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Original article

Sediments surrounding waterlogged archaeological wood: could arsenic be involved in preserving wooden pile dwelling from microbial decay? The Gran Carro case study (Lake Bolsena, central Italy)

M.C. Moscatelli[a,*], R. Marabottini[a], S. Tamantini[a], G.M. Di Buduo[a], G. Galotta[b], F. Antonelli[b], M. Romagnoli[a]

[a] Dept. for Innovation in Biological, Agro-food and Forest systems (DIBAF), University of Tuscia, Viterbo, 01100 Italy
[b] Biology Laboratory, Central Institute for Restoration (ICR), Ministry of Cultural Heritage, via di San Michele 25, Roma, 00153 Italy

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ABSTRACT

Waterlogged archaeological wood (WAW) conservation depends on several abiotic properties related to water and/or sediments (temperature, pH, redox potential, salinity, heavy metals or pollutants) and biotic factors (amount and type of microorganisms and their metabolic activity). All these factors may be altered by anthropic activities and/or climate changes thus accelerating or decreasing wood decay rates. Regardless of its rate, the decomposition and alteration of submerged material can be expected to leave traces in the surrounding sediment properties.

Gran Carro is an archaeological site, located at Lake Bolsena (central Italy) and characterised by the presence of wooden pile dwellings (WPD). According to the most recent archaeological evidence, the site dates back in the period spanning from Middle Bronze Age (XV century BC) to the Early Iron Age (IX century BC). The study of waterlogged WPD, particularly those located in Mediterranean areas, was the object of **WOODPDLAKE** project financed by JPI-CH19. Lake Bolsena sediments chemical, biochemical (enzymatic activities) and microbiological properties (EL-FAME profiles), discriminated in the portion in tight contact with WAW (WSed) and the sediment in the surrounding area (Sed), are reported in this article with the aim to highlight any “wood effect”.

Most properties showed a clear “wood effect” indicating that WSed was strongly influenced by the processes occurring within WAW. The chemical properties of Gran Carro sediments showed a consistent amount of arsenic deriving from the volcanic lithological substrate whose fractionation showed, however, that it was mostly present in stable and immobilized forms. The presence of sulphate reducing bacteria suggested, in fact, the use of arsenic to form insoluble sulphide mineral-like phases that precipitated in the sediment and in wood structure. Arsenic was also highly concentrated within WAW, negatively impacting microbial biomass and activity, in terms of enzymatic activities, which were significantly decreased in WSed. The geochemistry and mobility of arsenic ion depend on environmental factors that may be impacted by climate changes. Therefore, it is advisable to monitor the current variations of environmental matrices such as soil, sediments, water in contact with archaeological material to understand the potential effects on the conservation of cultural heritage.

1. Introduction

The conservation of buried cultural heritage is the result of an equilibrium between the nature of any archaeological material and the soil/sediment conditions surrounding it [1,2]. In freshwater ecosystems, sediment is characterised by an active layer of soil and water which may act as sink and/or source of physical, chemical, and biological materials. The degradation of waterlogged archaeological wood (WAW) has been widely described for shipwrecks in marine environment, as [3–9]. According to Björdal [10], nonbiological factors, such as salinity, temperature, nutrients, composition of sediment layers and mainly wood type can affect the microbial decay rate of WAW, though microbiological factors are more relevant [11]. Less studies have been performed in lakes environment, particularly in shallow waters [12–18], being these environments

* Corresponding author.
E-mail address: mcm@unitus.it (M.C. Moscatelli).

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more sensitive to the external environmental conditions. WAW is rather sensitive to biotic degradation depending on specific water and sediment properties such as redox potential, pH, organic matter content, pollutants, microbial biomass composition and activity.

Additionally, sediments are dynamic systems subjected to changes induced by natural and anthropic pressures such as climate changes, land use and management, pollution; this may speed up decay processes or may change the nature of these processes [12,14,19,20]. Climate changes may in fact impact water, and consequently sediment properties, through variations of average temperature, pH, redox potential, CO_2 concentration [21,22]. Furthermore, human activities may increase the concentration of nitrogen and phosphorus, favouring lake eutrophication, of pesticides and heavy metals [20].

The current climatic changes are leading to longer warm seasons, and the outburst of heatwaves impacts all natural ecosystems, lakes included. The rise of average water temperatures induces lake stratification, isolating deep water, as it decreases convective mixing, thus leading to the diminishing of dissolved oxygen (DO) concentration and of redox potential [23,24]. Water pH is also closely related to redox potential: under acidic conditions this tends to be lower, while the reverse occurs under alkaline conditions. These important modifications on water chemistry affect sediments thus inducing likely changes in their chemical, biochemical and microbiological properties. The sediment oxidation state is an important driver determining the rate of decomposition process, e.g. decomposition of woody materials is relatively slow in anaerobic conditions where sediments properties combined with water play an important role in affecting this process [25,26]. Microbes play an important role in the biogeochemical processes occurring in freshwater ecosystems and are extremely sensitive to environmental changes so that they may be considered sensors of external stress. At the same time, microbial decomposition activity deeply influences sediment chemical, biochemical and microbiological properties, particularly in the area in tight contact with the organic material [27,28].

The growth and distribution of microbial communities is strongly affected by the availability of carbon sources, nutrients, electron acceptors, pH and heavy metals content [12]. Variations in the sediment environment may impact microbial functions; persistent hypoxia can lead to changes in the structure and function of microbial communities which in turn can modify decomposition processes, drivers of biogeochemical cycles [29], while certain heavy metals concentration may inhibit microbial activity and biomass. Among these, arsenic, whose geochemical cycle is highly dependent on pH and redox potential, can have a direct influence on microbial biomass as it is toxic to almost all bacteria by inhibiting cellular functions linked with energy metabolism [30,31].

2. Research aim

Among the different types of WAW, wooden pile dwellings (WPD) inform on landscape evolution and cultural activities developed around lakes. The study of WPD in Mediterranean lakes was the object of WOODPDLAKE project financed by JPI-CH19. Its main objective was to assess the impact of extreme climate events and anthropic pressure on the conservation and safeguard of pile dwellings in Mediterranean lakes. This study was carried out at Gran Carro settlement (WPD dated back XV-IX century BC) at Lake Bolsena, central Italy and takes the form of an in-depth following previous research focused on wood decay [15]. The main goal of this paper was to focus attention on the environmental context surrounding the WAW. The analysis of the sediment in tight contact with WAW may inform on decay process rate. In particular, the hypothesis to be tested was that the sediment surrounding archaeological wood (from now on termed WSed) showed evidence of decay, a “wood effect”, in terms of microbial activity and biomass, chemical composition which differ from the sediment not interested by the presence of WAW (from now on termed Sed). The term “wood effect” indicates, therefore, the occurrence of significant variations of sediments chemical, biochemical and microbiological properties induced by the presence of WAW due to its likely decomposition.

Consequently, the aims of this study were to: i) characterise the sediment context of Gran Carro archaeological site, ii) demonstrate a likely “wood effect” due to the presence of WAW.

3. Materials and methods

3.1. Archaeological site description

Gran Carro archaeological site has been first discovered and described by Alessandro Fioravanti in 1959 [32,33]. The site consists of two predominant remains: the “Aiola” and the residential area. The former is an elliptical mound (60 × 80 m), a stone structure which only recently has been identified as a place of worship [33–35]. The residential area, located farther south, covers an area of 8000 m^2 and consists in the remains of a Villanovan village [33]. According to the most recent archaeological evidence, the site dates back in the period spanning from Middle Bronze Age (XV century BC) to the Early Iron Age (IX century BC). It lies at a depth of 4 - 5 m, about 100 m off the east coast of Lake Bolsena, Viterbo province, Central Italy (42°35′N 11°59′E) (Fig. 1a and b).

According to the Köppen–Geiger climate classification [36], Lake Bolsena falls within the Csa category, which denotes a Mediterranean climate with hot dry summers. However, due to its specific altitude (∼350 m a.s.l.), local conditions are attenuated. In fact, the climate is characterised by mild winters, typically extending from October through May, with the mean minimum temperature of the coldest month dropping slightly below 0 °C (–0.3 °C). Summers are moderately warm, with mean maximum temperatures remaining below 29 °C. Annual precipitation is moderately high, ranging from 954 to 1166 mm, with summer rainfall events contributing between 103 and 163 mm. Summer aridity is not particularly pronounced and is limited to July and August, supporting the classification of Bolsena’s climate as mesotemperate, sub-humid to humid ombrotypes and lower supramediterranean thermotype [37].

