_Cladosporiaceae;g_Cladosporium;s_Cladosporium_halotolerans
 k_Fungi;p_Basidiomycota;c_Malasseziomycetes;o_Malasseziales;f_unidentified;g_unidentified;s_unidentified
 k_Fungi;p_Basidiomycota;c_Tremellomycetes;o_Cystofilobasidiales;f_Cystofilobasidiaceae;g_Guehomyces;s_Guehomyces_pullulans
 k_Fungi;p_Basidiomycota;c_Agaricomycetes;o_Corticiales;f_Vuilleminia;s_Vuilleminia_comedens
 k_Fungi;p_Basidiomycota;c_Agaricomycetes;o_Hymenochaetales;f_Schizoporaceae;g_Hyphodontia;s_Hyphodontia_setulosa
 k_Fungi;p_Ascomycota;c_Sordariomycetes;o_Xylariales;f_Diatrypaceae;g_Diatrype;s_Diatrype_stigma
 k_Fungi;p_Ascomycota;c_Dothideomycetes;o_Capnodiales;f_Mycosphaerellaceae;g_Mycosphaerella;_
 k_Fungi;p_Basidiomycota;c_Agaricomycetes;o_Cantharellales;f_Cantharellales_fam_Incertae_sedis;g_Sistotrema;s_Sistotrema_brinkmannii
 k_Fungi;p_Basidiomycota;c_Agaricomycetes;o_Auriculariales;f_Exidiaceae;g_Exidia;s_Exidia_glandulosa
 k_Fungi;p_Mucoromycota;c_Mucoromycetes;o_Mucorales;f_Mucoraceae;g_Mucor;s_Mucor_circinelloides
 k_Fungi;p_Basidiomycota;c_Exobasidiomycetes;o_Tilletiales;f_Tilletiaceae;g_Tilletia;s_Tilletia_puccinelliae
 k_Fungi;p_Basidiomycota;c_Agaricomycetes;o_Hymenochaetales;f_Schizoporaceae;g_Xylodon;s_Xylodon_erastii
 k_Fungi;p_Basidiomycota;c_Agaricomycetes;o_Auriculariales;f_Exidiaceae;g_Exidia;s_unidentified
 k_Fungi;p_Basidiomycota;c_Agaricomycetes;o_Agaricales;f_Tricholomataceae;g_Mycena;s_Mycena_rhannicola
 k_Fungi;p_Basidiomycota;c_Agaricomycetes;o_Hymenochaetales;f_Schizoporaceae;g_Hyphodontia;s_Hyphodontia_radula
 k_Fungi;p_Basidiomycota;c_Agaricomycetes;o_Agaricales;f_Psathyrellaceae;g_Coprinopsis;_
 k_Fungi;p_Ascomycota;c_Saccharomycetes;o_Saccharomycetales;f_Pichiaceae;g_Pichia;s_Pichia_fermentans
 k_Fungi;p_Basidiomycota;c_Agaricomycetes;o_Polyporales;f_Coriolaceae;g_Trametes;s_Trametes_versicolor
 k_Fungi;p_Basidiomycota;c_Agaricomycetes;o_Corticiales;f_Corticaceae;g_Corticium;s_unidentified
 k_Fungi;p_Basidiomycota;c_Tremellomycetes;o_Tremellales;f_Tremellaceae;g_Cryptococcus;s_Cryptococcus_frias
 k_Fungi;p_Ascomycota;c_Dothideomycetes;o_Pleosporales;f_Didymellaceae;g_Didymella;_
 k_Fungi;p_Basidiomycota;c_Tremellomycetes;o_Tremellales;f_Bulleraceae;g_Bullera;s_Bullera_alba
 k_Fungi;p_Basidiomycota;c_Agaricomycetes;o_Russulales;f_Stereaceae;g_Stereum;s_Stereum_ostrea
 k_Fungi;p_Basidiomycota;c_Agaricomycetes;o_Polyporales;f_Coriolaceae;g_Trametes;_
 k_Fungi;p_Ascomycota;c_Dothideomycetes;o_Pleosporales;f_Phaeosphaeriaceae;g_Parastagonospora;s_unidentified
 k_Fungi;p_Ascomycota;c_Pezizomycetes;o_Pezizales;f_Morchellaceae;g_Morchella;_
 k_Fungi;p_Ascomycota;c_Sordariomycetes;o_Diaporthales;f_Diaporthaceae;g_Diaporthe;_
 k_Fungi;p_Basidiomycota;c_Agaricomycetes;o_Hymenochaetales;f_Schizoporaceae;g_Hyphodontia;_
 k_Fungi;p_Ascomycota;c_Leotiomycetes;o_Helotiales;f_Hyaloscyphaceae;g_Calycina;s_Calycina_marina

Figure 22. Stacked barcharts of the ITS taxonomic profile at the species level sorted by city (A) and sample name (B)

4.2.3. Biocidal activity *in vitro*

The tests carried out *in vitro* allowed to evaluate the effect of the three tested essential oils (cinnamon bark, C, wild thyme, WT, and common thyme, CT) on the fungal spore germination and on the mycelium vitality.

Tab. 15 summarizes the results of the tests carried out to establish the EOs' Minimum Inhibitory Concentration (MIC). As it can be observed, the C concentration 0.5% was effective against all the fungal strains except for *Aspergillus japonicus*, while the same concentration of CT and WT was effective only against *Stachybotrys chartarum*. The concentration 0.7% gave good results against *Chaetomium* sp. and *S. chartarum* for the two thymes but only against *A. japonicus* for C. The 1% and 3% concentrations of the three EOs were efficient against all the strains so it can be affirmed that the MIC for the three EOs is 1%.

Tab. 15. Estimation of the Minimum Inhibitory Concentration of the EOs. +: presence of growth; -: absence of growth

Essential oil	Concentration	<i>Chaetomium</i> sp.	<i>Fusarium</i> sp.	<i>Aspergillus japonicus</i>	<i>Stachybotrys chartarum</i>
C	3%	-	-	-	-
	1%	-	-	-	-
	0.7%	+	+	-	+
	0.5%	-	-	+	-
WT	3%	-	-	-	-
	1%	-	-	-	-
	0.7%	-	+	+	-
	0.5%	+	+	+	-
CT	3%	-	-	-	-
	1%	-	-	-	-
	0.7%	-	+	+	-
	0.5%	+	+	+	-

Tabs. 16 and 17 and Fig. 23 resume the results of ATP bioluminescence assays aimed to evaluate the biocidal activity of the established MIC on the mycelium of the selected fungal strains.

As the results clearly show, the three oils allowed obtaining a neat decrease of the fungal vitality of all the selected strains. C and WT caused an ATP decrease equal to 99.99% or 100% in all the tests. The ATP decrease registered for CT was 100% for *Chaetomium* and *Fusarium*, 99.99% for *A. japonicus*, and 98% for *Stachybotrys*. The results of cultural analyses showed that all the pieces of mycelium transferred on the new culture medium were not able to grow (Fig. 24) confirming that the biocides had fungicidal and not fungistatic activity.

The results of biochemical analyses obtained for the control tests carried out treating the mycelium with a solution of water and ethanol (WE) varied greatly depending on the fungal strain. For *Chaetomium* and *S. chartarum* 99.94% and 93% decrease were registered respectively while the vitality of *A. japonicus* was reduced by 37%. Instead, for *Fusarium* the amount of ATP registered after the treatment was higher with respect to the pre-treatment value. In spite of the contrasting biochemical results, the cultural analyses showed that all the mycelia treated with water and ethanol when transferred on a new culture medium were still able to grow (Fig. 24).

The tests carried out to evaluate the possible influence of the EOs on the ATP quantification showed that none of the three oils had a registrable effect. The standard ATP values registered with and without the oils addition were comparable, a variation of $\pm 1\%$ with respect to the pure standard was observed. This range of variation is the one usually observed in a set of repeated measures on the standard and so it can be considered as irrelevant.

Tab. 16. Evaluation of the biocidal activity of the EOs MIC on fungal mycelia through ATP bioluminescence assay. (C: cinnamon; WT: wild thyme; CT: common thyme; WE: water-ethanol; PT: pre-treatment, AT: after treatment).

	Cellular ATP (RLU)							
	C		WT		CT		WE	
	PT	AT	PT	AT	PT	AT	PT	AT
<i>Chaetomium</i> sp.	141,463,153	8,370	63,859,443	0	74,999,796	0	64,789,488	41,098
<i>Fusarium</i> sp.	238,026,707	4,700	184,490,878	16,528	94,931,472	4,707	125,084,954	179,711,160
<i>Aspergillus japonicus</i>	40,249,203	2,857	28,196,658	7,225	53,213,664	4,413	21,664,705	13,645,291
<i>Stachybotrys chartarum</i>	101,396,802	26,069	225,239,286	370,328	70,763,254	1,234,871	207,549,160	13,886,664

Tab. 17. Percent decrease of the amount of ATP extracted from the fungal mycelia after the treatment with EOs.

	ATP Decrease (%)			
	C	WT	CT	WE
<i>Chaetomium</i> sp.	99.99	100	100	99.94
<i>Fusarium</i> sp.	100	99.99	100	-43.67
<i>Aspergillus japonicus</i>	99.99	99.97	99.99	37.02
<i>Stachybotrys chartarum</i>	100	100	98	93



Figure 23. Evaluation through ATP bioluminescence assay of the biocidal activity of cinnamon (C), wild thyme (WT) and common thyme (CT) oils MIC against mycelium of *Chaetomium* sp., *Fusarium* sp., *Aspergillus japonicus* and *Stachybotrys chartarum*. Control tests were performed treating the mycelium with a solution of deionised water and ethanol (WE). (PT: pre-treatment, AT: after treatment).

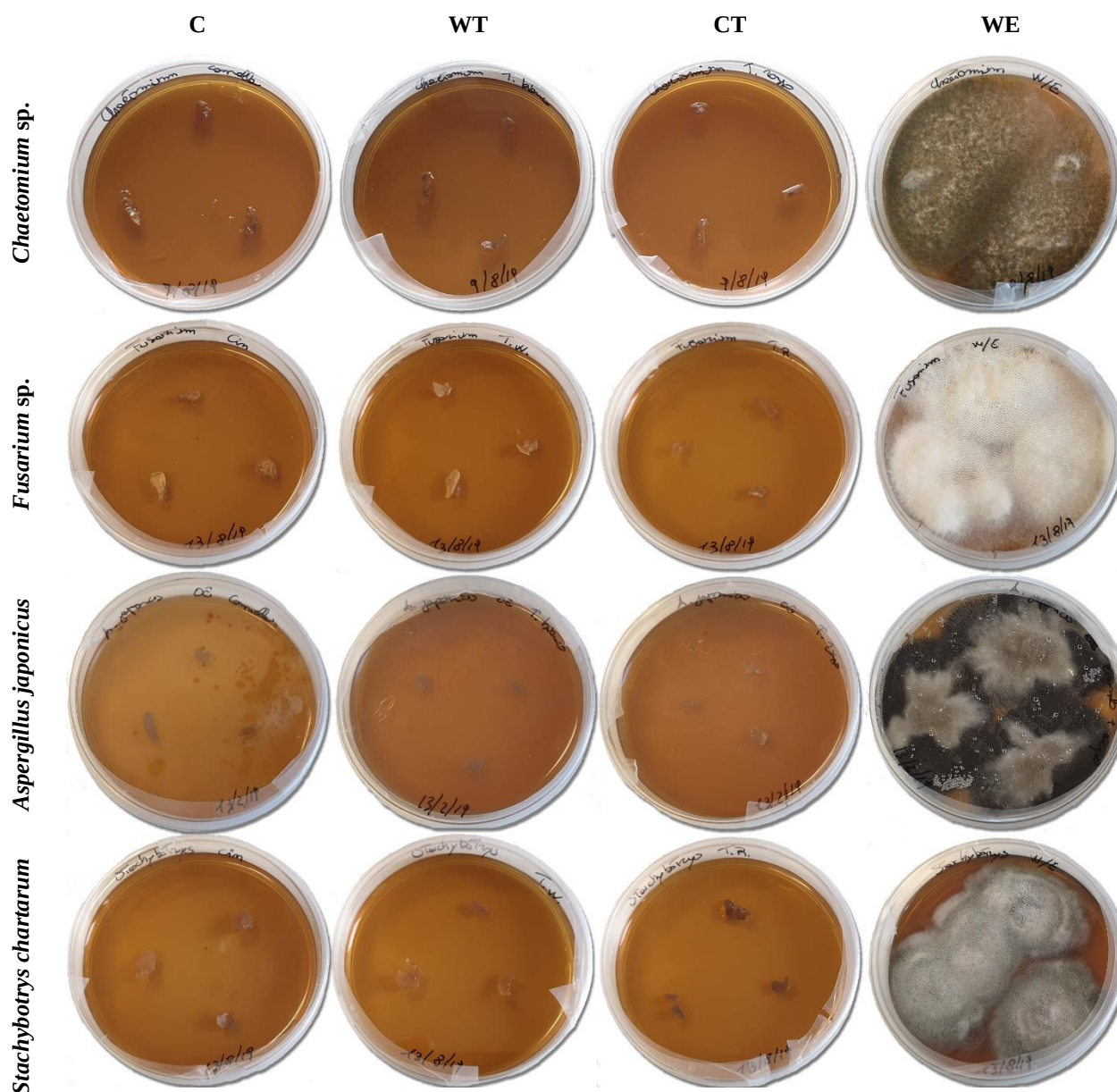


Figure 24. Results of cultural tests carried out on the fungal mycelia treated with the EOs MIC. (C: cinnamon; WT: wild thyme; CT: common thyme; WE: water-ethanol)

4.2.4. Waterlogged archaeological wood samples

The study carried out on archaeological waterlogged wood samples allowed to quantify the biocidal effect of the EOs MIC on the microbial communities present both in the storage water and the wood and to evaluate the influence of tested biocides on the fungal and bacterial communities.

The biochemical tests showed that the three oils had a positive effect on the decrease of the microbial vitality (Tabs. 18-19, Fig. 25). The tests carried out to evaluate the possible influence of wood extractives on ATP quantification showed that the presence of these compounds in the lysate suspension caused a decrease ranging between 15% and 20% of the standard ATP values. So, correction factors were calculated and applied to the data. Only the corrected results are showed in the text. The values registered for water and wood samples are comparable for C while for CT and WT the biocidal activity obtained on wood microbiota was slightly higher with respect that on water.

Cinnamon oil allowed to obtain the best results. A decrease by 100% on water and 99.95% on wood was registered with this oil. CT produced a vitality decrease by 95.38% on water and 99.53% on wood. The worst

results were obtained with WT, for this oil the after treatment residual microbial vitality was higher than 1.5% also for the wood.

The control tests carried out on the ethanol treated sample showed that the ATP values registered for the water after the biocidal treatment were higher with respect to the pre-treatment evaluation while the wood values decreased by 17.23%. For the untreated sample decrease by 79.25% and 57.85% were observed for water and wood respectively.

Tab. 18. Evaluation of the biocidal activity of the EOs MIC through ATP bioluminescence assays carried out on storage water and on waterlogged archaeological wood samples. (C: cinnamon; WT: wild thyme; CT: common thyme; E: ethanol; NT: not-treated control; PT: pre-treatment, AT: after treatment).

			Water	Wood
Cellular ATP (RLU)	C	PT	3,212,727	6,089,221
		AT	0	3,081
	WT	PT	1,363,737	7,365,543
		AT	62,212	133,924
	CT	PT	3,302,945	13,039,127
		AT	152,611	61,107
	E	PT	2,951,192	5,748,250
		AT	6,389,944	4,757,930
	NT	PT	2,740,069	9,799,995
		AT	568,597	4,130,445

Tab. 19. Percent decrease of the amount of ATP extracted from storage water and waterlogged archaeological wood samples after the treatment with EOs.