Lake water average temperature in the period 2014–2019 was 18.1 °C (± 6 °C) [38]. Furthermore, high resolution data were available from a probe (Multiparameter Probe TRIPOD, AQUALABO, Champigny-sur-Marne, France) installed in the vicinity of the archaeological site during the period July 2022-October 2023. During this period the average temperature was similar to that previously cited with a Tmax of 27.7 °C and a Tmin of 9.1 °C recorded in August 2023 and February 2023, respectively.

Presently, all research activities are coordinated by the Superintendence of Archaeology, Fine Arts and Landscape for Viterbo province and Southern Etruria. The wood identification, the physical-chemical properties and dating of oak poles are reported in Romagnoli et al. [17]. In particular, the identified species are *Quercus* section *robur* and *Quercus* section *cerris.*

3.2. Sediment sampling

Sediment samples were seasonally collected from Spring 2021 to Summer 2023 by the equipe of CRAS-APS (Centro Ricerche per l’Archeologia Subacquea) trying to exert as little disturbance as possible on the site’s archaeological structures. Sampling occurred randomly within the archaeological area discriminating between WSed, 12 replicates were taken in the vicinity of WAW, less than 5 cm from different poles and beams and up to 10 cm depth, and Sed, 12 replicates taken within the archaeological area but not

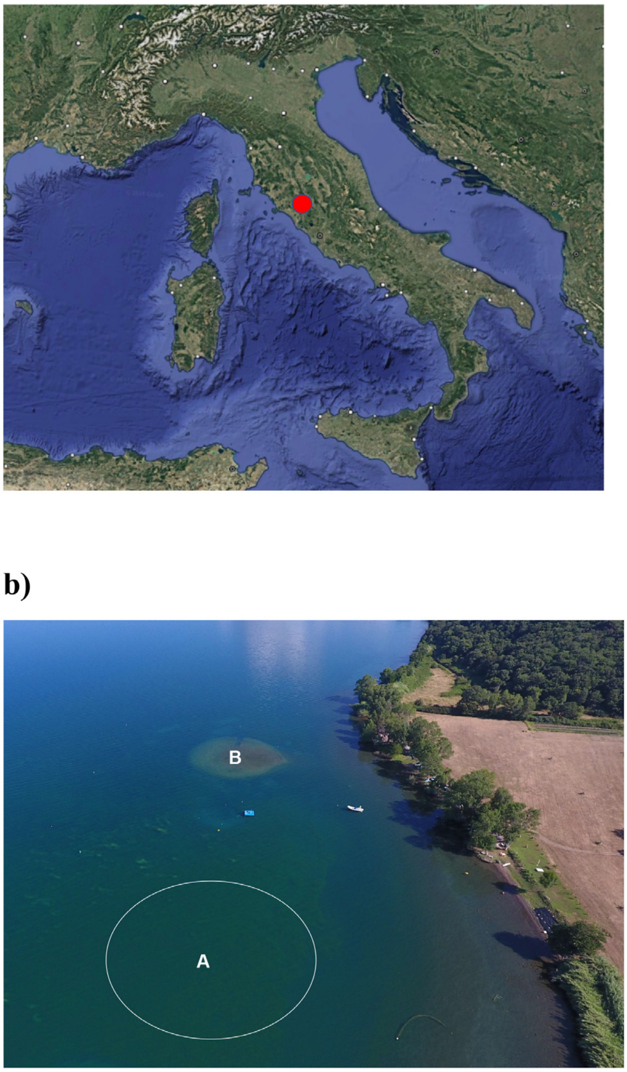

Fig. 1. **a)** Red dot, Lake Bolsena, Central Italy (42°35′N 11°59′E), **b)** Gran Carro archaeological site, Lake Bolsena, Central Italy, A: Submerged archaeological remains of Villanovian culture wooden pile dwellings and B: «Aiola» which served as a ritual site. Photo credits: Severi E., SABAP-VT-EM.

in contact with WAW. The 12 Sed samples were thus considered as controls. All samples were transferred in tightly sealed Falcon tubes and immediately frozen at −20 °C. Therefore, a total number of 24 sediment cores were taken at each sampling date.

3.3. Sediments physical and chemical analyses

Physical and chemical analyses of sediments included: texture, pH, redox potential (Eh), organic C (TOC), total and inorganic N content, inorganic P, total arsenic (As) content, total P, S, Fe, Al, Ca, Mg, Na, Si. Arsenic fractionation was also performed. pH, Eh, inorganic N and inorganic P were assessed at all sampling dates, total As content was determined in Spring and Summer 2021, Winter 2022 and Summer 2023 as well as all other total elements. Total organic C and N and texture were analysed only at the beginning of the study. Texture analysis was carried out in accordance with the Soil Survey Laboratory Methods Manual [39]. Soil pH was measured potentiometrically in a 1:2.5 (w/v) soil-deionised water suspension with a pH meter (Hanna Instruments) [40], while Eh was assessed in a 1:5 (w/v) soil-deionised water suspension by means of a commercially available combination oxidation–reduction potential (Redox (ORP)) electrode connected to a millivolt meter (METTLER TOLEDO©, Italia).

Total organic C (TOC) and nitrogen content (TN) were determined by means of an elemental analyser vario-MACRO cube (CHNOS ELEMENTAR ANALYZER, elemental Analyser system GmbH, Germany). The total content of elements (P, S, Fe, Al, Ca, Mg, Na, Si) was assessed by means of ICP-OES (inductively coupled plasma optical emission spectroscopy) (Perkin Elmer® OptimaTM 8000 DV) as reported by Sidoti et al. [15]. The chemical properties presented in this paper were averaged across all sampling dates for WSed and Sed to provide a comprehensive overview of sediment properties during the length of the project. Arsenic sequential extraction procedure (SEP), consisting in eight steps, was performed following Keon et al. [41] (Table 2) on sediments sampled in Summer 2023. In all the extraction steps, sediment to extractant ratio was 1:25 [42]. The sediment-extractant suspensions were shaken for the specified duration and then centrifuged for 40 min at 6000 xg to separate the supernatant. The sediment sample was washed with milliQ water for 30 min after each extraction and before the addition of the next extractant. As content was determined by means of ICP-OES (Perkin Elmer® OptimaTM 8000 DV) at 193.696 nm wavelength.

3.4. Enzymatic activities

The enzymatic activities were measured using the fluorogenic methylumbelliferyl (MUF)-substrates method [43] in Summer and Winter from 2021 to 2023. The following hydrolytic enzymes, involved in C, N, P and S biogeochemical cycles [44], were analysed: β-cellobiohydrolase (Cell, EC 3.2.1.91), N-acetyl-β-glucosaminidase (NAG, EC 3.2.1.30), β-glucosidase (β-gluc, EC 3.2.1.21), α-glucosidase (α-gluc, EC 3.2.1.20), β-xylosidase (Xylos, EC 3.2.2.27), acid phosphatase (Phosph, EC 3.1.3.2), arylsulphatase (Aryls, EC 3.1.6.1.) and leucine aminopeptidase (LAP, EC 3.4.11.1). The respective substrates were 4-MUF β-D-cellobioside, 4-MUF-N-acetyl-β-glucosaminide, 4-MUF β- D-glucoside, 4-MUF α-D-glucoside, 4-MUF-7-β-D-xyloside, 4-MUF-phosphate, 4-MUF-sulphate and L-leucine-7-amino-4-methylcoumarin (AMC). A suspension of soil was obtained by homogenizing 2 g of each sample with 50 ml sterile water using an Ultra Turrax (IKA, Staufen, Germany) at 9600 rpm for 3 min. Aliquots of 50 μl were withdrawn and dispensed into 96-well black microplates (three analytical replicates per sample per substrate). Sodium acetate buffer 0.5 M pH 5.5 was used for Cell, NAG, β- gluc, α-gluc, Xylos, Phosph and Aryls, whereas TrisHCl buffer 0.05 M pH 7.8 was used for LAP. One hundred μl of 1 mM substrate solution was added to all samples, obtaining a final substrate concentration of 500 μM. Plates were kept at 30 °C for 30 min and then fluorescence (excitation 360 nm; emission 450 nm) was measured with an automated fluorimetric plate-reader (Fluoroskan Ascent, Labsystem GmbH, Frankfurt, Germany) after 0, 30, 60, 120 and 180 min [45]. The results were expressed as nmoles of product (MUF or AMC) of each enzymatic reaction released per g of soil per unit of time in relation to a standard curve obtained with increasing MUF or AMC concentrations and incubated at the same experimental conditions. The S.E.I. (synthetic enzymatic index) represents the sum of all enzymatic activities.

3.5. EL-FAME analyses

Microbial biomass was estimated by means of ester linked fatty acid methyl ester (EL-FAME) technique in samples collected in Summer 2021 and Winter 2022. Microbial community structure was characterised by EL_FAME analysis, which employed a mild alkaline methanolysis method to extract ester-linked (EL) fatty acids but not free fatty acids [46]. To summarize, 16.6 ml of 0.2 M potassium hydroxide in methanol and 5 μl of internal standard solution (0.023 mg ml-1 methylnonadecanoate 19:0) were added into a 50 ml centrifuge tube containing 5 g of freeze-dried soil. The tubes were incubated at 37 °C for 1 hour in orbital shaker. Then, 6.6 ml of 1.0 M acetic acid were added to neutralize the pH of the tube contents. FAMEs were extracted by adding 10 ml of hexane. The mixture was vigorously mixed and centrifuged at 4500 rpm (2037.55 g) for 15 min. The hexane layer was transferred into a clean glass tube, dehydrated with sodium sulphate, filtered and evaporated under a steam of N_2. Finally, FAMEs were dissolved in 200 μl of dichloremethane and transferred to an amber vial for GC–MS quantification analysis. Mass spectra was recorded, using a QP-5050 (Shimadzu, Japan) spectrometer equipped with an AT 20 capillary column (0.25 mm i.d., 25 m) (Alltech, Deerfield). 14 methylated fatty acids were identified according to their mass spectra and using BAME 24 (47080 U) and 37 FAME Mix (47885-U, Sigma–Aldrich) as chemical standards. Data were expressed as relative amount, calculated as the area of each EL-FAME peak relative to the summed area of all EL-FAMEs peaks. Methylnonadecanoate, C19:0, at known concentrations, was used as internal standard. Standard nomenclature is used to describe FAs, which are designated by the total number of carbon atoms/number of double bonds, followed by the position of the double bond from the methyl (aliphatic) end (ω) of the molecule. The prefixes “a” and “i” refer to anteiso and isobranched FAs. The prefix “10Me” indicates a methyl group on the tenth carbon atom from the carboxyl end of the molecule and “cy” indicates cyclopropane FAs. Given the specific environmental matrix and the anaerobic environment, the following biomarkers were identified following Zhang et al. [13]. Unique fatty acids are indicative of specific groups of organisms, they were grouped into general bacteria [47], fungi [48], gram-positive bacteria (G+, i15:0, a15:0, i16:0, i17:0), gram-negative bacteria (G-, 16:1ω9, 18:1ω9t, 18:1ω9c, cy17:0, cy19:0) [47,49], sulfate-reducing bacteria (SRB) and other anaerobes [50,51] and algae [52,53]. The ratio 18:1ω 9t/18:1ω9c (trans/cis) was calculated as an index of microbial stress [54].

3.6. WAW sampling and manipulation for arsenic assessment

To assess WAW arsenic content, a wooden composite sample (derived from 6 sub-samples collected within the archaeological area) was thawed and oven dried at 103 °C ± 2 °C. After desiccation the sample was milled by means of an IKA MF10 blade mill at 0.1 mm of diameter. Three replicates, of approximately 0.2 g, each were placed into 100 mL PFA HP 500 Plus digestion vessel, and 7.5 mL of ultrapure HNO_3 (68 %), 0.5 ml HCl (37 %) and 2 mL of H_2O_2 (30 %) were added to each vessel and then were loaded into a microwave oven (MARSXpress, CEM Corporation, Matthews, North Carolina).

The heating program was as follows: from 25 °C to 200 °C in 37 min, hold at 200 °C for 15 min. In all analytical determinations, blanks and triplicate samples were used to ensure the quality and reproducibility of the results. The samples were removed to cool. After cooling, the samples were filtered and diluted to a volume of 50 mL with Milli-Q water.

As content was determined by means of ICP-OES (Perkin Elmer® OptimaTM 8000 DV, equipped with a Scott nebulizer) at 193.696 nm wavelength.

3.7. Statistical analyses

Statistical analyses were performed using the JMP11.0 (SAS Inst. Inc. Cary, NC, USA) software package. Two-way ANOVA and Tukey post hoc test were used to highlight differences between Sed and Wsed properties and between sampling dates.

Table 1
Sediments physical and chemical properties. WSed: sediment sampled in the vicinity of the WAW, Sed: sediment sampled far from WAW. Eh: redox potential. Data were averaged across sampling dates.

		Wsed	**Sed**	P value
Texture		Loamy sand	Sand	
Organic C	%	8.6	1.3	< 0.001
Total N	%	0.5	0.3	ns
pH		7.2	7.1	ns
Eh	(mV)	−14.3	−15.4	ns
$N\text{-}NH_4$	(mg kg^{-1})	4.0	3.9	ns
$N\text{-}NO_3$		8.8	8.1	ns
Inorg. P		11.5	6.1	<0.01
Total P		545	423	<0.01
Total S		779	337	<0.001
Total Fe		9300	11,308	<0.001
Total Al		14,292	15,661	ns
Total Ca		7472	10,633	<0.05
Total Mg		2543	4149	<0.001
Total Na		473	547	ns
Total Si		140	119	ns

4. Results

The sediment physical and chemical properties are reported in Table 1.

Sediment texture was almost sandy, with a slightly alkaline pH and a suboxic environment (ranging throughout the three years period from 6.4 to 7.8 pH and from −45 to +7 mV, respectively). The organic C content was significantly higher in WSed reaching 8.6 % as also inorganic and total P with 11.5 and 545 ppm, respectively, and total S (779 ppm).

As for Fe, Ca and Mg a significantly higher content is present in Sed samples with 11,308, 10,633, 4149 ppm, respectively.

Arsenic concentration was particularly high at Gran Carro site sediments (Fig. 2).

At all sampling dates its content was always significantly higher in WSed samples reaching more than 150 ppm in Spring 2021. Arsenic sequential fractionation showed a different distribution among the diverse fractions (Table 2).

In general, the largest contribution to the total content was represented by the strongly adsorbed fraction accounting for 37 and 29 % in WSed and Sed respectively, followed by As oxides coprecipitated with silicate clays accounting for 17 and 25 % and As coprecipitated with pyrite 10 and 9.5 %. All other fractions ranged from 10 to 0.9 % of the total As content. When considering As absolute values of all immobilized forms (precipitated into insoluble compounds) they were 68 $\mu g\ g^{-1}$ for WSed and 37 $\mu g\ g^{-1}$ for Sed. Arsenic forms interacting with sulphides were significantly higher in WSed either coprecipitated with pyrite and As_2S_3 or coprecipitated with carbonate and amorphous volatile sulphur compounds (36.4 vs 9.6 in WSed and Sed, respectively). Arsenic concentration in the archaeological wood showed a consistent amount, much higher with respect to sediments (ca. 5000 ppm) (Fig. 3).

EL-FAME total biomass reached the highest content in Winter 2022 in Sed samples accounting for about 4000 nmoles g^{-1}. EL-FAME content changed between seasons; in particular, total biomass was higher in Sed in Winter while it increased in Summer in WSed. At the two sampling dates a significant reduction was observed in WSed, up to −70 % in Winter 2022 (Table 3a).