ATP Decrease (%)					
	C	WT	CT	E	NT
Water	100	95.44	95.38	-116.52	79.25
Wood	99.95	98.18	99.53	17.23	57.85

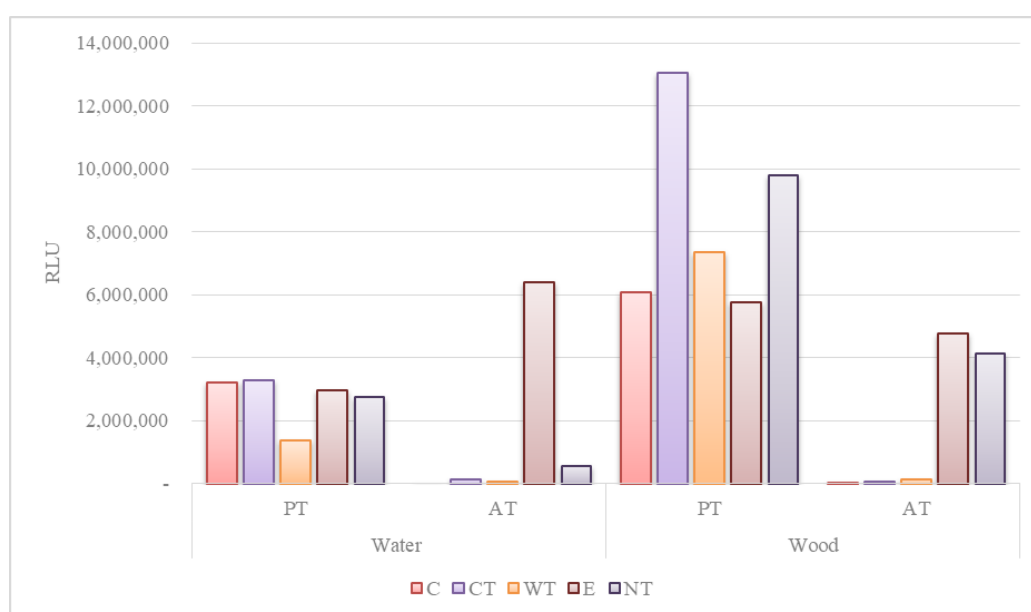


Figure 25. Evaluation of the biocidal activity of the EOs MIC on storage water and waterlogged archaeological wood samples through ATP bioluminescence assay. (C: cinnamon; WT: wild thyme; CT: common thyme; E: ethanol; NT: not-treated control; PT: pre-treatment, AT: after treatment).

With respect to the results obtained for the *in vitro* tests, the study carried out on wood samples showed differences in the biocidal activity of the three oils. In fact, while for *in vitro* tests the decrease of vitality was close to 100% for all oils on all fungal strains, with the only exception of CT on *S. chartarum*, in tests on storage water and wood only cinnamon oil allowed to obtain a decrease in the ATP values greater than 99.5%. An explanation of the obtained results can be linked to the setting up of the experimentation. During the tests, the same amount of ethanol was used for the three oils. Perhaps, this quantity was not sufficient to completely suspend WT and CT. On the surface of the storage water treated with these two oils, in fact, an oily patina was visible.

Another possible explanation is linked to the microbial communities present in the wood. The *in vitro* tests showed that the oils are effective against isolated strains but it can be possible that their effectiveness decreases in the presence of a complex community. Moreover, molecular investigations showed that the fungal taxa present in wood and storage water are different from those used for *in vitro* experimentation. The effectiveness of the EOs tested against these strains may be different.

Surely in order to explain the obtained results, the chemical composition of the oils must be taken into consideration. As shown in Tab. 4, the tested EOs are a complex mixture of molecules (mainly terpenes, terpenoids, and aromatic and/or aliphatic constituents characterized by low molecular weight). These molecules have a variety of targets in the microbial cells (mainly the membrane and the cytoplasm proteins) and can inhibit or slow the growth of bacteria and fungi, sometimes causing a complete alteration of the cell morphology (Bakkali et al., 2008; Nazzaro et al., 2013; Singh and Singh, 2012). Several studies, mainly conducted on human pathogens, analyzed the effect of the EOs and/or their constituents against microbial cells showing that usually the EOs biological effect is determined by the major components (Ipek et al., 2005). The cinnamon essential oil used in the experimentation is mainly composed by cinnamaldehyde, eugenol, caryophyllene, and linalool. The wild thyme oil is dominated by carvacrol, linalool and γ -terpinene while the common thyme is mainly composed of thymol, *p*-cymene, γ -terpinene, and linalool.

Phenylpropenes (cinnamaldehyde and eugenol) are derivatives of cinnamic acid, characterized by an aromatic phenol group and a three-carbon propene tail. Eugenol interacts with bacterial cell membranes affecting the transport of ions and ATP and inhibits different bacterial enzymes like ATPase, amylase and protease (Hyltdgaard et al., 2012; Thoroski et al., 1989; Wendakoon and Morihiko, 1995). Furthermore, it is considered a growth inhibitor in fungi, it limits protein production and DNA replication and causes the lysis of spores (Duke, 2002; Thobunluepop et al., 2009). The effect of cinnamaldehyde is linked to its concentration. Small amount of the molecule inhibit enzymes involved in important cell functions, at higher concentrations cinnamaldehyde is an ATPase inhibitor. Lethal concentrations of this molecule perturb cell membranes (Nazzaro et al., 2013). Tests carried out on wood decay fungi showed that cinnamaldehyde and eugenol have a synergistic effect being their combination effective at much lower concentrations than the molecules used alone (Hsu et al., 2007; Yen and Chang, 2008).

The antimicrobial activity of terpenoids (*p*-cymene, γ -terpinene, linalool, carvacrol, and thymol) is mainly linked to their functional groups, the hydroxyl group and the presence of delocalized electrons is particularly important for phenolic terpenoids. *P*-cymene, a monoterpene with a benzene ring without functional groups, can perturb microbial membranes, causing their expansion and a modification of membrane potential (Ultee et al., 2002). It does not directly affect the membrane permeability but can decrease its enthalpy and melting temperature (Cristani et al., 2007). Several tests showed that *p*-cymene is less effective of other terpenoids but it can increase the antimicrobial activity of other compounds, like carvacrol (Ben Arfa et al., 2006; Dorman and Deans, 2000; Rattanachaikunsopon and Phumkhachorn, 2010; Ultee et al., 2002). Linalool is a terpene alcohol, able to cause a rise in intracellular cAMP (Lis-Balchin and Hart, 1999). Thymol is a phenolic monoterpene structurally similar to carvacrol but having hydroxyl group at a distinct position on the phenolic ring. Thymol interacts with microbial outer and inner membranes causing structural and functional alterations (i.e. increase of permeability, release of K⁺ ions and ATP, release of lipopolysaccharides) and can interact with membrane proteins and intracellular targets (Juven et al., 1994; Lambert et al., 2001; Walsh et al., 2003; Xu et al., 2008). The phenolic monoterpene carvacrol, as thymol, alters the structure and function of cell membranes, causing an increase in permeability (Cristani et al., 2007; Lambert et al., 2001; Sikkema et al., 1995; Xu et al., 2008). Furthermore, carvacrol is one of the few components of EOs able to disintegrate the outer membrane of Gram-negative bacteria (La Stora et al., 2011). Both thymol and carvacrol have an antifungal activity linked to the formation of lesion in the cytoplasmic membranes and a reduction in ergosterol content (Pinto et al., 2006). Compared to others terpenoids present in EOs, carvacrol is a more effective antimicrobial agent (Ben Arfa et al., 2006; Dorman and Deans, 2000; Ultee et al., 2002).

As said, the biophysical and biological features of an essential oil reflect those of its major components. Anyway, even if no data are available on the biological activity of other minor components, studies evidenced that possibly the activity of the most abundant molecules may be modulated by the minor ones (Franzios et al., 1997; Hoet et al., 2006; Santana-Rios et al., 2001).

Considering the results obtained in the present experimentation, we can assess that the synergic action of cinnamaldehyde-eugenol present in cinnamon oil is more effective against the microbiota present in wood and storage water with respect to the molecule combinations of thyme oils. Furthermore, the CT composition has a greater antimicrobial activity than the WT ones despite the greater antimicrobial effect reported for carvacrol, of which this oil is enriched, compared to the other terpenoids.

The NGS analyses performed on treated and untreated wood and storage water allowed to evaluate the effect of the tested oils on the microbiota composition. Fig. 26 shows the alpha diversity, calculated as Shannon index, for 16S (A-B) and ITS (C-D). No differences in term of richness and biodiversity were observed between the wood and storage water microbial communities. The lower diversity observed for the water sample in the 16S sequencing was not significant. The indexes calculated by clustering the samples for treatment show that, for both 16S and ITS, C and E samples were characterized by the greater diversity. The biodiversity obtained for CT, WT and NT was lower with respect to C and E and no significant differences were registered among these treatments.

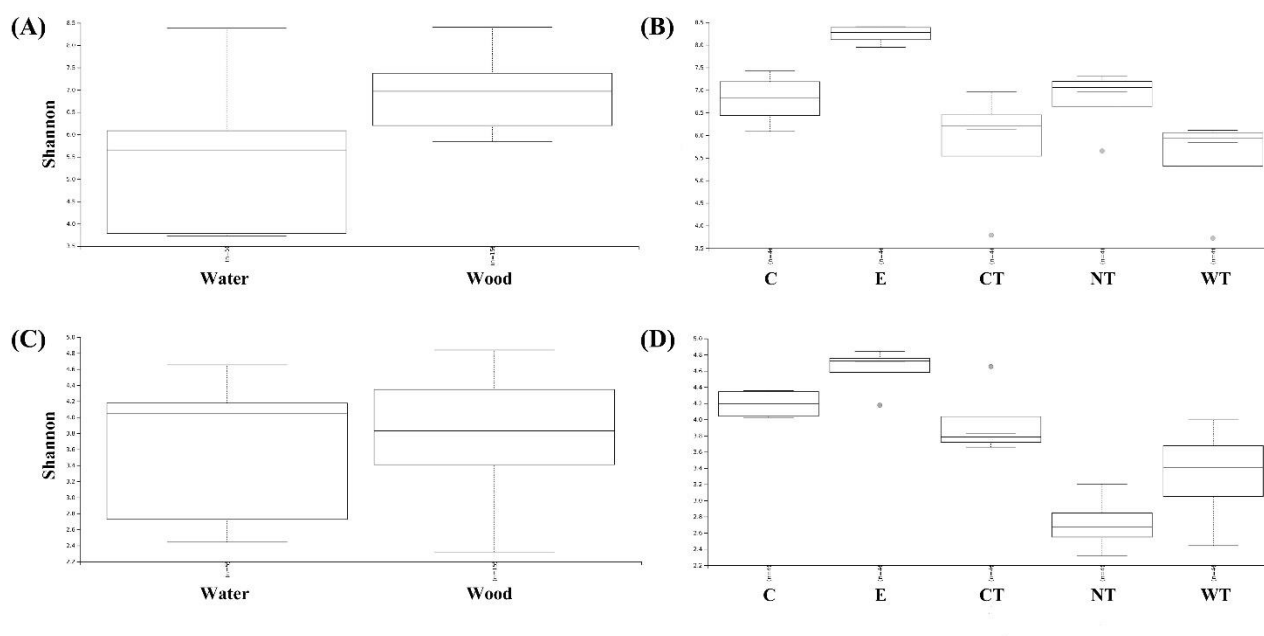


Figure 26. Boxplots of Alpha-diversity Index (calculated as Shannon index) (A-B) 16S. (C-D) ITS. (C: cinnamon; WT: wild thyme; CT: common thyme; E: ethanol; NT: not-treated control).

Fig. 27 reports the calculated beta diversity represented by Principle Coordinate Analysis Emperor plot on a Bray-Curtis distance matrix for 16S (A, C) and ITS (B, D). The quantitative diversity index (weighted UniFrac) for 16S (Fig. 27A) shows that the treatments can be clearly distinguished in five groups while the differences observed for ITS (Fig. 27B) were not so clear. The qualitative diversity indexes (unweighted UniFrac) show that NT and E samples, for 16S, and E samples, for ITS, group on the first axis, while no grouping could be observed for the other treatments.

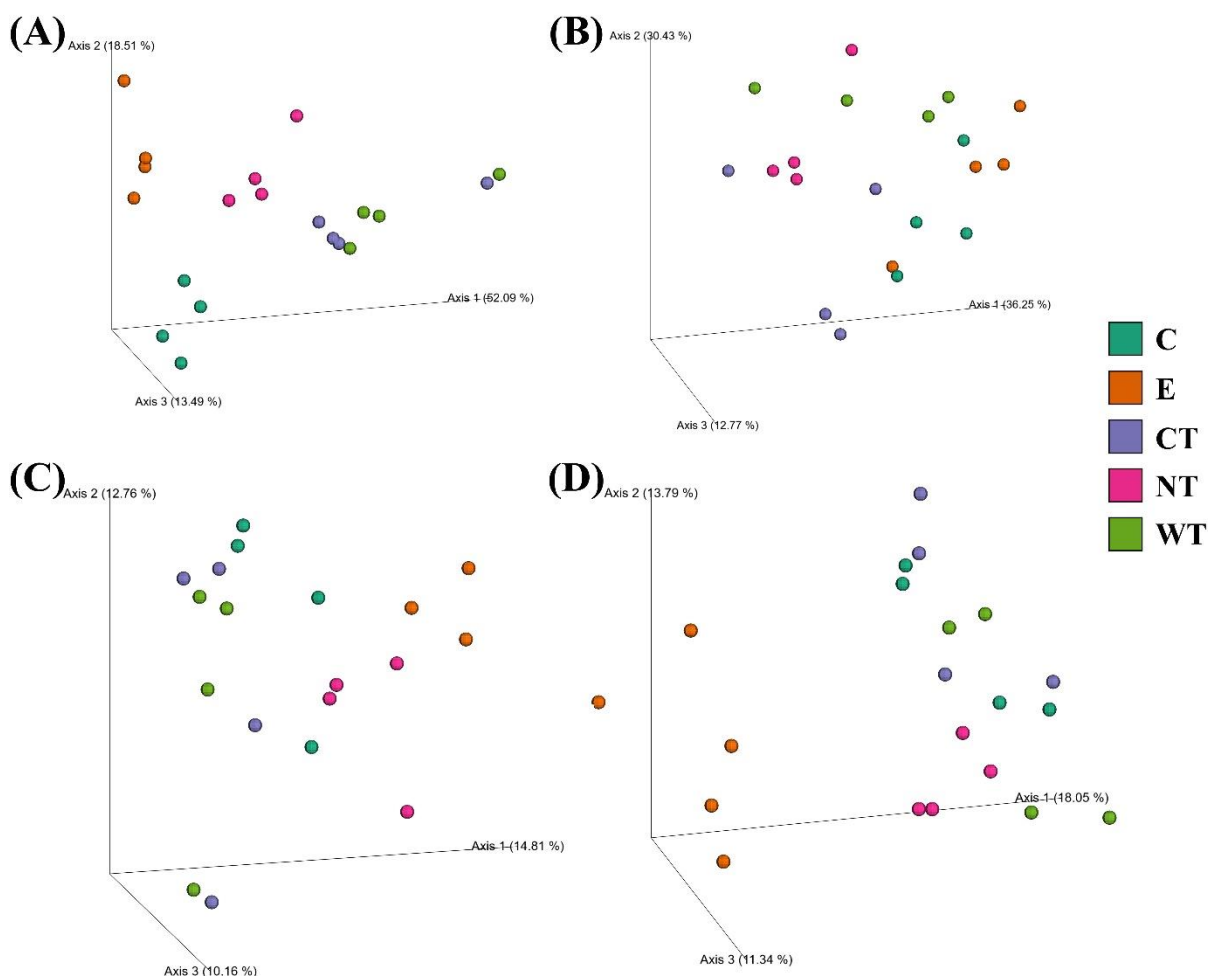


Figure 27. 16S (A, C) and ITS (B, D) beta diversity represented by Principle Coordinate Analysis Emperor plot on a Bray-Curtis distance matrix. (A-B) weighted UniFrac, (C-D) unweighted UniFrac. (C: cinnamon; WT: wild thyme; CT: common thyme; E: ethanol; NT: not-treated control).