The largest contribution within microbial population was represented by general bacteria accounting for an average 52 %, followed by G- 32 %, $G + 8$ %, fungi 4 % and algae 2 %, across sampling dates. All groups showed a significant reduction in WSed with respect to Sed at both sampling dates; this was more pronounced in Winter with −67, −75 and −72 and −64 and −68 % for general bacteria, G+, G-, fungi and algae, respectively. Table 3b shows the different

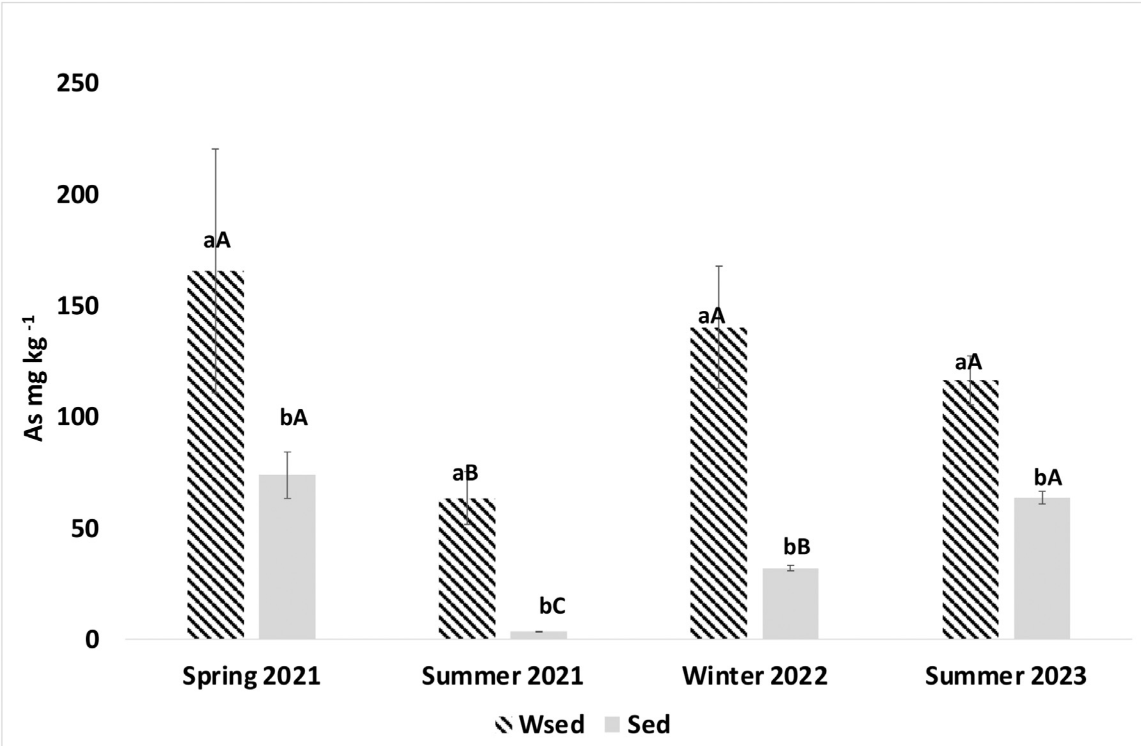

Fig. 2. Total arsenic in the sediments. Different letters mean significant difference, $P < 0.05$: capital letters indicate temporal variation, low case letters indicate variation between Sed and WSed. Bars represent standard error.

Table 2
Arsenic fractionation performed following Keon et al. [41] on sediments sampled in August 2023. Different letters mean significant difference ($P < 0.05$). As fractions are listed from the most loosely to the most strongly immobilized one. AVS: amorphous volatile sulphides.

	Sed	WSed
	As (μg/g)	
Total As	68.3 ± 3.5 b	129.7 ± 3.1 a
Sequential extraction procedure (SEP):		
Loosely adsorbed As	2.4 ± 0.0 b	5.3 ± 0.1 a
Strongly adsorbed As (Fe and Al)	19.4 ± 0.1 b	47.2 ± 0.7 a
As co-precipitated with AVS, carbonate, Mn oxide, and very amorphous Fe oxyhydroxides	3.3 ± 0.2 b	13.2 ± 0.1 a
As co-precipitated with amorphous Fe, Al and Mn oxyhydroxide	2.9 ± 0.1 b	5.3 ± 0.0 a
As co-precipitated with crystalline Fe, and Al oxyhydroxide	6.2 ± 0.1 b	12.2 ± 0.1 a
As oxide and As co-precipitated with silicates	16.7 ± 0.9 b	22.5 ± 0.7 a
As co-precipitated with pyrite and amorphous As_2S_3	6.3 ± 0.4 b	13.6 ± 0.4 a
Remaining residual strongly recalcitrant As minerals	1.4 ± 0.1 a	1.2 ± 0.1 a
Recovery (%)	*87.7 ± 6.9 %*	*93.27 ±3.5 %*

percentage distribution of biomarkers Cy:17 and 18:1w9 (*cis* and trans), within G- bacteria group. The percentage of these biomarkers, within G- group, increased in WSed by 117 % in Winter and 87 % and 44 % in Summer, respectively, although their total amount was decreased. Sulphate reducing bacteria (SRB), which together with other anaerobic bacteria are part of G- bacteria, decreased significantly in WSed samples only in Winter 2022 (Fig. 4a). Microbial stress index, obtained by calculating the ratio of 18:1w9t to 18:1w9c (trans/*cis*) is reported in Fig. 4b This index was significantly increased at both sampling dates in WSed and was positively and significantly correlated to total As content ($P < 0.01$, Fig. 4d). Finally, microbial structural diversity, measured by means of Shannon diversity index (H') showed a significant decrease in Summer 2021 in WSed samples (Fig. 4c).

Enzymatic activities grouped into C, N, P and S cycle are shown in Fig. 5a for Sed and 5b for WSed samples. All enzymatic activities showed seasonal fluctuations with a consistent decrease in Summer 2022. Apart from few exceptions, all enzymatic activities showed a significant decrease in WSed samples which accounted for an average of −23 % for C and P and −46 % for N and S cycles respectively, across all sampling dates. In particular, C cycling enzymes significantly decreased in WSed at all sampling dates, N cycling in Sept. 2022 and March 2023, P cycling in March and Sept. 2022 and August 2023 and S cycling at all dates except March 2023 ($P < 0.05$).

The relationship between As total content and the synthetic enzymatic index (S.E.I.) showed a negative correlation significant at P value <0.05 (Fig. 5c).

5. Discussion

5.1. Gran Carro sediments characterisation

Sediment properties may impact cultural heritage conservation in waterlogged conditions [1,14,26], particularly when dealing with organic materials, thus influencing their decay rate. This may also

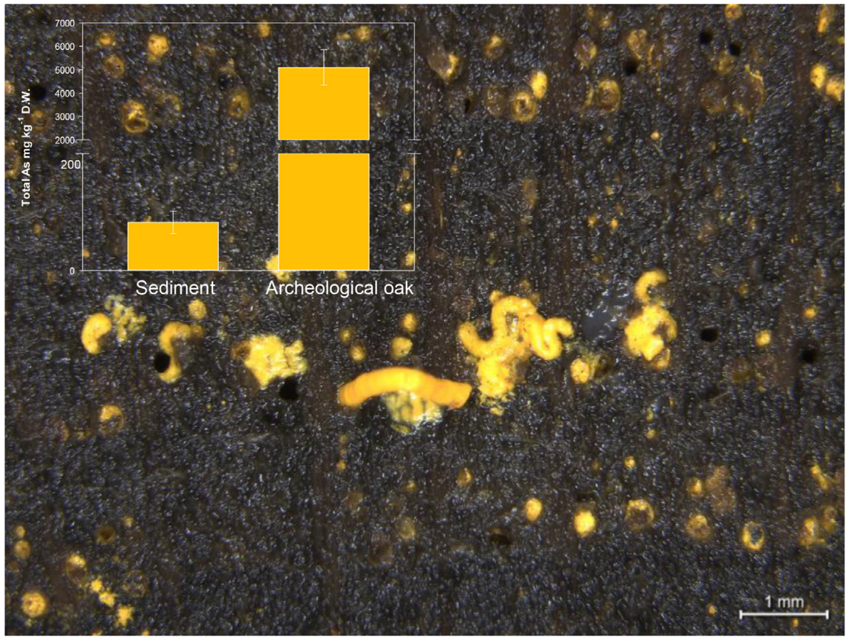

Fig. 3. Stereomicroscope (Leica M205C) transversal view of an oak sample of WAW. Yellow forms are As deposits of realgar which leak out from the vessels by gently squeezing the waterlogged sample. The graph shows the As content in the archaeological wood and in Gran Carro site sediments, averaged across sampling dates and Sed and WSed samples. Bars represent standard error.

Table 3
a) EL-FAME profiles determined in Sed and WSed sediments sampled in Summer 2021 and Winter 2022, **b)** percentage contribution of biomarkers *Cy 17:0, 18:1ω9t and 18:1ω9c* to total G- bacteria group. Different letters mean significant difference ($P < 0.05$), capital letters indicate temporal variation, low case letters indicate variation between Sed and WSed.

a)

	Summer 2021		Winter 2022	
	Sed	WSed	Sed	WSed
	nmol/g			
General bacteria	1334.3 ± 113.1 aA	1075.4 ± 102.7 bA	1895.0 ± 408.8 aA	610 ± 55.8 bB
G+	288.2 ± 24.2 aA	119.9 ± 17.7 bA	345.6 ± 85.3 aA	86.0 ± 8.6 bA
G-	992.8 ± 105.3 aB	548.5 ± 69.1 bA	1623.3 ± 406.8 aA	441.8 ± 51.9 bA
Fungi	107.2 ± 13.5 aA	80.7 ± 6.5 bA	111.0 ± 21.4 aA	40.3 ± 4.9 bB
Algae	57.2 ± 13.6 aA	21.3 ± 5.6 bA	98.3 ± 24.2 aA	30.7 ± 3.4 bA
Total biomass	2780 ± 258 aB	1846 ± 185 bA	4062 ± 943 aA	1203 ± 119 bB

b)

	Summer 2021		Winter 2022	
	SED	WSED	SED	WSED
	%			
Cy 17:0	10.90 ± 1.2 aA	12.10 ± 0.4 aA	5.30 ± 0.3 bB	11.50 ± 1 aA
18:1ω9t	11.60 ± 0.5 bA	21.70 ± 1.5 aB	11.70 ± 1.3 aA	12.70 ± 0.9 aA
18:1ω9c	16.90 ± 1.1 bA	24.30 ± 1.1 aB	16.90 ± 1.4 aA	17.30 ± 0.6 aA

occur in a reciprocal way meaning that constituents of organic materials may be incorporated into the sediments modifying their original properties. Moreover, any change induced by human impact (i.e., pollution) and climate changes (variations of pH, Eh and temperature) may affect the direction and intensity of decay processes [15].

Jordan (2001) [14] state that the specific properties of the site where WAW is located have not been systematically characterised and correlated to the types and causes of degradation. The same author underlines, therefore, the importance of considering the interactions between physical, chemical and biochemical processes with the deposited organic material (i.e., wood), as well as changes in these processes over extended periods of time.

At Gran Carro archaeological site, sediments properties reflect the interaction of different factors such as their depth, water quality, the specific nature of the lithological substrate, atmospheric deposition, and the biological processes carried out in the lake ecosystem.

The local climate conditions may certainly be the first cause of changes occurring in the lake ecosystem. In particular, the average air temperatures recorded at Lake Bolsena reported an increase of hot days (number of days when Tmax ≥ 30 °C) during the period 1990–2023 [24] showing a clear evidence of climate change.

Redox potential, together with pH, are important properties of the burial environment; neutral pH and Eh values between +100 and − 400 mV indicate good preservation of archaeological organic material [55]. The shallow waters characterising Gran Carro archaeological site, being the pile dwelling at an average depth of 4 m, did not completely inhibit oxygen circulation in the sediments that showed, during the three years of

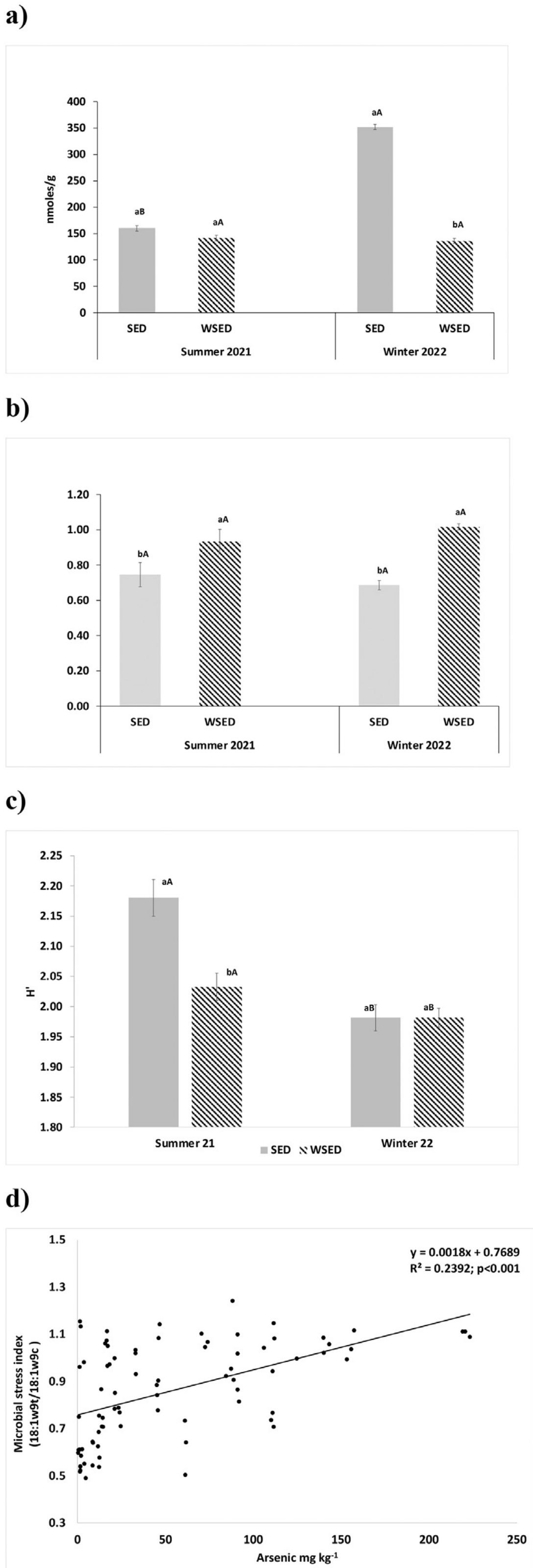

Fig. 4. **a)** Sulphate reducing (SRB) and other anaerobic bacteria, **b)** microbial stress index (18:1w9t/18:1w9c, trans/*cis*), **c)** Shannon diversity index (H') calculated with EL-FAME, **d)** correlation between sediment total Arsenic and microbial stress index, measured in Summer 2021 and Winter 2022 in Sed and WSed samples. Different letters mean significant difference ($P < 0.05$), capital letters indicate temporal variation, low case letters indicate variation between Sed and WSed. Bars represent standard error.