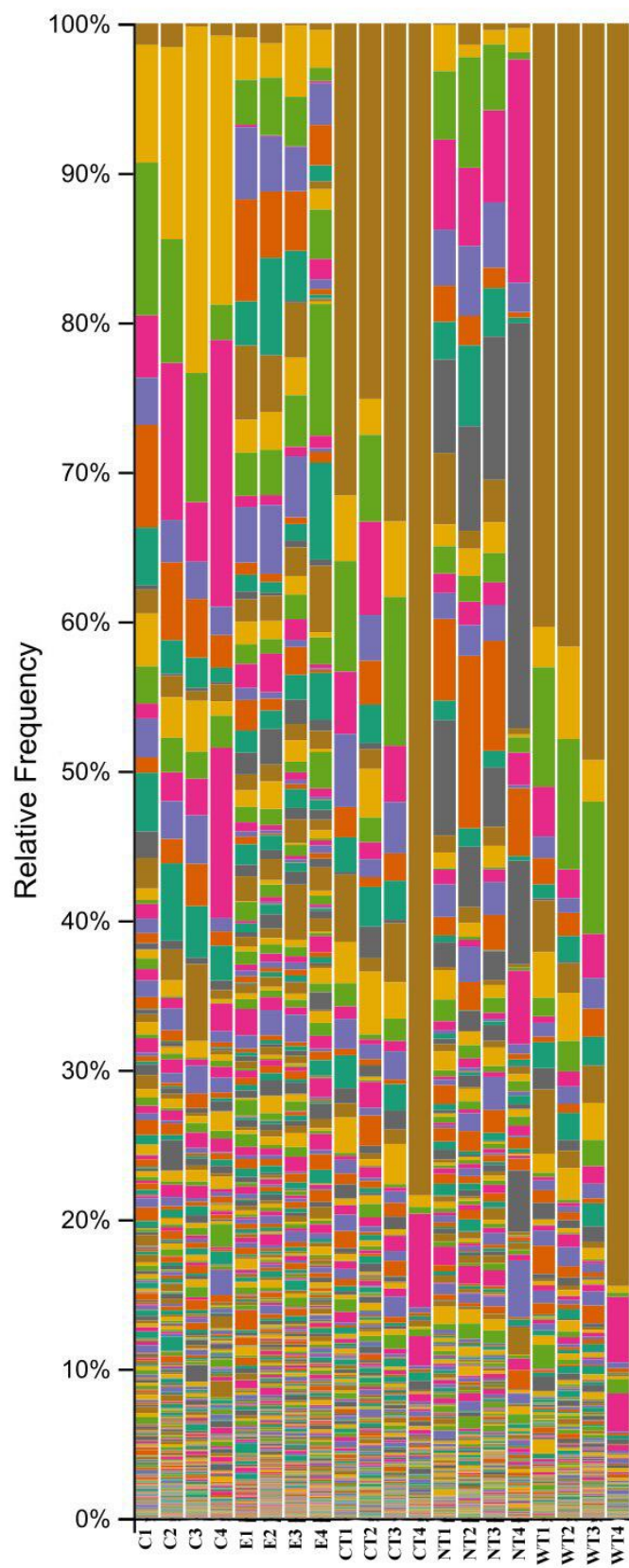
Figs. 28 and 29 report the stacked barcharts of the 16S and ITS taxonomic profiles at genus and species level sorted by treatment. The four samples reported for every treatment comprise the three wood replicates and the water sample.

The bacterial and fungal taxa identified during this part of the project confirm the community composition observed for this archaeological artifact (sample N9) during the preliminary tests carried out for the method development (§ 4.2.2.). Anyway, some differences could be highlighted among the different treatments in term of taxa relative frequencies.

For 16S at phylum level, it can be observed that Bacteroidetes bacteria are scarcely represented in all the oil treated samples. In fact, while in controls (both E and NT) the average relative frequency of this phylum is 6.6%, reaching even 13% in one of the E replicas, in treated wood and water it never exceeds 3%, with a mean value of 1.8%. The same trend was observed for the phylum Verrucomicrobia. In this case, average frequency values of 4.9% and 0.9% were registered in controls and treated samples respectively. Of note, the lowest values (always lower than 1%) were observed for the thymes, both CT and WT.

At family level, it is interesting to note that Pseudomonadaceae is enriched in thyme samples (mean frequency 47.9%, reaching 84.4% for WT and 78.4% for CT) while it represents less than 0.8% in all other analyzed samples. Obviously, this same trend was observed for the genus *Pseudomonas* (mean frequencies 47.9% for CT and WT and 0.9% the others).

Of note, Caulobacteraceae and Magnetospirillaceae are enriched in NT while they are present in trace in all the other samples.



D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Pseudomonadales;D_4_Pseudomonadaceae;D_5_Pseudomonas

D_0_Bacteria;D_1_Patescibacteria;D_2_Parcubacteria;D_3_Candidatus Adlerbacteria; ;

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Sphingomonadales;D_4_Sphingomonadaceae; ;

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhodobacterales;D_4_Rhodobacteraceae; ;

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Burkholderiaceae; ;

D_0_Bacteria;D_1_Planctomycetes;D_2_Planctomycetacia;D_3_Pirellulales;D_4_Pirellulaceae;D_5_uncultured

D_0_Bacteria;D_1_Planctomycetes;D_2_Planctomycetacia;D_3_Pirellulales;D_4_Pirellulaceae;D_5_Pir4 lineage

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhodospirillales;D_4_Magnetospirillaceae;D_5_Magnetospirillum

D_0_Bacteria;D_1_Patescibacteria;D_2_Parcubacteria;D_3_Candidatus Moranbacteria;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Planctomycetes;D_2_Planctomycetacia;D_3_Pirellulales;D_4_Pirellulaceae; ;

D_0_Bacteria; ; ; ; ;

D_0_Bacteria;D_1_Planctomycetes;D_2_Planctomycetacia;D_3_Planctomycetales;D_4_Schlesneriaceae;D_5_Planctopirus

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Xanthobacteraceae; ;

D_0_Bacteria;D_1_Bacteroidetes;D_2_Alphaproteobacteria;D_3_Caulobacterales;D_4_Caulobacteraceae;D_5_Brevundimonas

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Azospirillales;D_4_Azospirillaceae;D_5_Azospirillum

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Peptococcaceae;D_5_Desulfohalobium

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Methylophilaceae; ;

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Amb-16S-1323;D_5_uncultured bacterium

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria; ; ;

D_0_Bacteria;D_1_Verrucomicrobia;D_2_Verrucomicrobiae;D_3_Verrucomicrobiales;D_4_Verrucomicrobiaceae;D_5_Prostheco bacter

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales; ; ;

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Xanthobacteraceae;D_5_Pseudolabrys

D_0_Bacteria;D_1_Planctomycetes;D_2_Planctomycetacia;D_3_Gemmatales;D_4_Gemmataceae;D_5_Gemmata

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Xanthobacteraceae;D_5_uncultured

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Chitinophagales;D_4_Saprospiraceae;D_5_uncultured

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Rhizobiaceae;D_5_Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium

D_0_Bacteria;D_1_Planctomycetes;D_2_Planctomycetacia;D_3_Planctomycetales;D_4_Rubinisphaeraceae;D_5_SH-PL14

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Hyphomicrobiaceae;D_5_Hyphomicrobium

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Legionellales;D_4_Legionellaceae;D_5_Legionella

D_0_Bacteria;D_1_Actinobacteria;D_2_Actinobacteria;D_3_Micrococcales; ; ;

D_0_Bacteria;D_1_Verrucomicrobia;D_2_Verrucomicrobiae;D_3_Pedospiraales;D_4_Pedospiraaceae; ;

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria; ; ; ;

D_0_Bacteria;D_1_Acidobacteria;D_2_Subgroup 6; ; ; ;

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Devesiaceae;D_5_Devesia

D_0_Bacteria;D_1_Chloroflexi;D_2_Anaerolineae;D_3_Caldilineales;D_4_Caldilineaceae;D_5_uncultured

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Flavobacteriales;D_4_Flavobacteriaceae;D_5_Flavobacterium

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Parvibaculales;D_4_Parvibaculaceae;D_5_Parvibaculum

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Xanthobacteraceae;D_5_Xanthobacter

D_0_Bacteria;D_1_Planctomycetes;D_2_Planctomycetacia;D_3_Pirellulales;D_4_Pirellulaceae;D_5_Pirellula

D_0_Bacteria;D_1_Planctomycetes;D_2_Planctomycetacia;D_3_Planctomycetales;D_4_uncultured; ;

D_0_Bacteria;D_1_Patescibacteria;D_2_Parcubacteria;D_3_Candidatus Nomurabacteria;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Rhizobiales Incertae Sedis;D_5_uncultured

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_R7C24;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Rhizobiaceae; ;

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria; ; ; ;

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Desulfuromonadales;D_4_Geobacteraceae;D_5_Geobacter

D_0_Bacteria;D_1_Acidobacteria;D_2_Aminicenantia;D_3_Aminicenantales; ; ;

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Prolixibacteraceae;D_5_WCHB1-32

D_0_Bacteria;D_1_Verrucomicrobia;D_2_Verrucomicrobiae;D_3_Opitutales;D_4_Opitutaceae; ;

D_0_Bacteria;D_1_Planctomycetes;D_2_Phycisphaerae;D_3_Tepidisphaerales;D_4_WD2101 soil group;D_5_uncultured bacterium

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Cytophagales;D_4_Microscillaceae;D_5_uncultured

D_0_Bacteria;D_1_Patescibacteria;D_2_Parcubacteria;D_3_Rhizobiales;D_4_Devesiaceae; ;

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Myxococcales;D_4_Sandaracinaceae;D_5_uncultured

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Caulobacterales;D_4_Caulobacteraceae; ;

D_0_Bacteria;D_1_Chlamydiae;D_2_Chlamydiae;D_3_Chlamydiales; ; ;

D_0_Bacteria;D_1_Bacteroidetes;D_2_Ignavibacteria;D_3_SJA-28;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Chloroflexi;D_2_Anaerolineae;D_3_SBR1031;D_4_A4b; ;

D_0_Bacteria;D_1_Actinobacteria;D_2_Actinobacteria;D_3_Micrococcales;D_4_Microbacteriaceae; ;

D_0_Bacteria;D_1_Patescibacteria;D_2_Parcubacteria;D_3_Candidatus Adlerbacteria;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Chitinophagales;D_4_Chitinophagaceae;D_5_Ferruginibacter

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Prolixibacteraceae;D_5_uncultured

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Caulobacterales;D_4_Hyphomonadaceae;D_5_Hirschia

D_0_Bacteria;D_1_Patescibacteria;D_2_Parcubacteria;D_3_Candidatus Kaiserbacteria; ; ;

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Oceanospirillales;D_4_Pseudohongiellaceae;D_5_Bfyi10

Unassigned; ; ; ; ;

D_0_Bacteria;D_1_Acidobacteria;D_2_Thermoanaerobaculia;D_3_Thermoanaerobaculales;D_4_Thermoanaerobaculaceae;D_5_Subgroup 10

D_0_Bacteria;D_1_Planctomycetes;D_2_Planctomycetacia;D_3_Isosphaerales;D_4_Isosphaeraceae;D_5_uncultured

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Family XIII;D_5_Anaerovorax

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Paracaeidbacteriales;D_4_Paracaeidbacteriaceae;D_5_Candidatus Paracaeidbacter

D_0_Bacteria;D_1_Patescibacteria;D_2_Microgenomatia;D_3_Candidatus Collierbacteria;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Sva0485; ; ;

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_A0839;D_5_uncultured bacterium

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Caulobacterales;D_4_Caulobacteraceae;D_5_Phenylobacterium

D_0_Bacteria;D_1_Verrucomicrobia;D_2_Verrucomicrobiae;D_3_Verrucomicrobiales;D_4_Verrucomicrobiaceae;__

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Hydrogenophilaceae;D_5_Thiobacillus

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Sphingobacteriales;__

D_0_Bacteria;D_1_Armatimonadetes;D_2_Fimbriimonadia;D_3_Fimbriimonadales;D_4_Fimbriimonadaceae;__

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Cytophagales;D_4_Microscillaceae;__

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;__

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Hyphomicrobiaceae;__

D_0_Bacteria;D_1_Planctomycetes;D_2_Planctomycetacia;D_3_Planctomycetales;D_4_uncultured;D_5_metagenome

D_0_Bacteria;D_1_Firmicutes;D_2_Negativicutes;D_3_Selenomonadales;D_4_Veillonellaceae;D_5_Anaerospira

D_0_Bacteria;D_1_Verrucomicrobia;D_2_Verrucomicrobiae;D_3_Methylacidiphilales;D_4_Methylacidiphilaceae;D_5_uncultured

D_0_Bacteria;D_1_Verrucomicrobia;D_2_Verrucomicrobiae;D_3_Chthoniobacteriales;D_4_Chthoniobacteraceae;D_5_Chthoniobacter

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Hyphomicrobiaceae;D_5_Pedomicrobium

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Bdellovibrionales;D_4_Bdellovibrionaceae;D_5_Bdellovibrio

D_0_Bacteria;D_1_Proteobacteria;__

D_0_Bacteria;D_1_Planctomycetes;D_2_Planctomycetacia;D_3_Gemmatales;D_4_Gemmataceae;D_5_uncultured

D_0_Bacteria;D_1_Hydrogenedentes;D_2_Hydrogenedentia;D_3_Hydrogenedentiales;D_4_Hydrogenedensaceae;D_5_metagenome

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Caulobacterales;D_4_Caulobacteraceae;D_5_Caulobacter

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_TRA3-20;__

D_0_Bacteria;D_1_Planctomycetes;D_2_Planctomycetacia;D_3_Pirellulales;D_4_Pirellulaceae;D_5_Blastopirellula

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_CCD24;__

D_0_Bacteria;D_1_Planctomycetes;D_2_vadinHA49;D_3_metagenome;D_4_metagenome;D_5_metagenome

D_0_Bacteria;D_1_Acidobacteria;D_2_Holophagae;D_3_Subgroup 7;__

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Burkholderiaceae;D_5_Rhizobacter

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Gammaproteobacteria Incertae Sedis;D_4_Unknown Family;__

D_0_Bacteria;D_1_Actinobacteria;D_2_Actinobacteria;D_3_PeM15;__

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Diplorickeetsiales;D_4_Diplorickeetsiaceae;D_5_Aquicella

D_0_Bacteria;D_1_Planctomycetes;D_2_Planctomycetacia;__

D_0_Bacteria;D_1_Actinobacteria;D_2_Acidimicrobiia;D_3_Microtrichales;D_4_Illumatobacteraceae;D_5_Illumatobacter

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Cytophagales;D_4_Microscillaceae;D_5_OLB12

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Sphingomonadales;D_4_Sphingomonadaceae;D_5_Sphingourantiacus

D_0_Bacteria;D_1_Verrucomicrobia;D_2_Verrucomicrobiae;D_3_Verrucomicrobiales;D_4_Rubritaleaceae;D_5_Luteolibacter

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Chitinophagales;D_4_Saprospiraceae;D_5_Haliscomenobacter

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_R7C24;__

D_0_Bacteria;D_1_Patescibacteria;D_2_Parcubacteria;__

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Caulobacterales;D_4_Hyphomonadaceae;D_5_SWB02

D_0_Bacteria;D_1_Planctomycetes;D_2_Phycisphaerae;D_3_Tepidisphaerales;D_4_Tepidisphaeraeae;D_5_uncultured bacterium

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Burkholderiaceae;D_5_Delftia

D_0_Bacteria;D_1_Verrucomicrobia;D_2_Verrucomicrobiae;D_3_Opitutales;D_4_Opitutaceae;D_5_Opitutus

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Sphingomonadales;D_4_Sphingomonadaceae;D_5_Sphingomonas

D_0_Bacteria;D_1_Chloroflexi;D_2_Anaerolineae;D_3_Anaerolineales;D_4_Anaerolineaceae;D_5_uncultured

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Desulfarculales;D_4_Desulfarculaceae;D_5_uncultured

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Dongiales;D_4_Dongiaceae;D_5_Dongia

D_0_Bacteria;D_1_Spirochaetes;D_2_Spirochaetia;D_3_Spirochaetales;D_4_Spirochaetaceae;D_5_Spirochaeta 2

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;__

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;__

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Gracilbacteraceae;D_5_Lutispora

D_0_Bacteria;D_1_Nitrospirae;D_2_Thermodesulfobivibronia;D_3_uncultured;D_4_uncultured Nitrospirae bacterium;D_5_uncultured Nitrospirae bacterium

D_0_Bacteria;D_1_Actinobacteria;D_2_Acidimicrobiia;D_3_Microtrichales;D_4_Illumatobacteraceae;D_5_CL500-29 marine group

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Cellvibrionales;D_4_Cellvibrionaceae;D_5_Cellvibrio

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Myxococcales;__

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Chitinophagales;__

D_0_Bacteria;D_1_Verrucomicrobia;D_2_Verrucomicrobiae;D_3_Chthoniobacteriales;D_4_Chthoniobacteraceae;__

D_0_Bacteria;D_1_Planctomycetes;D_2_Planctomycetacia;D_3_Gemmatales;D_4_Gemmataceae;D_5_Fimbrigliobus

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Burkholderiaceae;D_5_Achromobacter

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Reyranellales;D_4_Reyranellaceae;D_5_Reyranella

D_0_Bacteria;D_1_Patescibacteria;D_2_Gracilbacteria;D_3_Absconditabacteriales (SR1);__

D_0_Bacteria;D_1_Acidobacteria;D_2_Acidobacteriia;D_3_Solibacterales;D_4_Solibacteraceae (Subgroup 3);D_5_Paludibaculum

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;__

D_0_Bacteria;D_1_Atribacteria;D_2_JS1;D_3_uncultured bacterium;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Bacteroidaceae;D_5_Bacteroides

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Rhodocyclaceae;__

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Micavibrionales;D_4_uncultured;__

D_0_Bacteria;D_1_Chloroflexi;__

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Diplorickeetsiales;D_4_Diplorickeetsiaceae;D_5_uncultured

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Myxococcales;D_4_Nannocystaceae;D_5_Nannocystis

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Rhizobiales Incertae Sedis;D_5_Bauldia

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Syntrophobacteriales;D_4_Syntrophaceae;D_5_uncultured

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Muribaculaceae;D_5_uncultured bacterium

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_CCD24;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Xanthobacteraceae;D_5_Bradyrhizobium

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Myxococcales;D_4_Iliangiaceae;D_5_Iliangium

D_0_Bacteria;D_1_Patescibacteria;D_2_Parcubacteria;D_3_Candidatus Adlerbacteria;D_4_metagenome;D_5_metagenome

D_0_Bacteria;D_1_Chloroflexi;D_2_Dehalococcoidia;D_3_661239;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Holosporales;D_4_Holosporaceae;D_5_uncultured

D_0_Bacteria;D_1_Fibrobacteres;D_2_Fibrobacteria;D_3_Fibrobacterales;D_4_Fibrobacteraceae;D_5_possible genus 04

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Flavobacteriales;D_4_Flavobacteriaceae;D_5_uncultured

D_0_Bacteria;D_1_Plantomycetes;D_2_Plantomycetacia;D_3_uncultured;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Sphingomonadales;D_4_Sphingomonadaceae;D_5_Sphingobium

D_0_Bacteria;D_1_Patescibacteria;D_2_Gracilibacteria;D_3_Candidatus Peribacteria;D_4_metagenome;D_5_metagenome

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Chitinophagales;D_4_Chitinophagaceae;D_5_Terrimonas

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhodospirillales;D_4_Magnetospiraceae;D_5_uncultured

D_0_Bacteria;D_1_Chloroflexi;D_2_TK10;D_3_ ; ;

D_0_Bacteria;D_1_Verrucomicrobia;D_2_Verrucomicrobiae;D_3_Verrucomicrobiales;D_4_Rubritaleaceae;D_5_uncultured

D_0_Bacteria;D_1_Nitrospirae;D_2_Nitrospira;D_3_Nitrospirales;D_4_Nitrospiraceae;D_5_Nitrospira

D_0_Bacteria;D_1_Chloroflexi;D_2_Dehalococcoidia;D_3_t0.6.f;D_4_ ; ;

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Papillibacter

D_0_Bacteria;D_1_Patescibacteria;D_2_Microgenomatia;D_3_Candidatus Levybacteria;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Armatimonadetes;D_2_uncultured;D_3_ ; ;

D_0_Bacteria;D_1_Chloroflexi;D_2_Dehalococcoidia;D_3_t0.6.f;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Actinobacteria;D_2_Acidimicrobiia;D_3_IMCC26256;D_4_ ; ;

D_0_Bacteria;D_1_Chloroflexi;D_2_Dehalococcoidia;D_3_S085;D_4_metagenome;D_5_metagenome

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Clostridiaceae I;D_5_Clostridium sensu stricto 13

D_0_Bacteria;D_1_Patescibacteria;D_2_WWE3;D_3_ ; ;

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Xanthomonadales;D_4_Rhodanobacteraceae;D_5_uncultured

D_0_Bacteria;D_1_Verrucomicrobia;D_2_Verrucomicrobiae;D_3_Verrucomicrobiales;D_4_Verrucomicrobiaceae;D_5_Roseimicrobium

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Xanthomonadales;D_4_Rhodanobacteraceae;D_5_uncultured

D_0_Bacteria;D_1_Gemmatimonadetes;D_2_Gemmatimonadetes;D_3_Gemmatimonadales;D_4_Gemmatimonadaceae;D_5_uncultured

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Gammaproteobacteria Incertae Sedis;D_4_Unknown Family;D_5_Candidatus Berkiella

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Myxococcales;D_4_Brii41;D_5_uncultured

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Nitrosomonadaceae;D_5_IS-44

D_0_Bacteria;D_1_Elusimicrobia;D_2_4-29;D_3_uncultured bacterium;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Dependentiae;D_2_Babeliae;D_3_Babeliales;D_4_ ; ;

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Micropepsales;D_4_Micropepsaceae;D_5_uncultured

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Faecalibacterium

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Peptococcaceae;D_5_uncultured

D_0_Bacteria;D_1_Plantomycetes;D_2_Plantomycetacia;D_3_Isosphaerales;D_4_Isosphaeraceae;D_5_Aquisphaera

D_0_Bacteria;D_1_Verrucomicrobia;D_2_Verrucomicrobiae;D_3_Verrucomicrobiales;D_4_DEV007;D_5_uncultured

D_0_Bacteria;D_1_Chloroflexi;D_2_KD4-96;D_3_uncultured bacterium;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Chloroflexi;D_2_JG30-KF-CM66;D_3_uncultured Chloroflexi bacterium;D_4_uncultured Chloroflexi bacterium;D_5_uncultured Chloroflexi bacterium

D_0_Bacteria;D_1_Acidobacteria;D_2_Acidobacterii;D_3_Solibacterales;D_4_Solibacteraceae (Subgroup 3);D_5_Bryobacter

D_0_Bacteria;D_1_Verrucomicrobia;D_2_Verrucomicrobiae;D_3_Verrucomicrobiales;D_4_Verrucomicrobiaceae;D_5_uncultured

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Sphingomonadales;D_4_Sphingomonadaceae;D_5_Sphingopyxis

D_0_Bacteria;D_1_Chlamydiae;D_2_Chlamydiae;D_3_Chlamydiales;D_4_Parachlamydiaceae;D_5_Candidatus Protochlamydia

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Burkholderiaceae;D_5_Massilia

D_0_Bacteria;D_1_Armatimonadetes;D_2_Fimbriimonadia;D_3_Fimbriimonadales;D_4_Fimbriimonadaceae;D_5_uncultured bacterium

D_0_Bacteria;D_1_Plantomycetes;D_2_Phycisphaerae;D_3_mle1-8;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Beijerinckiaceae;D_5_Bosea

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Caulobacteriales;D_4_Caulobacteraceae;D_5_uncultured

D_0_Bacteria;D_1_Plantomycetes;D_2_Phycisphaerae;D_3_Phycisphaerales;D_4_Phycisphaeraceae;D_5_uncultured

D_0_Bacteria;D_1_Plantomycetes;D_2_Plantomycetacia;D_3_Pirellulales;D_4_Pirellulaceae;D_5_Pir1 lineage

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Myxococcales;D_4_P3OB-42;D_5_metagenome

D_0_Bacteria;D_1_Acidobacteria;D_2_Blastocatellia (Subgroup 4);D_3_Blastocatellales;D_4_Blastocatellaceae;D_5_uncultured

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Burkholderiaceae;D_5_Pisciniibacter

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Sphingobacteriales;D_4_Sphingobacteriaceae;D_5_uncultured

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Sva0485;D_4_uncultured delta proteobacterium;D_5_uncultured delta proteobacterium

D_0_Bacteria;D_1_Spirochaetes;D_2_Leptospirae;D_3_Leptospirales;D_4_Leptospiraceae;D_5_Leptospira

D_0_Bacteria;D_1_Patescibacteria;D_2_Parcubacteria;D_3_GWA2-38-13b;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Cytophagales;D_4_ ; ;

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Muribaculaceae;D_5_uncultured

D_0_Bacteria;D_1_Actinobacteria;D_2_Acidimicrobiia;D_3_Microtrichales;D_4_uncultured;D_5_uncultured

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rickettsiales;D_4_Rickettsiaceae;D_5_Candidatus Megaira

D_0_Bacteria;D_1_Plantomycetes;D_2_OM190;D_3_ ; ;

D_0_Bacteria;D_1_Plantomycetes;D_2_Plantomycetacia;D_3_Plantomycetales;D_4_Rubinisphaeraceae;D_5_uncultured

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Caulobacteriales;D_4_Hyphomonadaceae;D_5_Hyphomonas

D_0_Bacteria;D_1_Chloroflexi;D_2_Anaerolineae;D_3_SBR1031;D_4_A4b;D_5_uncultured soil bacterium

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Myxococcales;D_4_Phaselicystidaceae;D_5_Phaselicystis

D_0_Bacteria;D_1_Patescibacteria;D_2_Microgenomatia;D_3_Candidatus Amesbacteria;D_4_Candidatus Amesbacteria bacterium GW2011_GWA2_42_12;D_5_Candidatus Amesbacteria b

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Micavibrionales;D_4_Micavibrionaceae;D_5_uncultured

D_0_Bacteria;D_1_Actinobacteria;D_2_Acidimicrobiia;D_3_Microtrichales;D_4_ ; ;

D_0_Bacteria;D_1_Actinobacteria;D_2_WCHB1-81;D_3_ ; ;

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Xanthobacteraceae;D_5_Pseudorhodoplanes

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Rhodocyclaceae;D_5_Sulfuritalea

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Sphingobacteriales;D_4_KD3-93;D_5_metagenome

D_0_Bacteria;D_1_Chloroflexi;D_2_OLB14;D_3_ ; ;

D_0_Bacteria;D_1_Plantomycetes;D_2_Phycisphaerae;D_3_Tepidisphaerales;D_4_WD2101 soil group;__

D_0_Bacteria;D_1_Actinobacteria;D_2_Acidimicrobia;D_3_Microtrichales;D_4_Jamiaceae;D_5_Jamia

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Myxococcales;D_4_Blfdi19;__

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Acetobacterales;D_4_Acetobacterales Incertae Sedis;D_5_uncultured

D_0_Bacteria;D_1_Chlamydiae;D_2_Chlamydiae;D_3_Chlamydiales;D_4_Parachlamydiaceae;__

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Desulfobacterales;D_4_Desulfobulbaceae;D_5_Desulfurivibrio

D_0_Bacteria;D_1_Acidobacteria;D_2_Subgroup 6;D_3_Unknown Order;D_4_Unknown Family;D_5_Luteitalea

D_0_Bacteria;D_1_Acidobacteria;D_2_Acidobacteriia;D_3_Solibacterales;D_4_Solibacteraceae (Subgroup 3);__

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Bdellovibrionales;D_4_Bacteriovoracaceae;D_5_Peredibacter

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Cytophagales;D_4_Spirosomaceae;D_5_Runella

D_0_Bacteria;D_1_TA06; ; ; ;__

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Eubacteriaceae;__

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Sphingomonadales;D_4_Sphingomonadaceae;D_5_Altererythrobacter

D_0_Archaea;D_1_Crenarchaeota;D_2_Bathyarchaea; ; ; ;__

D_0_Bacteria;D_1_Chloroflexi;D_2_OLB14;D_3_uncultured bacterium;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Steroidobacterales;D_4_Steroidobacteraceae;__

D_0_Bacteria;D_1_Patescibacteria;D_2_Parcubacteria;D_3_Candidatus Kaiserbacteria;D_4_metagenome;D_5_metagenome

D_0_Bacteria;D_1_Chloroflexi;D_2_Dehalococcoidia; ; ; ;__

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Flavobacteriales; ; ; ;__

D_0_Bacteria;D_1_Actinobacteria;D_2_WCHB1-81;D_3_uncultured bacterium;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Chloroflexi;D_2_KD4-96; ; ; ;__

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Paracaeidibacteriales;D_4_Paracaeidibacteraceae;D_5_uncultured

D_0_Bacteria;D_1_Patescibacteria;D_2_Parcubacteria;D_3_Candidatus Nomurabacteria; ; ; ;__

D_0_Archaea;D_1_Crenarchaeota;D_2_Bathyarchaea;D_3_uncultured methanogenic archaeon;D_4_uncultured methanogenic archaeon;D_5_uncultured methanogenic archaeon

D_0_Bacteria;D_1_Kiritimatiellaota;D_2_Kiritimatiellae;D_3_WCHB1-41;D_4_metagenome;D_5_metagenome

D_0_Bacteria;D_1_Gemmatimonadetes;D_2_Longimicrobia;D_3_Longimicrobiales;D_4_Longimicrobiaceae;D_5_uncultured bacterium

D_0_Bacteria;D_1_Cyanobacteria;D_2_Melainabacteria;D_3_Obscuribacterales;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Chitinophagales;D_4_Chitinophagaceae;D_5_Flavihumibacter

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Blaulia

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Micavibrionales;D_4_uncultured;D_5_metagenome

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Chitinophagales;D_4_Chitinophagaceae;D_5_uncultured

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Nitrosomonadaceae;D_5_Nitrosomonas

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_A21b;__

D_0_Bacteria;D_1_Verrucomicrobia;D_2_Verrucomicrobiae;D_3_Opitutales;D_4_Opitutaceae;D_5_Lacunisphaera

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Cytophagales;D_4_Spirosomaceae;D_5_Emticicia

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Prevotellaceae;D_5_Prevotella 9