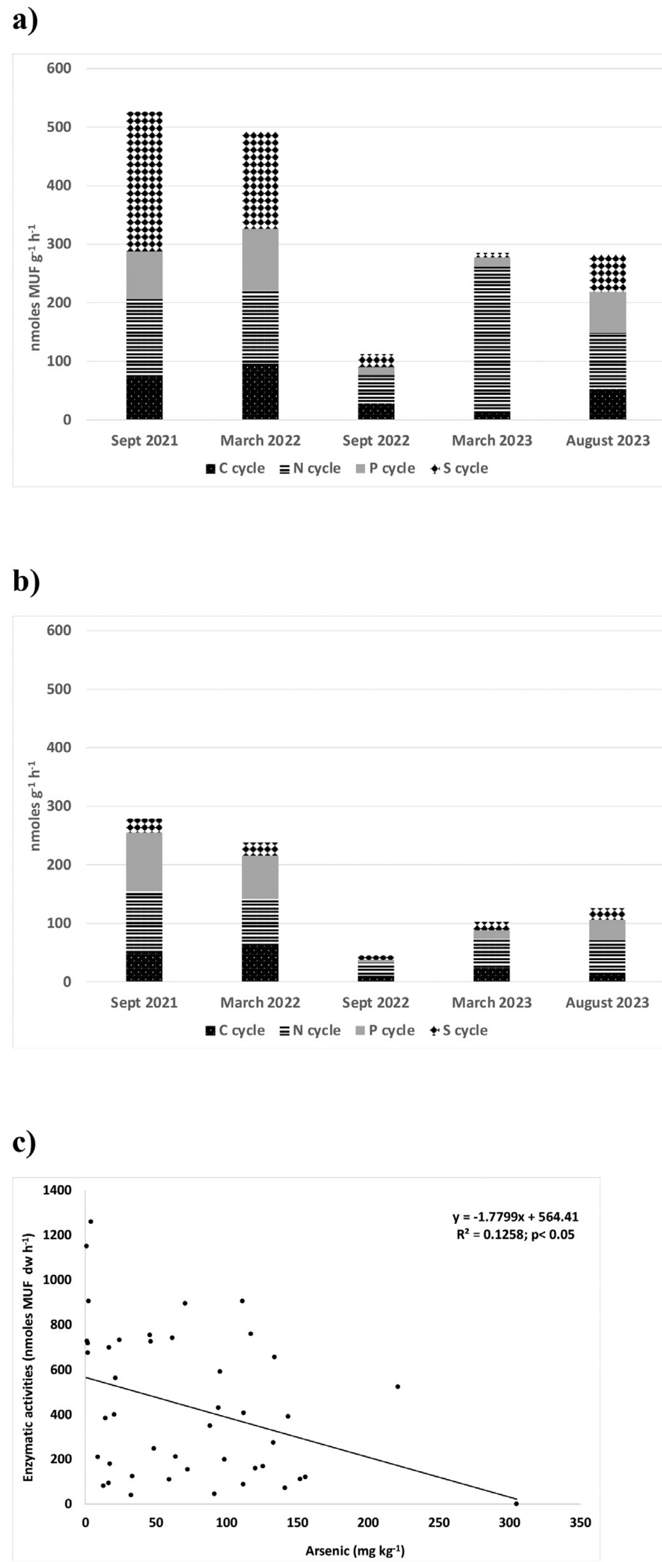

Fig. 5. **a)** C, N, P and S cycle enzymatic activities measured in Sed samples at all sampling dates **b)** C, N, P and S cycle enzymatic activities measured in WSed samples at all sampling dates, **c)** correlation between sediment total Arsenic and S.E.I. Different letters mean significant difference ($P < 0.05$). The significance of the decrease of enzymatic activities in WSed with respect to Sed samples is reported in the results section.

investigation, suboxic conditions (ranging between −35 mV and + 8 mV).

In the period of study, the fungal biomass and algae presence, accounting for about 4 and 2 % of total biomass, were assessed through EL-FAME profiles (see next paragraph) and nitrate ion was also present indicating that oxidation processes were not completely hindered. At Gran Carro the total phosphorus content in sediments ranged from 422 to 545 mg kg^{-1}; this may inform on lake eutrophication conditions as these values are index of nominal/moderate pollution according to the United States Environmental Protection Agency [13,56].

Arsenic is naturally present in the surrounding soils, since they derive from the Vulsini volcanic deposits [57], and in the groundwaters due to geogenic contamination [58,59]. The presence of arsenic in the lake waters could also be locally and variably derived by interaction with thermal springs linked to deep geothermal water circulation, as well known in the nearby Viterbo area [60,61].

Sediments total As content was particularly elevated and exceeded, at all sampling dates, the threshold value of 33 mg/kg^{-1} indicated by Mac Donald et al. [62] for freshwater sediments.

As reported by Shaheen et al. [22] the increase of As concentration in the water, and consequently in the sediments, is the result of increasing reducing conditions where the As III prevails over As V, as well as the associated reductive/acidic dissolution of Fe and Mn oxides. However, at Gran Carro site, lake water As concentration did never exceed 25 ppb as average value during the project period (data not shown) confirming the results reported by Mosello et al. [21] and thus excluding a potential environmental hazard being 150 ppb the threshold for As chronic pollution [63]. Therefore, the high content found in the sediments may be the result of an immobilization process through a likely adsorption/fixation or precipitation process either on sediment mineral/organic fractions or on wood tissues.

Arsenic chemistry in anaerobic environments depends on pH, Eh and Fe oxidation state, presence of labile organic C [64]. At the environmental conditions characterising Lake Bolsena sediments, such as neutral–subalkaline pH and suboxic Eh, arsenic is supposed to be mainly present as As III and in the form of H_3AsO_3 [65]. It may be hypothesized that, under the ongoing climate changes the rise of average water temperatures may favour thermal stratification of lake waters. This event decreases convective mixing and leads to the diminishing of dissolved oxygen concentration and of redox potential thus favouring accumulation of reduced As forms (AsIII).

Microbial sulphate reduction is the major pathway for anaerobic degradation of organic matter in marine sediments [29]. Sulphate reducing bacteria (SRB) mainly use sulphate as their terminal electron acceptor and deeply affect biogeochemical cycling of As [66].

As III is a particularly mobile form and it can be hypothesized that it may react with hydrogen sulphides, released by (SRBs), microorganisms that were particularly consistent in Lake Bolsena sediments. These hydrogen sulphides, in the presence of Fe and As ions, are not stable and transform into iron and arsenic sulphides that precipitate and may be immobilised into wood porous structure [64,67,68]. Sun et al. [69] reported that SRBs can use arsenic to form insoluble sulphide mineral-like phases in the form of an orpiment-like phase (As_2S_3), a realgar-like phase (AsS), an arsenopyrite-like phase (FeAsS) [68,70,71]. At Gran Carro site, the precipitation of As in the form of realgar, one of the insoluble forms of As, and its further accumulation into WAW, was confirmed by Sidoti et al. [15] through the XRD analysis.

The metabolism of SRB is strictly involved into As transformations under anaerobic environments as these microorganisms depend on sulphate availability as a source of energy [22]. Oxidized chemical species, such as sulphates, are consumed under anoxic conditions by bacteria such as sulphate-reducing bacteria that further lower the redox potential when adequate supplies of nutrients and sulphate are present [14]. At Gran Carro site, arylsulfatase activity, an enzyme produced by bacteria, fungi and algae [72] and which releases sulphate ion from organic substrates, was activated at all sampling dates indicating a microbial demand for sulphate ion.

The immobilization of As into the sediments was further studied through the sequential fractionation procedure [41]. As hypothesized, the results of SEP confirm that most of the arsenic is immobilised into strongly adsorbed forms (about 30 %) and co-precipitated with crystalline Fe, S, and silicates (about 48 %). The scarce content of loosely adsorbed As (< 5 % of the total content) explains the lack of water contamination.

Mrozik et al. [73] report scarcity of information about the procaryotic distribution and community structure in the lake sediments. The extraction of membrane phospholipids (EL-FAME technique), performed with the aim to characterise microbial biomass in lake/marine sediments, has been reported by [13,22,29,74,75]; however, no studies aimed to relate microbial population content and, mainly, enzymatic activities in the presence of waterlogged archaeological wood seem to be published.

The EL-FAME analysis can provide insight into the composition of the microbial community structure according to the relative abundance of different groups of microorganisms [76–79]. Bolsena sediments, at Gran Carro archaeological site, showed a consistent amount of microbial biomass with an average content, across dates and samples, of about 2500 nmoles g^{-1}. A similar range of total PLFA were obtained by Steger et al. [80] in boreal lake sediments. Despite the suboxic environment, this content may be also ascribable to the availability of carbon, particularly in the vicinity of WAW, and mineral nutrients such as N and P. Furthermore, the variation of these factors induced by climatic fluctuations (water temperature, redox potential, etc.) are responsible of the significant variations found between the two sampling dates (Summer 2021 and Winter 2022).

At Gran Carro site, apart from general bacteria, the highest contribution was represented by G- bacteria, followed by G+ bacteria, fungi, and algae. Wood in aquatic environments is usually attacked by bacteria [81,82], here erosion, tunnelling and cavitation bacteria, belonging to G- group, degrade secondary wall layers and deplete cellulose and hemicellulose from the wood [82].

Yan et al. [64] reported that fatty acids *cy17:0*, *18:1ω9*trans and *18:1ω9*cis are indicative of bacterial species using Fe(III), As(V) and SO_4^{2-} as electron acceptors to produce Fe(II), As(III) and HS^-. In particular, microbial species characterised by these fatty acids were observed in arsenic rich regions as reported by [83–85]. At Gran Carro site, microbial species containing the above-mentioned biomarkers contributed to the total G- bacteria group for an amount ranging from 34 to 58 %. Honschopp et al., Bentley and Chasteen, and Shaheen et al. [22,86,87] show evidence of bacterial species, belonging to Gram negative group, able to cope with high concentrations of As also by means of arsenic biomethylation mechanisms.