D_0_Bacteria;D_1_Plantomycetes;D_2_Plantomycetacia;D_3_Plantomycetales; ; ; ;__

D_0_Bacteria;D_1_Patescibacteria;D_2_Saccharimonadia;D_3_Saccharimonadales; ; ; ;__

D_0_Bacteria;D_1_Patescibacteria;D_2_Microgenomatia;D_3_Candidatus Levybacteria; ; ; ;__

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_uncultured; ; ; ;__

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Peptococcaceae;D_5_BRH-c8a

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Chitinophagales;D_4_Chitinophagaceae;__

D_0_Bacteria;D_1_Patescibacteria;D_2_Parcubacteria;D_3_Candidatus Kaiserbacteria;D_4_uncultured organism;D_5_uncultured organism

D_0_Bacteria;D_1_Cyanobacteria;D_2_Oxyphotobacteria;D_3_Chloroplast; ; ; ;__

D_0_Bacteria;D_1_Patescibacteria;D_2_Parcubacteria;D_3_Candidatus Vogelbacteria;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Rhizobiales Incertae Sedis;D_5_Nordella

D_0_Bacteria;D_1_Acidobacteria;D_2_Blastocatellia (Subgroup 4);D_3_11-24; ; ; ;__

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Coxiellales;D_4_Coxiellaceae;D_5_Coxiella

D_0_Bacteria;D_1_Hydrogenedentes;D_2_Hydrogenedentia;D_3_Hydrogenedentiales;D_4_Hydrogenedensaceae;__

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Reyranellales;D_4_Reyranellaceae;D_5_uncultured

D_0_Bacteria;D_1_Chloroflexi;D_2_TK10;D_3_metagenome;D_4_metagenome;D_5_metagenome

D_0_Bacteria;D_1_Plantomycetes;D_2_Phycisphaerae;D_3_Phycisphaerales;D_4_Phycisphaeraceae;D_5_SM1A02

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Acetobacterales;D_4_Acetobacteraceae;__

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Xanthomonadales;D_4_Xanthomonadaceae;__

D_0_Bacteria;D_1_Acidobacteria;D_2_Blastocatellia (Subgroup 4);D_3_Blastocatellales;D_4_Blastocatellaceae;D_5_Aridibacter

D_0_Bacteria;D_1_Actinobacteria;D_2_Actinobacteria;D_3_Corynebacteriales;D_4_Mycobacteriaceae;D_5_Mycobacterium

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;__

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Burkholderiaceae;D_5_Aquabacterium

D_0_Bacteria;D_1_Chloroflexi;D_2_Dehalococcoidia;D_3_GIF9;D_4_AB-539-J10;D_5_SCGC-AB-539-J10

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Beijerinckiaceae;__

D_0_Bacteria;D_1_Patescibacteria;D_2_Parcubacteria;D_3_Candidatus Spechtbacteria;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Plantomycetes;D_2_Plantomycetacia;D_3_Pirellulales;D_4_Pirellulaceae;D_5_Rhodopirellula

D_0_Bacteria;D_1_Actinobacteria;D_2_Acidimicrobia; ; ; ;__

D_0_Bacteria;D_1_Chloroflexi;D_2_Chloroflexia;D_3_Thermomicrobiales;D_4_JG30-KF-CM45;D_5_metagenome

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rickettsiales;D_4_Mitochondria;D_5_Andalucia godoyi

D_0_Bacteria;D_1_Dependentiae;D_2_Babeliae;D_3_Babeliales;D_4_Vermiphilaceae;__

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Sphingobacteriales;D_4_NS11-12 marine group;__

D_0_Bacteria;D_1_Actinobacteria;D_2_Thermoleophilia;D_3_Gaiellales;D_4_Gaiellaceae;D_5_Gaiella

D_0_Bacteria;D_1_Acidobacteria;D_2_Subgroup 17; ; ; ;__

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Cytophagales;D_4_Spirosomaceae;D_5_Dyadobacter

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Muribaculaceae;D_5_uncultured Bacteroidales bacterium

D_0_Bacteria;D_1_Gemmatimonadetes;D_2_Gemmatimonadetes;D_3_Gemmatimonadales;D_4_Gemmatimonadaceae;__

D_0_Bacteria;D_1_Actinobacteria;D_2_Actinobacteria;D_3_Propionibacteriales;D_4_Nocardioidaceae;D_5_Nocardioides

D_0_Bacteria;D_1_Chloroflexi;D_2_Anaerolineae;D_3_SBR1031; ; ;

D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Bacillales;D_4_Paenibacillaceae; ;

D_0_Bacteria;D_1_Chloroflexi;D_2_Anaerolineae;D_3_SBR1031;D_4_A4b;D_5_metagenome

D_0_Bacteria;D_1_Plantomycetes;D_2_Plantomycetacia;D_3_Isosphaerales;D_4_Isosphaeraceae;D_5_Singulisphaera

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Christensenellaceae;D_5_uncultured

D_0_Bacteria;D_1_Chlamydiae;D_2_Chlamydiae;D_3_Chlamydiales;D_4_cvE6;D_5_uncultured bacterium

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Bdellovibrionales;D_4_Bacteriovoracaceae;D_5_Bacteriovorax

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_[Eubacterium] hallii group

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_KF-JG30-B3;D_5_uncultured bacterium

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Sphingobacteriales;D_4_Sphingobacteriaceae;D_5_Pedobacter

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Syntrophobacteriales;D_4_Syntrophaceae;D_5_Desulfomonile

D_0_Bacteria;D_1_Plantomycetes;D_2_Phycisphaerae;D_3_Tepidisphaerales;D_4_Tepidisphaeraceae;D_5_Tepidisphaera

D_0_Bacteria;D_1_Actinobacteria;D_2_Actinobacteria;D_3_Bifidobacteriales;D_4_Bifidobacteriaceae;D_5_Bifidobacterium

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Cellvibrionales;D_4_Porticocaceae;D_5_C1-B045

D_0_Bacteria;D_1_Acidobacteria;D_2_Aminicenantia;D_3_Aminicenantes;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Thermoanaerobacteriales;D_4_SRB2;D_5_uncultured bacterium

D_0_Bacteria;D_1_Actinobacteria;D_2_Thermoleophilina;D_3_Gaiellales;D_4_uncultured; ;

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Azospirillales;D_4_Azospirillales Incertae Sedis;D_5_Stella

D_0_Bacteria;D_1_Patescibacteria;D_2_Parcubacteria;D_3_Candidatus Kaiserbacteria;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Plantomycetes;D_2_Plantomycetacia;D_3_Gemmatales;D_4_Gemmataceae;D_5_Telmatocola

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Immundisolibacteriales;D_4_Immundisolibacteriaceae;D_5_Immundisolibacter

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Myxococcales;D_4_mle1-27;D_5_metagenome

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Sphingobacteriales;D_4_env.OPS 17; ;

D_0_Bacteria;D_1_Plantomycetes;D_2_Plantomycetacia;D_3_Plantomycetales;D_4_uncultured;D_5_uncultured bacterium

D_0_Bacteria;D_1_Cyanobacteria;D_2_Sericytochromatia; ; ;

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Chitinophagales;D_4_Chitinophagaceae;D_5_Sediminibacterium

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Tannerellaceae;D_5_Parabacteroides

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Gammaproteobacteria Incertae Sedis;D_4_Unknown Family;D_5_Acidibacter

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Bdellovibrionales;D_4_Bdellovibrionaceae;D_5_OM27 clade

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Acetobacteriales;D_4_Acetobacteraceae;D_5_Roseomonas

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhodospirillales;D_4_Rhodospirillaceae;D_5_uncultured

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Chitinophagales;D_4_Saprospiraceae; ;

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Chitinophagales;D_4_Chitinophagaceae;D_5_Dinghuibacter

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Prevotellaceae;D_5_Alloprevotella

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Burkholderiaceae;D_5_Curvibacter

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Desulfarculales;D_4_Desulfarculaceae;D_5_Desulfocarbo

D_0_Bacteria;D_1_Chloroflexi;D_2_Anaerolineae;D_3_SBR1031;D_4_uncultured organism;D_5_uncultured organism

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Rikenellaceae;D_5_Alistipes

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4 Beijerinckiaceae;D_5_Methylobacterium

D_0_Bacteria;D_1_Plantomycetes;D_2_Plantomycetacia;D_3_Plantomycetales;D_4_Gimesiaceae;D_5_uncultured

D_0_Bacteria;D_1_Actinobacteria;D_2_Acidimicrobiia;D_3_IMCC26256;D_4_uncultured Acidimicrobiidae bacterium;D_5_uncultured Acidimicrobiidae bacterium

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Oligoflexales;D_4_0319-6G20; ;

D_0_Bacteria;D_1_Plantomycetes;D_2_Plantomycetacia;D_3_uncultured; ; ;

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Enterobacteriales;D_4_Enterobacteriaceae;D_5_Serratia

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Desulfuromonadales; ; ;

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_MBNT15; ; ;

D_0_Bacteria;D_1_Armatimonadetes;D_2_Armatimonadia;D_3_Armatimonadales; ; ;

D_0_Bacteria;D_1_Chloroflexi;D_2_Anaerolineae;D_3_MSB-5B2;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales; ; ;

D_0_Bacteria;D_1_Plantomycetes;D_2_Plantomycetacia;D_3_Isosphaerales;D_4_Isosphaeraceae; ;

D_0_Bacteria;D_1_Chloroflexi;D_2_Anaerolineae; ; ; ;

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Gallionellaceae; ;

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Hyphomicrobiaceae;D_5_uncultured

D_0_Bacteria;D_1_Actinobacteria;D_2_Acidimicrobiia;D_3_Actinomarinales;D_4_uncultured; ;

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminiclostridium 1

D_0_Bacteria;D_1_Chloroflexi;D_2_JG30-KF-CM66; ; ; ;

D_0_Bacteria;D_1_Acidobacteria;D_2_Acidobacteriia;D_3_Solibacteriales;D_4_Solibacteraceae (Subgroup 3);D_5_Elev-16S-1166

D_0_Bacteria;D_1_Lentisphaerae; ; ; ; ;

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_NB1-j;D_4_uncultured organism;D_5_uncultured organism

D_0_Bacteria;D_1_Plantomycetes;D_2_Plantomycetacia;D_3_Gemmatales;D_4_Gemmataceae; ;

D_0_Bacteria;D_1_Plantomycetes;D_2_Phycisphaerae; ; ; ;

D_0_Archaea;D_1_Crenarchaeota;D_2_Bathyarchaeia;D_3_uncultured archaeon;D_4_uncultured archaeon;D_5_uncultured archaeon

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhodobacterales;D_4_Rhodobacteraceae;D_5_Pseudorhodobacter

D_0_Bacteria;D_1_Spirochaetes;D_2_Leptospirae;D_3_Leptospirales;D_4_Leptospiraceae;D_5_Turneriella

D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Bacillales;D_4_Paenibacillaceae;D_5_Ammophilus

D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Bacillales;D_4_Alicyclobacillaceae; ;

D_0_Bacteria;D_1_Actinobacteria;D_2_Actinobacteria;D_3_Propionibacteriales;D_4_Propionibacteriaceae;D_5_Propionicicella

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Sneathiellales;D_4_Sneathiellaceae;D_5_Ferrovibrio

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Subdoligranulum

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Clostridiaceae 1; ;

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Burkholderiaceae;D_5_Parasutterella

D_0_Bacteria;D_1_Modulibacteria;D_2_Moduliflexia;D_3_Moduliflexales;D_4_Moduliflexaceae;D_5_uncultured bacterium

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Desulfobacterales;D_4_Desulfobulbaceae;D_5_Desulfobulbus

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Coprococcus 3

D_0_Bacteria;D_1_Chloroflexi;D_2_Dehalococcoidia;D_3_S085; ;

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Heliobacteriaceae;D_5_Hydrogenispora

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Nitrosomonadaceae;D_5_mle1-7

D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Lactobacillales;D_4_Streptococcaceae;D_5_Streptococcus

D_0_Bacteria;D_1_Firmicutes;D_2_Erysipelotrichia;D_3_Erysipelotrichales;D_4_Erysipelotrichaceae;D_5_Erysipelotrichaceae UCG-003

D_0_Bacteria;D_1_Plantomycetes;D_2_Phycisphaerae;D_3_Phycisphaerales;D_4_Phycisphaeraceae;D_5_CL500-3

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Dorea

D_0_Bacteria;D_1_Actinobacteria; ; ; ;

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_TRA3-20;D_5_metagenome

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Azospirillales;D_4_Azospirillaceae;D_5_uncultured

D_0_Bacteria;D_1_Actinobacteria;D_2_Acidimicrobiia;D_3_Microtrichales;D_4_Microtrichaceae;D_5_uncultured

D_0_Bacteria;D_1_Chloroflexi;D_2_Chloroflexia;D_3_Thermomicrobiales;D_4_JG30-KF-CM45; ;

D_0_Bacteria;D_1_Actinobacteria;D_2_Acidimicrobiia;D_3_uncultured; ; ;

D_0_Bacteria;D_1_Hydrogenedentes;D_2_Hydrogenedentia;D_3_Hydrogenedentiales;D_4_Hydrogenedensaceae;D_5_uncultured bacterium

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Prevotellaceae; ;

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhodovibrionales; ; ;

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcus 2

D_0_Bacteria;D_1_Spirochaetes;D_2_Spirochaetia;D_3_Spirochaetales;D_4_Spirochaetaceae; ;

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhodobacterales;D_4_Rhodobacteraceae;D_5_Rhodobacter

D_0_Bacteria;D_1_Firmicutes;D_2_Negativicutes;D_3_Selenomonadales;D_4_Acidaminococcaceae;D_5_Phascolartobacterium

D_0_Bacteria;D_1_Bacteroidetes;D_2_Ignavibacteria;D_3_SJA-28; ; ;

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Cytophagales;D_4_Spirosomaceae;D_5_Larkinella

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Gammaproteobacteria Incertae Sedis;D_4_Unknown Family;D_5_Candidatus Ovatusbacter

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Acidiferrobacterales;D_4_Acidiferrobacteraceae;D_5_Sulfurifustis

D_0_Bacteria;D_1_Chloroflexi;D_2_Anaerolineae;D_3_SBR1031;D_4_A4b;D_5_OLB13

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Oligoflexales;D_4_Oligoflexaceae;D_5_uncultured

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_TSAC18;D_5_uncultured bacterium

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_KF-JG30-B3; ;

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Micavibrionales; ; ;

D_0_Bacteria;D_1_Patescibacteria;D_2_Parcubacteria;D_3_Candidatus Nomurabacteria;D_4_uncultured soil bacterium;D_5_uncultured soil bacterium

D_0_Bacteria;D_1_Plantomycetes;D_2_Plantomycetacia;D_3_Plantomycetales;D_4_Rubinisphaeraceae; ;

D_0_Bacteria;D_1_Gemmatimonadetes;D_2_Gemmatimonadetes;D_3_Gemmatimonadales;D_4_Gemmatimonadaceae;D_5_Gemmatimonas

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_[Eubacterium] ruminantium group

D_0_Bacteria;D_1_Actinobacteria;D_2_Acidimicrobiia;D_3_Microtrichales;D_4_Microtrichaceae;D_5_IMCC26207

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Prevotellaceae;D_5_Paraprevotella

D_0_Bacteria;D_1_Plantomycetes;D_2_Phycisphaerae;D_3_Phycisphaerales;D_4_AKAU3564 sediment group; ;

D_0_Bacteria;D_1_Chloroflexi;D_2_Dehalococcoidia;D_3_vadinBA26;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Plantomycetes;D_2_Phycisphaerae;D_3_Phycisphaerales;D_4_Phycisphaeraceae; ;

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Sphingobacteriales;D_4_AKYH767;D_5_uncultured bacterium

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_[Eubacterium] coprostanoligenes group

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Agathobacter

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Flavobacteriales;D_4_Crocinitomicaceae;D_5_Crocinitomix

D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Bacillales;D_4_Paenibacillaceae;D_5_Paenibacillus

D_0_Bacteria;D_1_Armatimonadetes;D_2_uncultured;D_3_metagenome;D_4_metagenome;D_5_metagenome

D_0_Bacteria;D_1_Actinobacteria;D_2_Coriobacteriia;D_3_OPB41; ; ;

D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Bacillales;D_4_Bacillaceae;D_5_Bacillus

D_0_Bacteria;D_1_Acidobacteria;D_2_Acidobacteriia;D_3_Solibacterales;D_4_Solibacteraceae (Subgroup 3);D_5_PAUC26f

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Thalassobactulales;D_4_Thalassobaculaceae;D_5_Thalassobaculum

D_0_Bacteria;D_1_Chloroflexi;D_2_Dehalococcoidia;D_3_MSBL5;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Chloroflexi;D_2_Chloroflexia;D_3_Thermomicrobiales;D_4_JG30-KF-CM45;D_5_uncultured bacterium

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Barnesiellaceae;D_5_Barnesiella

D_0_Bacteria;D_1_Chloroflexi;D_2_Anaerolineae;D_3_SBR1031;D_4_A4b;D_5_uncultured organism

D_0_Bacteria;D_1_Actinobacteria;D_2_Thermoleophilia;D_3_Gaiellales; ; ;

D_0_Bacteria;D_1_Chloroflexi;D_2_Chloroflexia;D_3_Chloroflexales;D_4_Roseiflexaceae;D_5_uncultured

D_0_Bacteria;D_1_Patescibacteria;D_2_Parcubacteria;D_3_uncultured bacterium;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Burkholderiaceae;D_5_Hydrogenophaga

D_0_Bacteria;D_1_Plantomycetes;D_2 BD7-11;D_3_uncultured Plantomycetaceae bacterium;D_4_uncultured Plantomycetaceae bacterium;D_5_uncultured Plantomycetaceae bacterium

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Bdellovibrionales;D_4_Bacteriovoraceae; ;

D_0_Bacteria;D_1_Chloroflexi;D_2_Anaerolineae;D_3_ADurb.Bin180;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_uncultured;D_4_uncultured Acetobacteraceae bacterium;D_5_uncultured Acetobacteraceae bacterium

D_0_Bacteria;D_1_Gemmatimonadetes;D_2_Gemmatimonadetes;D_3_Gemmatimonadales;D_4_Gemmatimonadaceae;D_5_Gemmatirosa

D_0_Bacteria;D_1_Armatimonadetes;D_2_Chthonomonadetes;D_3_Chthonomonadales;D_4_Chthonomonadaceae;D_5_Chthonomonas

D_0_Bacteria;D_1_WPS-2;D_2_metagenome;D_3_metagenome;D_4_metagenome;D_5_metagenome

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Enterobacteriales;D_4_Enterobacteriaceae; ;

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Myxococcales;D_4_Polyangiaceae;D_5_Pajarollobacter

D_0_Bacteria;D_1_Firmicutes; ; ; ;

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Roseburia

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Muribaculaceae;D_5_CAG-873

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminiclostridium

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Myxococcales;D_4_Sandaracinaceae;D_5_Sandaracinus

D_0_Bacteria;D_1_Chloroflexi;D_2_Dehalococcoidia;D_3_FS117-23B-02; ;

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Nitrosomonadaceae;D_5_Ellin6067

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Sneathiellales;D_4_Sneathiellaceae; ;

D_0_Bacteria;D_1_Patescibacteria;D_2_Parcubacteria;D_3_Candidatus_Campbellbacteria;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Planctomycetes;D_2_Phycisphaerae;D_3_mle1-8;D_4_uncultured organism;D_5_uncultured organism

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Burkholderiaceae;D_5_Rhodiferax

D_0_Bacteria;D_1_Chloroflexi;D_2_Anaerolineae;D_3_MSB-5B2; ;

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Gammaproteobacteria Incertae Sedis;D_4_Unknown Family;D_5_uncultured

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_PB19;D_4_metagenome;D_5_metagenome

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-014

D_0_Bacteria;D_1_Cyanobacteria;D_2_Melainabacteria;D_3_Vampirotvibrionales;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Actinobacteria;D_2_Actinobacteria;D_3_Micrococcales;D_4_Micrococcaceae; ;

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Nitrosomonadaceae;D_5_MND1

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Myxococcales;D_4_MidBa8;D_5_uncultured delta proteobacterium

D_0_Archaea;D_1_Crenarchaeota;D_2_Bathyarchaea;D_3_uncultured Thermoprotei archaeon;D_4_uncultured Thermoprotei archaeon;D_5_uncultured Thermoprotei archaeon

D_0_Bacteria;D_1_Latescibacteria;D_2_Latescibacteria;D_3_Latescibacteriales;D_4_Latescibacteraceae;D_5_uncultured bacterium

D_0_Bacteria;D_1_Planctomycetes;D_2_Phycisphaerae;D_3_Pla1 lineage;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Muribaculaceae;D_5_Muribaculum

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Enterobacteriales;D_4_Enterobacteriaceae;D_5_Escherichia-Shigella

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Sphingomonadales;D_4_Sphingomonadaceae;D_5_Novosphingobium

D_0_Bacteria;D_1_Planctomycetes;D_2_Phycisphaerae;D_3_Tepidisphaerales;D_4_CPlA-3 termite group;D_5_uncultured planctomycete

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Gracilibacteraceae;D_5_Gracilibacter

D_0_Bacteria;D_1_Verrucomicrobia;D_2_Verrucomicrobiae;D_3_Verrucomicrobiales;D_4_Akkermansiaceae;D_5_Akkermansia

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Oscillibacter

D_0_Bacteria;D_1_Chloroflexi;D_2_JG30-KF-CM66;D_3_uncultured bacterium;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Actinobacteria;D_2_Actinobacteria;D_3_Corynebacteriales;D_4_Nocardiaceae;D_5_Rhodococcus

D_0_Bacteria;D_1_Firmicutes;D_2_Negativicutes;D_3_Selenomonadales;D_4_Veillonellaceae;D_5_Anaeroglobus

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Chitinophagales;D_4_37-13;D_5_uncultured bacterium

D_0_Bacteria;D_1_Planctomycetes;D_2_vadinHA49; ; ;

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Sphingobacteriales;D_4_KD3-93; ;

D_0_Bacteria;D_1_Actinobacteria;D_2_Actinobacteria;D_3_Propionibacteriales;D_4_Propionibacteriaceae; ;

D_0_Bacteria;D_1_Verrucomicrobia;D_2_Verrucomicrobiae; ; ;

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Myxococcales;D_4_Polyangiaceae;D_5_Polyangium

D_0_Bacteria;D_1_Chloroflexi;D_2_Dehalococcoidia;D_3_GIF3;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Chitinophagales;D_4_37-13; ;

D_0_Bacteria;D_1_Patescibacteria;D_2_ABY1;D_3_Candidatus_Magasanikbacteria;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Calditrichaeota;D_2_Calditrichia;D_3_Calditrichales;D_4_Calditrichaceae; ;

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_PLTA13; ; ;

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Nitrosomonadaceae; ;

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhodospirillales;D_4_Magnetospirillaceae;D_5_uncultured

D_0_Bacteria;D_1_Actinobacteria;D_2_Actinobacteria;D_3_Micrococcales;D_4_Microbacteriaceae;D_5_Microbacterium

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rickettsiales;D_4_ABI1; ;

D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Bacillales;D_4_Bacillaceae; ;

D_0_Bacteria;D_1_Actinobacteria;D_2_Coriobacteriia;D_3_Coriobacteriales;D_4_Coriobacteriaceae;D_5_Collinsella

D_0_Bacteria;D_1_Actinobacteria;D_2_Actinobacteria; ; ; ;

D_0_Bacteria;D_1_Acidobacteria;D_2_Subgroup 21; ; ; ;

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Burkholderiaceae;D_5_Cupriavidus

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Oligoflexales;D_4_Oligoflexaceae;D_5_Oligoflexus

D_0_Bacteria;D_1_Chloroflexi;D_2_Gitt-GS-136;D_3_uncultured bacterium;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Cytophagales;D_4_Cyclobacteriaceae; ;

D_0_Bacteria;D_1_Rokubacteria;D_2_NC10;D_3_Methylomirabilales;D_4_Methylomirabilaceae;D_5_Z114MB74

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Methylophilaceae;D_5_MM2

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Xanthomonadales; ; ;

D_0_Bacteria;D_1_Planctomycetes; ; ; ; ;

D_0_Bacteria;D_1_Patescibacteria;D_2_WWE3;D_3_uncultured candidate division WWE3 bacterium;D_4_uncultured candidate division WWE3 bacterium;D_5_uncultured candidate division

D_0_Bacteria;D_1_Firmicutes;D_2_Erysipelotrichia;D_3_Erysipelotrichales;D_4_Erysipelotrichaceae;D_5_Holdemanella

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Peptococcaceae;D_5_Desulfurispora

D_0_Bacteria;D_1_Actinobacteria;D_2_Thermoleophilia;D_3_Solirubrobacteriales;D_4_67-14; ;

D_0_Bacteria;D_1_Verrucomicrobia;D_2_Verrucomicrobiac;D_3_Chthoniobacteriales;D_4_Terrimicrobiaceae;D_5_Terrimicrobium

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Caulobacteriales;D_4_Parvularculaceae;D_5_Amphiplicatus

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Prevotellaceae;D_5_Prevotellaceae NK3B31 group

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Diploricetisiales;D_4_Diploricetisaceae;D_5_Rickettsiella

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Azospirillales;D_4_Azospirillaceae; ;

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_[Eubacterium] xylanophilum group

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Cytophagales;D_4_Microscillaceae;D_5_Ohtaekwangia

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Beijerinckiaceae;D_5_Microvirga

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminiclostridium 9

D_0_Bacteria;D_1_Actinobacteria;D_2_Actinobacteria;D_3_Frankiales; ; ;

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Methylophilaceae;D_5_Methylotenera

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Desulfarculales;D_4_Desulfarculaceae;D_5_Desulfatiglans

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Butyricoccus

D_0_Bacteria;D_1_Chloroflexi;D_2_OLB14;D_3_metagenome;D_4_metagenome;D_5_metagenome

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Cytophagales;D_4_Spirosomaceae;D_5_Lacihabitans

D_0_Bacteria;D_1_Armatimonadetes;D_2_DG-56;D_3_uncultured bacterium;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Peptostreptococcaceae;D_5_Romboutsia

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Coproccoccus 2

D_0_Bacteria;D_1_Cyanobacteria;D_2_Melainabacteria;D_3_Gastranaerophilales;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Verrucomicrobia;D_2_Verrucomicrobiae;D_3_Opitutales;D_4_Opitutaceae;D_5_IMCC26134

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Desulfobacterales;D_4_Desulfobacteraceae;D_5_uncultured bacterium

D_0_Bacteria;D_1_Chloroflexi;D_2_OLB14;D_3_uncultured Chloroflexi bacterium;D_4_uncultured Chloroflexi bacterium;D_5_uncultured Chloroflexi bacterium

D_0_Bacteria;D_1_Bacteroidetes;D_2_Rhodothermia;D_3_Rhodothermales;D_4_Rhodothermaceae;D_5_uncultured bacterium

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Chitinophagales;D_4_Saprospiraceae;D_5_Aureispira

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Burkholderiaceae;D_5_Ralstonia

D_0_Bacteria;D_1_Omnitrophicaeota;D_2_ ; ; ;

D_0_Bacteria;D_1_Cyanobacteria;D_2_Oxyphotobacteria;D_3_Chloroplast;D_4_uncultured rhodophyte;D_5_uncultured rhodophyte

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Cytophagales;D_4_Hymenobacteraceae;D_5_Adhaeribacter

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Desulfovibrionales;D_4_Desulfovibrionaceae;D_5_Desulfovibrio

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminiclostridium 5

D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Lactobacillales;D_4_Lactobacillaceae;D_5_Lactobacillus

D_0_Bacteria;D_1_FBP;D_2_uncultured bacterium;D_3_uncultured bacterium;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Chloroflexi;D_2_Anaerolineae;D_3_Anaerolineales;D_4_Anaerolineaceae;D_5_Pelolinea

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Prevotellaceae;D_5_Prevotellaceae UCG-003

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Marinifilaceae;D_5_Odoribacter

D_0_Bacteria;D_1_BRC1;D_2_metagenome;D_3_metagenome;D_4_metagenome;D_5_metagenome

D_0_Bacteria;D_1_Armatimonadetes;D_2_Fimbriimonadia;D_3_Fimbriimonadales;D_4_ ; ;

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Myxococcales;D_4_Sandaracinaceae;D_5_uncultured bacterium

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhodospirillales;D_4_Magnetospiraceae;D_5_uncultured bacterium

D_0_Bacteria;D_1_Planctomycetes;D_2_Phycisphaerae;D_3_MSBL9;D_4_SG8-4;D_5_ ; ;

D_0_Bacteria;D_1_Patescibacteria;D_2_Saccharimonadia;D_3_Saccharimonadales;D_4_Saccharimonadaceae;D_5_uncultured bacterium

D_0_Bacteria;D_1_Patescibacteria;D_2_Gracilibacteria;D_3_Candidatus Peribacteria;D_4_ ; ;

D_0_Bacteria;D_1_Latescibacteria;D_2_Latescibacteria;D_3_Latescibacteriales;D_4_Latescibacteraceae;D_5_uncultured bacterium

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Anaerostipes

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Sphingobacteriales;D_4_AKYH767;D_5_ ; ;

D_0_Bacteria;D_1_Patescibacteria;D_2_Parcubacteria;D_3_wastewater metagenome;D_4_wastewater metagenome;D_5_wastewater metagenome

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_[Eubacterium] eligens group

D_0_Bacteria;D_1_Cyanobacteria;D_2_Oxyphotobacteria;D_3_Chloroplast;D_4_Epipyxis sp. PR26KG;D_5_Epipyxis sp. PR26KG

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Chitinophagales;D_4_Chitinophagaceae;D_5_Flavitalea

D_0_Bacteria;D_1_BRC1;D_2_ ; ; ;

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhodospirillales;D_4_Magnetospiraceae;D_5_Magnetospira

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-002

D_0_Bacteria;D_1_Actinobacteria;D_2_Coriobacteriia;D_3_Coriobacteriales;D_4_Eggerthellaceae;D_5_uncultured bacterium

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Burkholderiaceae;D_5_Herminiimonas

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Myxococcales;D_4_MidBa8;D_5_ ; ;

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Paracaedibacteriales;D_4_Paracaedibacteraceae;D_5_uncultured bacterium

D_0_Bacteria;D_1_Nitrospirae;D_2_Thermodesulfovibrionia;D_3_uncultured;D_4_ ; ;

D_0_Bacteria;D_1_Firmicutes;D_2_Negativicutes;D_3_Selenomonadales;D_4_Veillonellaceae;D_5_Veillonella

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Chitinophagales;D_4_Saprospiraceae;D_5_Phaeodactylibacter

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Chitinophagales;D_4_37-13;D_5_metagenome

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Leelaceae;D_5_uncultured bacterium

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rickettsiales;D_4_ ; ;

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rickettsiales;D_4_Rickettsiaceae;D_5_uncultured bacterium

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhodospirillales;D_4_ ; ;

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Puniceispirillales;D_4_EF100-94H03;D_5_uncultured bacterium

D_0_Bacteria;D_1_Chloroflexi;D_2_Dehalococcoidia;D_3_GIF9;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Myxococcales;D_4_P3OB-42;D_5_uncultured delta proteobacterium