Many factors, individually or in interaction, influence extracellular enzymatic activities in nearly anaerobic environments as sediments such as temperature, pH, water table, nutrients supply, quality of organic matter, and anthropogenic impact [88]. In this study, enzymatic activities showed seasonal fluctuations that reflect all the factors mentioned above. In particular, Summer 2022 was characterised by drought conditions where high air/water temperatures combined to a low level of dissolved oxygen [24] may have hampered microbial activity and consequent hydrolytic processes. When grouping enzymatic activities into geochemical cycles, averaging across sampling dates, it emerged the following ranking N>S>P>C and N>P>C>S for Sed and WSed samples, respectively. Low activities of C cycling enzymes with respect to N and P cycles were also found by Mahmoudi et al. [89] in marine, Perlinski et al. [90] in coastal river estuary and Zoppini et al. [91] in riverine sediments. Souza et al. [92] similarly found an enhancement of aminopeptidases in sandy sediments in Texas and hypothesized that N-cycling enzymes are constitutive cell enzymes and that microorganisms tend to maintain a regular synthesis of this enzyme because of high cellular demand for nitrogen. Also, it may be argued that at Lake Bolsena the presence of lake DOC (dissolved organic C produced by algae biomass) may provide carbon substrates for microbes thus reducing C-cycling enzymes.

5.2. The sediments surrounding WAW: is there a wood effect?

At Gran Carro site, the sediments that surrounded waterlogged archaeological wood showed a clear “wood effect” for most of their properties, either chemical, biochemical, or microbiological ones. In general, in the vicinity of the wood poles, a higher amount of organic C, P (inorganic and total), and S derived from decomposition activity was found.

Either total As or all its fractions, except the recalcitrant one, showed a consistent and significant increase in WSed samples. Arsenic insoluble forms, as realgar, accumulated in WAW porous structure. A likely explanation for this mechanism could be that the insoluble forms of As, originated by its reaction with hydrogen sulphide, are initially precipitated and trapped within wood porous structure, either into cell lumina or within the voids originated by celluloses and hemicellulose degradation. Subsequently, following the slow WAW degradation, insoluble As forms migrate by means of organic debris towards the surrounding sediments as already stated by Sidoti et al. [15].

Arsenic (As) is toxic to almost all soil microorganisms by inhibiting cellular functions linked with energy metabolism [30,31,93].

Total EL-FAME significantly decreased in WSed samples by 34 and 70 % respectively in Summer 2021 and Winter 2022, suggesting a negative effect on microbial biomass induced by enhanced As concentration in the vicinity of WAW. The negative impact of As on microbial communities is widely reported as it may alter cellular functions and microbial metabolism [30,31]. In this study, a positive significant correlation was observed between an index of microbial stress (*18:1w9t* to *18:1w9c* fatty acids ratio) and total As concentration. A similar inverse relationship was found by Yan et al. [64] at Datong basin in China.

However, when looking at a group of biomarkers such as *cy17:0*, $18{:}1\omega9$trans and $18{:}1\omega9$cis, whose presence seems to be related to As presence [64], their percentage contribution to the total G-bacteria content recorded a significant increase in WSed. In particular, for *cy17:0* it increased by 117 % in Winter 2022 while for $18{:}1\omega9$trans and $18{:}1\omega9$cis it was enhanced by 87 and 44 % in Summer 2021, respectively. This may indicate that the elevated concentrations of As, found in WSed samples, exerted a selective pressure within microbial biomass in favour of As-resistant species.

Microbial structural diversity, measured by means of EL-FAME analysis, showed a significant decrease of Shannon index (H’) in Summer 2021 in WSed samples. It may be hypothesized that the above discussed selective pressure induced by elevated As concentrations, combined to the elevated summer temperatures, may have produced a shift within microbial community composition.

Arsenic, as most heavy metals, may inactivate enzymes by reacting with sulfhydryl groups or interacting with the enzyme–substrate complex [44]. Hydrolysing enzymes were reported to be inversely correlated to As [31,94]. At Gran Carro archaeological site all enzymatic activities reported a decrease in WSed samples and the significant inverse relationship between S.E.I. and total As confirms the inhibitory effect on decomposition activity.

Microbial biomass content and enzymatic activities decreased in WSed indicating not favourable conditions for decomposition processes; this may be ascribable to the high arsenic concentration found within the wood poles structure and in the sediment surrounding them. The significant relationships of As with microbial stress index and with enzymatic activities could point to the role of this metalloid at Gran Carro as a possible «protection factor» for archaeological wood from microbial degradation. This confirms what observed in a previous study conducted on the poles of the Gran Carro, that showed highest arsenic values in the less decayed wood [15].

As recommended by Williams [55] this can be a starting point to develop a monitoring activity aimed to assess any significant change of the environmental context. According to Mosello [21] Lake Bolsena is a monomictic lake, meaning that the complete overturn occurs once a year in Winter, while Summer is characterized by a thermal stratification that prevents water mixture. Therefore, it can be proposed to collect during these two periods data on water properties (temperature, pH, dissolved oxygen, redox potential, salinity, As concentration) and sediment chemical properties (pH, Eh, As concentration and its likely distribution into the different fractions). In this way it can be ascertained whether climate changes produce significant variations that may hamper WAW conservation. However, these last indications are specifically formulated for the environmental context and the peculiar chemical composition of Lake Bolsena sediments due its volcanic origin. This constitutes a limitation to extending these recommendations to Mediterranean lakes.

6. Conclusions

At Lake Bolsena, arsenic biogeochemical cycling deserves particular attention being related to a natural contamination process. Also, As speciation and mobility is highly dependent on environmental conditions and on the presence of sulphate reducing bacteria. Global warming can induce future thermal stratification that may impact sediment and water quality leading to the decrease of redox potential thus influencing As speciation and geochemical cycle.

Monitoring sediment properties in these peculiar archaeological sites, under the current climate changes, will help to understand their potential effects on the conservation of cultural heritage.

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The authors herewith declare that they did not use either generative AI or AI-assisted technologies during the writing process.

References

[1] M. Kibblewhite, G. Tóth, T. Hermann, Predicting the preservation of cultural artefacts and buried materials in soil, Sci. Total Environ. 529 (2015) 249–263, doi:10.1016/j.scitotenv.2015.04.036.

[2] H. Walkington, Soil science applications in archaeological contexts: a review of key challenges, Earth. Sci. Rev. 103 (2010) 122–134, doi:10.1016/j.earscirev.2010.09.002.

[3] C. Capretti, N. Macchioni, B. Pizzo, G. Galotta, G. Giachi, D. Giampaola, The characterization of waterlogged archaeological wood: the three roman