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Puniceispirillales;D_4_uncultured;D_5_metagenome

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Acetobacteriales;D_4_Acetobacteraceae;D_5_Rhodovastum

D_0_Bacteria;D_1_Gemmatimonadetes;D_2_BD2-11 terrestrial group;D_3_ ; ;

D_0_Bacteria;D_1_Elusimicrobia;D_2_Elusimicrobia;D_3_Lineage IV;D_4_ ; ;

D_0_Bacteria;D_1_Armatimonadetes;D_2_ ; ; ;

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Paracaedibacteriales;D_4_Paracaedibacteraceae;D_5_Candidatus Captivus

D_0_Bacteria;D_1_Firmicutes;D_2_Negativicutes;D_3_Selenomonadales;D_4_Veillonellaceae;D_5_Anaerospromusa

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidetes VC2.1 Bac22;D_4_metagenome;D_5_metagenome

D_0_Bacteria;D_1_Armatimonadetes;D_2_uncultured;D_3_uncultured organism;D_4_uncultured organism;D_5_uncultured organism

D_0_Archaea;D_1_Nanoarchaeaeta;D_2_Nanoarchaeota;D_3_Aenigmarchaeales;D_4_uncultured archaeon;D_5_uncultured archaeon

D_0_Bacteria;D_1_Chloroflexi;D_2_Dehalococcoidia;D_3_GIF9;D_4_AB-539-J10;D_5_ ; ;

D_0_Bacteria;D_1_Chloroflexi;D_2_Anaerolineae;D_3_uncultured;D_4_ ; ;

D_0_Bacteria;D_1_Chloroflexi;D_2_Anaerolineae;D_3_Anaerolineales;D_4_Anaerolineaceae;D_5_uncultured bacterium

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Cytophagales;D_4_Cyclobacteriaceae;D_5_Algoriphagus

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Thalassobaculales;D_4_uncultured;D_5_uncultured bacterium

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_uncultured;D_5_uncultured bacterium

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Clostridiales vadinBB60 group;D_5_ ; ;

D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Lactobacillales;D_4_Carnobacteriaceae;D_5_Carnobacterium

D_0_Bacteria;D_1_Actinobacteria;D_2_Coriobacteriia;D_3_OPB41;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Desulfobacterales; ;

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Tistrellales;D_4_Geminicoccaceae;D_5_Candidatus Alysiosphaera

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhodospirillales;D_4_Magnetospirillaceae; ;

D_0_Bacteria;D_1_Plantomycetes;D_2_Phycisphaerae;D_3_MSBL9;D_4_SG8-4;D_5_uncultured plantomycete

D_0_Bacteria;D_1_Firmicutes;D_2_Negativicutes;D_3_Selenomonadales;D_4_Acidaminococcaceae;D_5_Acidaminococcus

D_0_Bacteria;D_1_Firmicutes;D_2_Erysipelotrichia;D_3_Erysipelotrichales;D_4_Erysipelotrichaceae;D_5_Erysipelothrix

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Syntrophomonadaceae; ;

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Clostridiales vadinBB60 group;D_5_uncultured bacterium

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Xanthobacteraceae;D_5_Starkeya

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Puniceispirillales;D_4_EF100-94H03; ;

D_0_Bacteria;D_1_Patescibacteria;D_2_Microgenomatia;D_3_Candidatus Collierbacteria; ; ;

D_0_Bacteria;D_1_Zixibacteria;D_2_uncultured Caldithrix sp.;D_3_uncultured Caldithrix sp.;D_4_uncultured Caldithrix sp.;D_5_uncultured Caldithrix sp.

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Rhodocyclaceae;D_5_Methyloversatilis

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Myxococcales;D_4_Nannocystaceae;D_5_uncultured

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Myxococcales;D_4_Bld119;D_5_uncultured bacterium

D_0_Bacteria;D_1_Plantomycetes;D_2_Phycisphaerae;D_3_Phycisphaerales;D_4_AKAU3564 sediment group;D_5_uncultured organism

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Clostridiaceae 1;D_5_Clostridium sensu stricto 1

D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Bacillales;D_4_Staphylococcaceae;D_5_Staphylococcus

D_0_Bacteria;D_1_Acidobacteria;D_2_Subgroup 18; ; ;

D_0_Archaea;D_1_Euryarchaeota;D_2_Methanomicrobia;D_3_Methanocellales;D_4_Methanocellaceae; ;

D_0_Bacteria;D_1_WS2;D_2_uncultured bacterium;D_3_uncultured bacterium;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_MBNT15;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Deltaproteobacteria Incertae Sedis;D_4_Syntrophorhabdaceae;D_5_Syntrophorhabdus

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_UBA1819

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_[Eubacterium] ventriosum group

D_0_Bacteria;D_1_Elusimicrobia;D_2_Elusimicrobia;D_3_Lineage IV;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Chloroflexi;D_2_Dehalococcoidia;D_3_MSBL5; ; ;

D_0_Bacteria;D_1_Chloroflexi;D_2_Anaerolineae;D_3_Ardenticatenales;D_4_uncultured;D_5_uncultured organism

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Desulfovibrionales;D_4_Desulfovibrionaceae;D_5_Halodesulfobivrio

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rickettsiales;D_4_Mitochondria; ;

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Azospirillales;D_4_Inquilinaceae;D_5_Inquilinus

D_0_Bacteria;D_1_Plantomycetes;D_2_Phycisphaerae;D_3_Phycisphaerales;D_4_AKAU3564 sediment group;D_5_uncultured bacterium

D_0_Bacteria;D_1_Patescibacteria;D_2_ABY1;D_3_Candidatus Magasanikbacteria; ; ;

D_0_Bacteria;D_1_Firmicutes;D_2_Erysipelotrichia;D_3_Erysipelotrichales;D_4_Erysipelotrichaceae;D_5_Turicibacter

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_SB-5; ;

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Marinilabiliaceae; ;

D_0_Bacteria;D_1_BHI80-139;D_2_uncultured bacterium;D_3_uncultured bacterium;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Archaea;D_1_Euryarchaeota;D_2_Methanomicrobia;D_3_Methanocellales;D_4_Methanocellaceae;D_5_Rice Cluster 1

D_0_Bacteria;D_1_WS2; ; ; ;

D_0_Bacteria;D_1_Spirochaetes;D_2_Leptospirae;D_3_Leptospirales;D_4_Leptospiraceae;D_5_RBG-16-49-21

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Myxococcales;D_4_P3OB-42;D_5_uncultured bacterium

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_NRL2;D_4_uncultured alpha proteobacterium;D_5_uncultured alpha proteobacterium

D_0_Bacteria;D_1_Patescibacteria;D_2_WS6 (Dojkabacteria); ; ; ;

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_ASF356

D_0_Bacteria;D_1_Elusimicrobia;D_2_Elusimicrobia;D_3_MVP-88;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Cyanobacteria;D_2_Melainabacteria;D_3_Gastranaerophilales; ; ;

D_0_Bacteria;D_1_Actinobacteria;D_2_Acidimicrobia;D_3_Actinomarinales;D_4_uncultured;D_5_uncultured Actinomycetales bacterium

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Pseudomonadales; ; ;

D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Nitrosomonadaceae;D_5_Nitrosospira

D_0_Bacteria;D_1_Plantomycetes;D_2_Plantomycetacia;D_3_uncultured;D_4_uncultured plantomycete;D_5_uncultured plantomycete

D_0_Bacteria;D_1_GN01;D_2_uncultured bacterium;D_3_uncultured bacterium;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-013

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Anaerotruncus

D_0_Archaea;D_1_Euryarchaeota;D_2_Thermoplasmata;D_3_Marine Benthic Group D and DHVEG-I;D_4_uncultured Thermoplasmata archaeon;D_5_uncultured Thermoplasmata archaeon

D_0_Bacteria;D_1_Zixibacteria; ; ; ; ;

D_0_Bacteria;D_1_Verrucomicrobia;D_2_Verrucomicrobiae;D_3_Verrucomicrobiales;D_4_DEV007;D_5_uncultured bacterium

D_0_Bacteria;D_1_Rokubacteria;D_2_NC10;D_3_Methylomirabilales;D_4_Methylomirabilaceae;D_5_Sh765B-TzT-35

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Syntrophobacterales;D_4_Syntrophaceae;D_5_Desulfobacca

D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Bradymonadales; ; ;

D_0_Bacteria;D_1_Plantomycetes;D_2_Phycisphaerae;D_3_DG-20;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Plantomycetes;D_2_Phycisphaerae;D_3_CCM11a;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Plantomycetes;D_2_BD7-11;D_3_uncultured bacterium;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Patescibacteria;D_2_Microgenomatia;D_3_Candidatus Pacebacteria;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Firmicutes;D_2_Negativicutes;D_3_Selenomonadales;D_4_Veillonellaceae; ;

D_0_Bacteria;D_1_Firmicutes;D_2_Negativicutes;D_3_Selenomonadales;D_4_Veillonellaceae;D_5_Megamonas

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-009

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Flavobacteriales;D_4_Flavobacteriaceae;D_5_Arenibacter

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Cytophagales;D_4_Spirosomaceae; ;

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Dysgonomonadaceae;D_5_Dysgonomonas

D_0_Bacteria;D_1_Acidobacteria;D_2_Subgroup 18;D_3_uncultured bacterium;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Archaea; ; ; ; ;

D_0_Bacteria;D_1_WS2;D_2_uncultured soil bacterium;D_3_uncultured soil bacterium;D_4_uncultured soil bacterium;D_5_uncultured soil bacterium

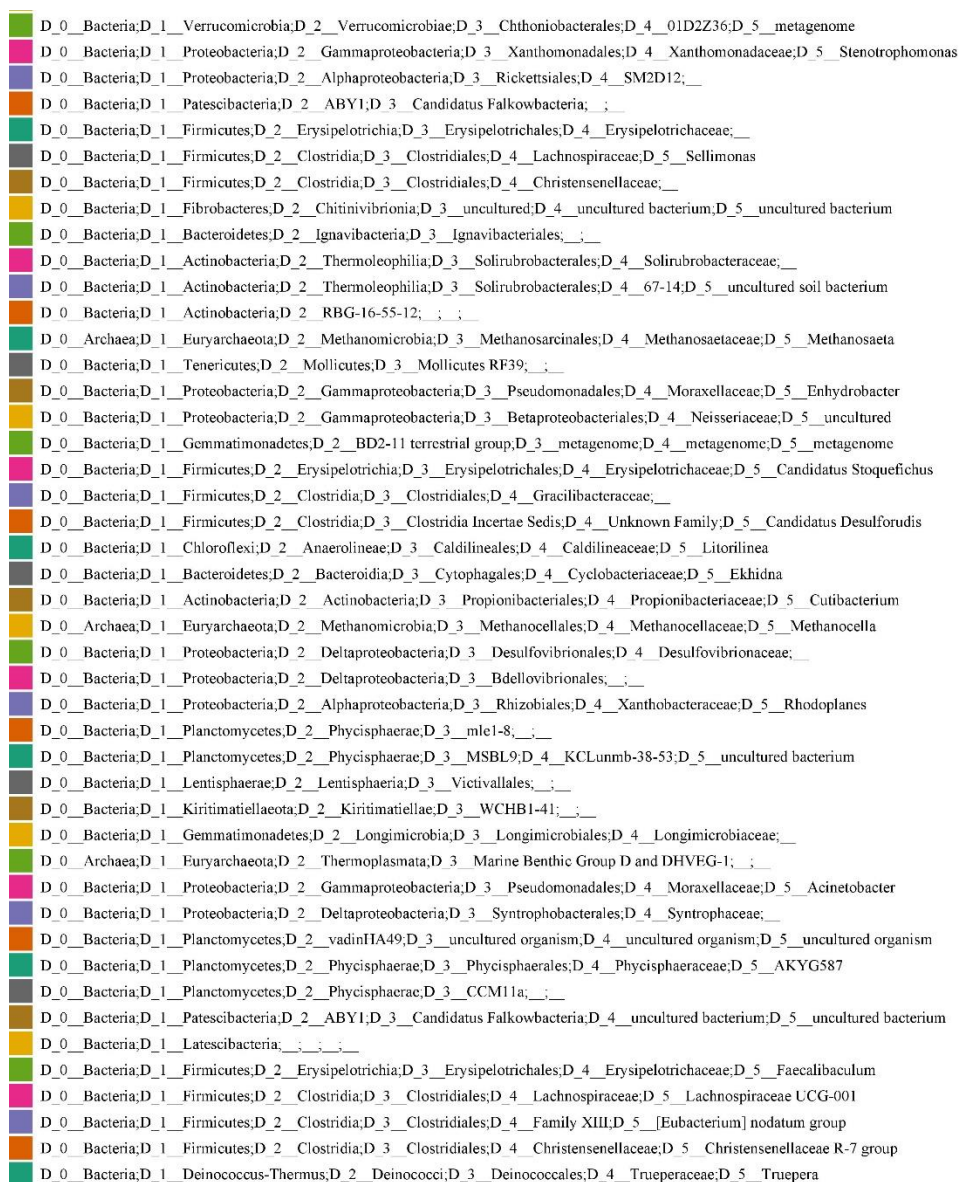


Figure 28. Stacked barcharts of the 16S taxonomic profile at the genus level sorted by treatment. The four samples reported for every treatment comprise the three wood replicates and the water sample. (C: cinnamon; WT: wild thyme; CT: common thyme; E: ethanol; NT: not-treated control).

The analysis of ITS taxonomic profile showed that the treated wood and water were enriched in sequences belonging to the kingdom Chromista and to the supergroup Rhizaria. The relative frequencies of these taxa with respect to the fungal sequences are higher with respect those observed during the preliminary tests carried out on sample N9 (§ 4.2.2.). This result may be attributed to the effect of the oils that acted as biocides on the fungi but not on these microorganisms.

Regarding the identified fungal species (Fig. 28), no clear differences in term of fungal community composition could be highlighted among the differently treated samples. The only species whose distribution could be attributed to the treatment is *Reticulascus clavatus*. In fact, its mean relative frequencies were 34.6%, for NT, 19.8% for CT and ca 1.2% for the other treatments.

The obtained results could let us hypothesizing that the oils had a differential effect on the bacterial taxa present in wood and water leading to the differently composed bacterial communities. While, the different fungal species have been affected more homogeneously by the action of biocides, and this has led us to observe more similar fungal communities.

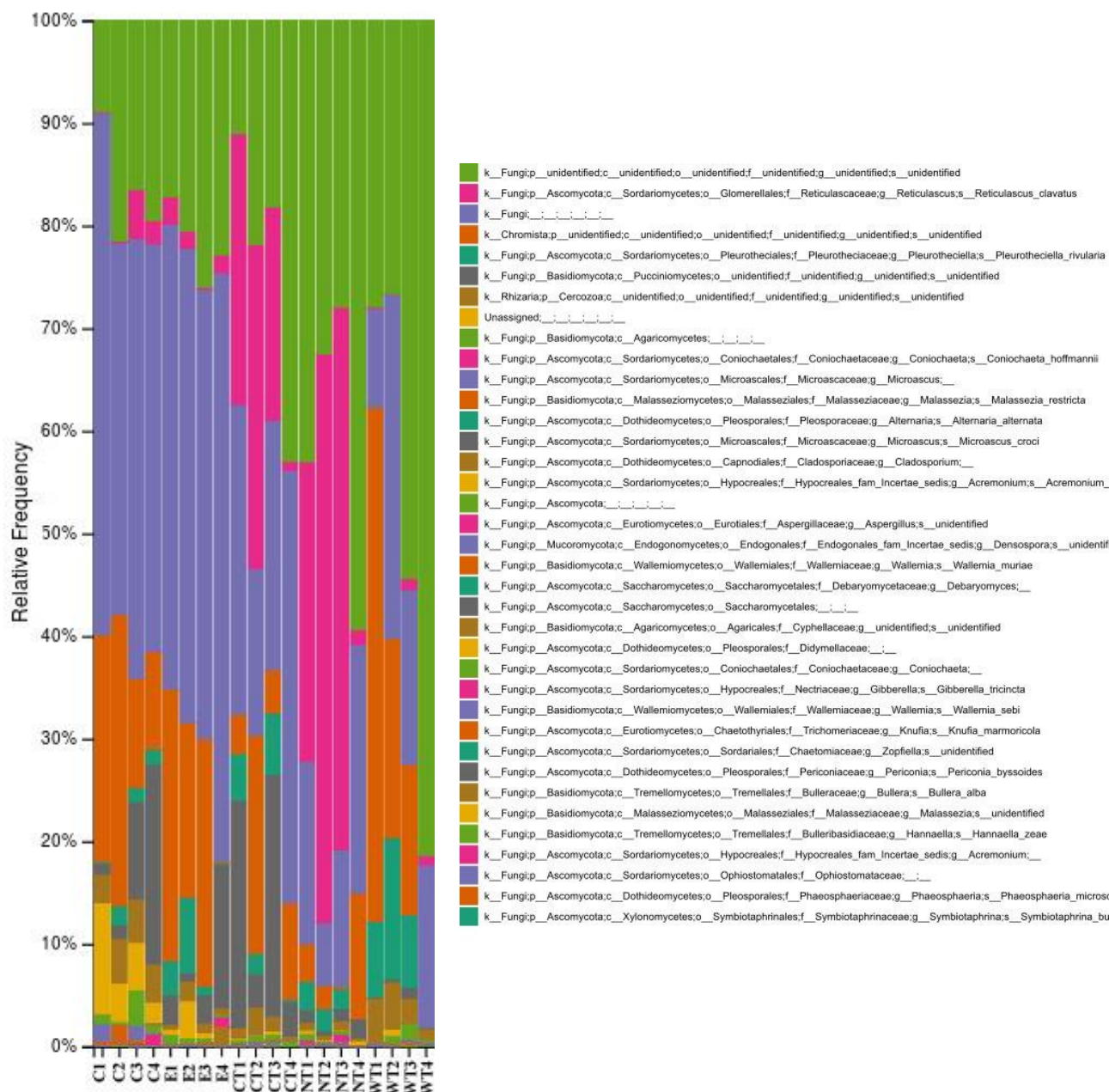


Figure 29. Stacked barcharts of the ITS taxonomic profile at the species level sorted by treatment. The four samples reported for every treatment comprise the three wood replicates and the water sample. (C: cinnamon; WT: wild thyme; CT: common thyme; E: ethanol; NT: not-treated control).

4.2.5. Waterlogged archaeological wood remains

The tests performed in the frame of the thesis project testing the consolidation activity of the lactitol/trehalose mixture allowed evaluating the biocidal effect of cinnamon essential oil during an actual restoration procedure and to compare it to a biocide currently used in the restoration laboratories.

The results of biochemical tests (Tab. 20 and Fig. 30) and cultural analyses (Fig. 31) showed that a neat decrease in the microbial vitality and in the contamination of the impregnation baths was obtained with both biocides. Of note, the tests carried out to evaluate the possible influence of lactitol-trehalose and of Preventol RI80 on the ATP quantification showed no remarkable effect. The standard ATP values registered in the presence of the mixtures lactitol-trehalose and lactitol-trehalose-Preventol RI80 and without them were

comparable. The observed variation with respect to the pure standard measures is comparable to that usually observed in a set of repeated measures on the standard and so it can be considered as irrelevant.

Tab. 20. Evaluation of the biocidal activity of Preventol RI80 and cinnamon essential oil (C) through bioluminescence assays. (PT: pre-treatment, AT: after treatment).

Tank	Biocide	Cellular ATP (RLU)	
		PT	AT
V2	Preventol RI80	1,134,529	307
V3	Preventol RI80	6,183,092	16,118
V4	C	1,123,270	11,553
V5	C	2,431,215	23,251

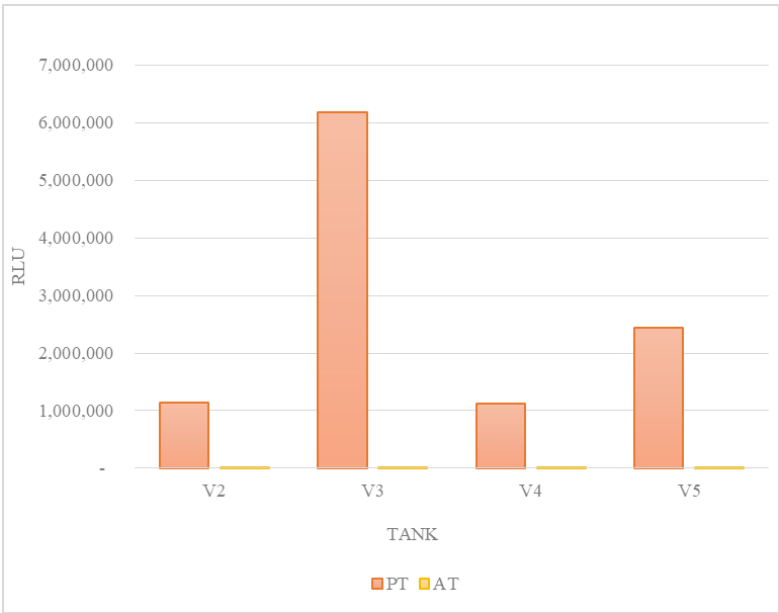


Figure 30. Evaluation of the biocidal activity of Preventol RI80 (V2 and V3) and cinnamon oil (V4 and V5) through ATP bioluminescence assay. (PT: pre-treatment, AT: after treatment).

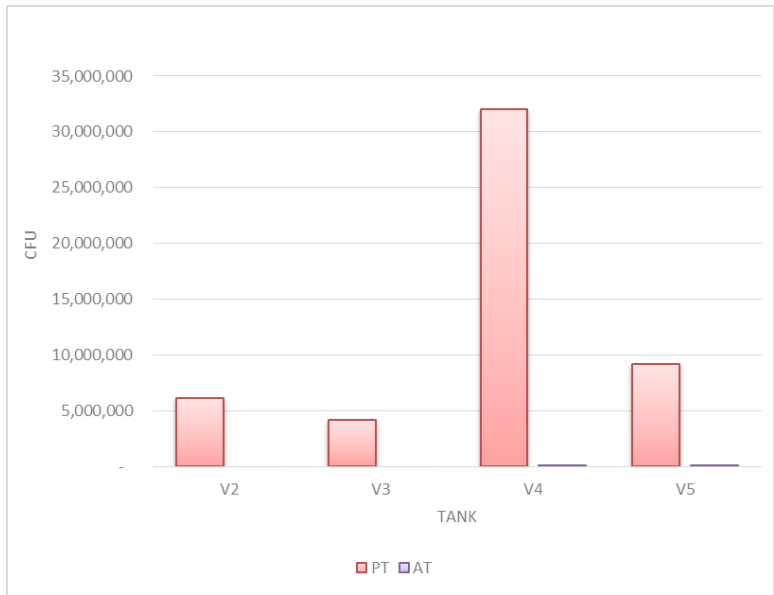


Figure 31. Evaluation of the biocidal activity of Preventol RI80 (V2 and V3) and cinnamon oil (V4 and V5) through cultural analysis. (PT: pre-treatment, AT: after treatment).

Tab. 21 resumes the values of ATP and CFU percent decreases obtained with Preventol RI80 and cinnamon EO. The results obtained with Preventol RI80 are slightly better with respect to cinnamon oil. In fact, for the commercial biocide the ATP decrease was higher than 99.7% for both tanks and the CFU decreased by 99.99% in tank V2 and by 100% for tank V3. Instead, for cinnamon oil a residual ATP value corresponding to ca 1% of the pre-treatment quantity was registered for both tanks and only in V4 a CFU decrease higher than 99% was observed.

It is interesting to note that the vitality decrease obtained with cinnamon during this experimentation is slightly lower than that observed on the wood samples (§ 4.2.4.). This discrepancy could be explained considering that an amount of ethanol equal to the oil volume was added to C. Probably, this volume was not sufficient to correctly dissolve the EO in the tank's water. Further tests with larger quantities of ethanol should be done to evaluate the influence of this factor. Another possible explanation, also to be evaluated with additional experiments, is that an interaction with the consolidants present in the tank's water decreased the biocidal effect of the oil.

Finally, in view of an application of cinnamon oil as a biocide in the field of restoration of archaeological waterlogged wood, it must be taken into account that the oil has a solvent effect on some plastic materials. In fact, during the experimentation the pumps used for the bath recirculation and the plastic accessories of the instruments used to evaluate the density of the suspensions suffered damages due to the presence of the biocide while the plastic of the tanks has remained unchanged.

Tab. 21 Percent decrease of the amount of ATP and Colony Forming Units (CFU) after the biocidal treatment.

Tank	Biocide	ATP Decrease (%)	CFU Decrease (%)
V2	Preventol RI80	99.97	99.99
V3	Preventol RI80	99.74	100
V4	C	98.97	99.98
V5	C	99.04	98.68

5. Conclusions and Future Perspectives

The aim of the PhD project was to evaluate the effectiveness of alternative bio-inspired materials for the consolidation and preservation of waterlogged archaeological wood.

Regarding the three nano-scale consolidants based on cellulose and lignin, the obtained results did not allow for discriminating significant differences regarding their efficacy but they laid a profound base for more focused tests in future.

The problems in color changing observed for LNPs and BC could be solved by modifying the impregnation baths. For BC, a pigment compatible with the treated material could be added to the bath. For LNPs, a careful selection of the wood source for lignin isolation could give nanoparticles of different color grades matching the color of the consolidated object (Zikeli et al., 2019).

Regarding the different penetration of the tested consolidants, no relationship was found neither with the state of preservation of wood nor with the shape and dimension of the nanoparticles. Thus, the different behavior must be related to chemical interactions between the nanoparticles themselves and between them and the wood. LNPs and CNCs penetration problems could be solved by modifying the impregnation conditions in the first case to facilitate penetration, in the second to prevent the gel formation. For BC a satisfying penetration was observed but the consolidating effect was not substantial. A best result could be achieved by modifying the shape of the nanocellulose or combining filamentous with spherical nanoparticles in order to obtain web-like structures more suitable to fill the voids and to support the cell wall, exploiting the potential of nano-scale consolidants to enter wood ultrastructure.

The excellent results obtained in testing essential oils as biocides for the preservation of waterlogged archaeological wood revealed the potential of these products during the delicate conservation and restoration phases of wooden artifacts. In particular, the results obtained for cinnamon and common thyme showed that adding 1% of the EO to the wood storage water allows obtaining a decrease by almost 100% of the vitality of the microbiota present inside wood.

The work carried out during the three years of the project allowed to observe a first application fallout, the cinnamon essential oil was used as biocide during an actual restoration process of archaeological wooden

artifacts. The obtained results were very satisfactory but further experiments can be planned to improve the biocide application. Tests with different amount of ethanol are needed to evaluate the volume necessary to obtain a correct solution of the oil. Furthermore, the interaction of the EO with the plastics (and eventually other materials) used during restoration must be investigated in order to set the limit of application.

The very good results obtained applying biochemical quantification of ATP showed the usefulness of this technique in monitoring the biological contamination of wood after the recovery, during the storage and restoration/preservation phases. Thus, this method could be considered as an actual alternative to culture-based methods in the field of conservation of waterlogged archaeological wood. Finally, the analysis of the microbiota composition through NGS allowed observing the presence of cellulolytic and/or ligninolytic species underlining the high biological risk to which wood is subjected during these delicate conservation phases. Furthermore, this innovative technique allowed highlighting the differential effect of the tested biocides on the bacterial and fungal communities present in waterlogged archaeological wood.

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“No amount of experimentation can ever prove me right; a single experiment can prove me wrong”
Albert Einstein

Author Contributions

For the chapters on waterlogged wood consolidation (from the paper: - Antonelli, F., Galotta, G., Sidoti, G., Zikeli, F., Nisi, R., Davidde Petriaggi, B., Romagnoli, M. Cellulose and lignin nano-scale consolidants for waterlogged archaeological wood. *Frontiers in materials. Nanoscience and Nanomaterials for the Knowledge and Conservation of Cultural Heritage. Accepted, in press*):

Antonelli Federica, Galotta Giulia and Romagnoli Manuela conceived and planned the experiments. Zikeli Florian (Department for Innovation in Biological, Agro-Food and Forestry Systems (DIBAF), Tuscia University, Viterbo, Italy) and Nisi Rossella (BioFaber srl, Mesagne, Italy) produced and provided the nanoparticles used in the experimentation. Sidoti Giancarlo (Testing Materials Laboratory, Istituto Superiore per la Conservazione e il Restauro (ISCR), Rome, Italy), Zikeli Florian and Nisi Rossella contributed to define the settings of the tests. Romagnoli Manuela and Davidde Petriaggi Barbara (Underwater Archaeological Operations Unit, Istituto Superiore per la Conservazione ed il Restauro (ISCR), Rome, Italy) helped supervise the project. All the authors contributed to the discussion. Antonelli Federica and Galotta Giulia wrote the chapters with inputs from all authors.

For the chapters on Next Generation Sequencing:

Antonelli Federica, Guerrieri Francesca (Center for Life NanoScience@Sapienza, Istituto Italiano di Tecnologia, Rome, Italy and Epigenetics and epigenomic of hepatocellular carcinoma, Cancer Research Center of Lyon (CRCL), U1052, Lyon, France), Romagnoli Manuela and Galotta Giulia conceived and planned the experiments. Antonelli Federica collected the samples and collaborated with Guerrieri Francesca in the DNA extraction, library preparation and sequencing. Esposito Alfonso (Department of Cellular, Computational and Integrative Biology – CIBIO, University of Trento, Trento, Italy) and Piazza Silvano (Department of Cellular, Computational and Integrative Biology – CIBIO, University of Trento, Trento, Italy) performed Sequencing Data Analysis. Antonelli Federica, Guerrieri Francesca, and Esposito Alfonso wrote the text with inputs from Galotta Giulia and Romagnoli Manuela.

For the chapters on ATP preliminary tests:

Antonelli Federica, Bartolini Marco (Biology Laboratory, Istituto Superiore per la Conservazione e per il Restauro (ISCR), Rome, Italy), Michela Grimaldi (External collaborator Biology Laboratory, Istituto Superiore per la Conservazione e per il Restauro (ISCR), Rome, Italy), Perego Silvia (Promega Italia s.r.l.) and Mondal Subhanjan (Promega Italia s.r.l.) conceived and planned the experiments. Antonelli Federica, and Michela Grimaldi carried out the tests. Antonelli Federica, Bartolini Marco and Michela Grimaldi wrote the text with inputs from Perego Silvia and Mondal Subhanjan.

For the chapters on the evaluation of the biocidal activity of the essential oils:

Antonelli Federica, Bartolini Marco (Biology Laboratory, Istituto Superiore per la Conservazione e per il Restauro (ISCR), Rome, Italy) and Galotta Giulia conceived and planned the experiments. Di Vito Maura

(Italian Society for Research on Essential Oils – SIROE) and Barboni Marta (FLORA srl) provided valuable advices on the experimentation. Antonelli Federica carried out the tests on fungal spores and mycelium and on waterlogged archaeological wood samples. Antonelli Federica and Pedone Cristian (graduand at ISCR restoration school) carried out the experimentation on waterlogged archaeological wood remains. Antonelli Federica wrote the text with inputs from Bartolini Marco, Galotta Giulia and Romagnoli Manuela.

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