ships found in Naples (Italy), Archaeometry 50 (2008) 855–876, doi:10.1111/j.1475-4754.2007.00376.x.
[4] M. Belasus, A. Daly, A.M. Martins, The Bremen-Cog: environmental issues, Archaeonautica (2021) 257–261, doi:10.4000/archaeonautica.1860.
[5] L.I. Elding, Ten years of Vasa research-review and outlook, in: M. Ek (Ed.), Proc. Int. Conf. Shipwrecks 2011, Roy. Inst. of Technology, KTH, Stockholm, Oct 18-21, 2011, Chemistry and Preservation of Waterlogged Wooden Shipwrecks, 2011, pp. 86–93. www.shipwrecks2011.com.
[6] P. Hoffmann, A. Singh, Y.S. Kim, S.G. Wi, I.J. Kim, U. Schmitt, The Bremen Cog of 1380 - an electron microscopic study of its degraded wood before and after stabilization with PEG, Holzforschung 58 (2004) 211–218, doi:10.1515/HF.2004.033.
[7] Q. Li, L. Cao, W. Wang, H. Tan, T. Jin, G. Wang, G. Lin, R. Xu, Analysis of the bacterial communities in the waterlogged wooden cultural relics of the Xiaobaijiao No. 1 shipwreck via high-throughput sequencing technology, Holzforschung 72 (2018) 609–619, doi:10.1515/hf-2017-0132.
[8] A. Vorobyev, G. Almkvist, N.P. Van DIjk, E.K. Gamstedt, Relations of density, polyethylene glycol treatment and moisture content with stiffness properties of Vasa oak samples, Holzforschung 71 (2017) 327–335, doi:10.1515/hf-2016-0202.
[9] A. Vorobyev, O. Arnould, D. Laux, R. Longo, N.P. Van Dijk, E.K. Gamstedt, Characterisation of cubic oak specimens from the Vasa ship and recent wood by means of quasi-static loading and resonance ultrasound spectroscopy (RUS), Holzforschung 70 (2016) 457–465, doi:10.1515/hf-2015-0073.
[10] C.G. Björdal, Microbial degradation of waterlogged archaeological wood, J. Cult. Herit. 13 (2012) S118–S122, doi:10.1016/J.CULHER.2012.02.003.
[11] A.P. Singh, Y.S. Kim, R.R. Chavan, Advances in understanding microbial deterioration of buried and waterlogged archaeological woods: a review, Forests 13 (2022) 394, doi:10.3390/f13030394.
[12] W. Wang, Y. Yang YujunYi, Y. Zhou, S. Zhang, X. Wang, Z. Yang, Impact of anthropogenic activities on the sediment microbial communities of Baiyangdian shallow lake, Int. J. Sediment Res. 35 (2020) 180–192, doi:10.1016/j.ijsrc.2019.10.006.
[13] X. Zhang, Q. Chen, C. Wang, H. Zhang, Y. Zhao, L. Zhang, B. Liu, Z. Wu, Q. Zhou, Characteristic analysis of phospholipid fatty acids (PLFAs) in typical nutrient polluted lake sediment in Wuhan, Int. J. Sediment Res. 36 (2021) 221–228, doi:10.1016/j.ijsrc.2020.09.002.
[14] B.A. Jordan, Site characteristics impacting the survival of historic waterlogged wood: a review, Int. Biodeterior. Biodegrad. 47 (2001) 47–54, doi:10.1016/S0964-8305(01)00035-X.
[15] G. Sidoti, F. Antonelli, G. Galotta, M.C. Moscatelli, D. Kržišnik, V. Vinciguerra, S. Tamantini, R. Marabottini, N. Macro, M. Romagnoli, Inorganic component in oak waterlogged archaeological wood and volcanic lake compartments, Biogeosciences 20 (2023) 3137–3149, doi:10.5194/bg-20-3137-2023.
[16] F. Menotti, Living on the Lake in Prehistoric Europe: 150 Years of Lake-Dwelling Research, Routledge Taylor & Francis Group, London, 2004, doi:10.4324/9780203583203.
[17] M. Romagnoli, G. Galotta, F. Antonelli, G. Sidoti, M. Humar, D. Kržišnik, K. Čufar, B. Davidde Petriaggi, Micro-morphological, physical and thermogravimetric analyses of waterlogged archaeological wood from the prehistoric village of Gran Carro (Lake Bolsena-Italy), J. Cult. Herit. 33 (2018) 30–38 https://www.sciencedirect.com/science/article/pii/S1296207417306623. accessed October 15, 2018.
[18] S. Tamantini, G. Sidoti, F. Antonelli, G. Galotta, M.C. Moscatelli, D. Kržišnik, V. Vinciguerra, R. Marabottini, N. Macro, M. Romagnoli, The WOODPDLAKE project. Lakes, wood and sediment: natural and Cultural Heritage affected by climate changes, in: EGU23, the 25th EGU General Assembly, Held 23-28 April 2023 in Vienna, Austria and Online, Vien, 2023, pp. EGU–15116, doi:10.5194/egusphere-egu23-15116.
[19] E.I. Kretschmar, H. Keijer, P. Nelemans, N. Lamersdorf, Investigating physicochemical sediment conditions at decayed wooden pile foundation sites in Amsterdam, Int. Biodeterior. Biodegrad. 61 (2008) 85–95, doi:10.1016/j.ibiod.2007.07.003.
[20] Z. Huang, C. Liu, X. Zhao, J. Dong, B. Zheng, Risk assessment of heavy metals in the surface sediment at the drinking water source of the Xiangjiang River in South China, Environ. Sci. Eur. 32 (2020) 23, doi:10.1186/s12302-020-00305-w.
[21] R. Mosello, P. Bruni, M. Rogora, G. Tartari, C. Dresti, Long-term change in the trophic status and mixing regime of a deep volcanic lake (Lake Bolsena, Central Italy), Limnologica 72 (2018) 1–9, doi:10.1016/j.limno.2018.07.002.
[22] S.M. Shaheen, A. El-Naggar, V. Antoniadis, F.S. Moghanm, Z. Zhang, D.C.W. Tsang, Y.S. Ok, J. Rinklebe, Release of toxic elements in fishpond sediments under dynamic redox conditions: assessing the potential environmental risk for a safe management of fisheries systems and degraded waterlogged sediments, J. Environ. Manage 255 (2020) 109778, doi:10.1016/j.jenvman.2019.109778.
[23] R. Adrian, C.M. O'Reilly, H. Zagarese, S.B. Baines, D.O. Hessen, W. Keller, D.M. Livingstone, R. Sommaruga, D. Straile, E. Van Donk, G.A. Weyhenmeyer, M. Winder, Lakes as sentinels of climate change, Limnol. Oceanogr. 54 (2009) 2283–2297, doi:10.4319/lo.2009.54.6_part_2.2283.
[24] S. Tamantini, M.C. Moscatelli, B. Barbaro, E. Severi, F. Antonelli, G. Galotta, M. Ciabattoni, F. Cappelli, M. Romagnoli, Climatic change and possible impact on the conservation of wooden pile dwelling in Bolsena Lake. Assessing climate proxy data with high-frequency water monitoring in the year, Hydrology (2022).
[25] D.J. Huisman, E.I. Kretschmar, N. Lamersdorf, Characterising physicochemical sediment conditions at selected bacterial decayed wooden pile foundation sites in the Netherlands, Germany, and Italy, Int. Biodeterior. Biodegrad. 61 (2008) 117–125, doi:10.1016/j.ibiod.2007.07.008.
[26] C.G. Björdal, J. Elam, Bacterial degradation of nine wooden foundation piles from Gothenburg historic city center and correlation to wood quality, environment, and time in service, Int. Biodeterior. Biodegrad. 164 (2021) 105288, doi:10.1016/j.ibiod.2021.105288.
[27] M.C. Moscatelli, M. Romagnoli, S. Cenfi, A. Lagomarsino, A. Di Tizio, S. Spina, S. Grego, Wood-soil interactions in soil bioengineering slope stabilization works, IForest 2 (2009) 187–191, doi:10.3832/ifor0513-002.
[28] K. Klinka, L.M. Lavkulich, Q. Wang, M.C. Feller, Influence of decaying wood on chemical properties of forest floors and surface mineral soils: a pilot study, Ann. Sci. For. 52 (1995) 523–533, doi:10.1051/forest:19950601.
[29] N. Mahmoudi, M.S. Robeson, H.F. Castro, J.L. Fortney, S.M. Techtmann, D.C. Joyner, C.J. Paradis, S.M. Pfiffner, T.C. Hazen, Microbial community composition and diversity in Caspian Sea sediments, FEMS Microbiol. Ecol. 91 (2015) 1–11, doi:10.1093/femsec/fiu013.
[30] R. Marabottini, S.R. Stazi, R. Papp, S. Grego, M.C. Moscatelli, Mobility and distribution of arsenic in contaminated mine soils and its effects on the microbial pool, Ecotoxicol. Environ. Saf. 96 (2013) 147–153, doi:10.1016/j.ecoenv.2013.06.016.
[31] S.R. Stazi, M.C. Moscatelli, R. Papp, S. Crognale, S. Grego, M. Martin, R. Marabottini, A multi-biological assay approach to assess microbial diversity in arsenic (As) contaminated soils, Geomicrobiol. J. 34 (2017) 183–192, doi:10.1080/01490451.2016.1189015.
[32] P. Tamburini, Un abitato villanoviano perilacustre. Il "Gran Carro" sul Lago di Bolsena (1959-1985), Rome, 1995.
[33] B. Barbaro, The protohistorical village of the «Gran Carro»: a settlement and a Cultual Complex on the Lake of Bolsena. Preliminary Research Report 2020-2021, Archaeol. Marit. Mediterr. 18 (2021) 13–34, doi:10.19272/202104501002.
[34] M. Porcaro, B. Barbaro, C. Canovaro, G. Artioli, C. Lucarelli, F. Lugli, A. Depalmas, A. Brunetti, A multi analytical characterization of a small bronze figurine from Gran Carro site (Bolsena Lake, Italy), J. Archaeol. Sci. Rep. 51 (2023) 104230, doi:10.1016/j.jasrep.2023.104230.
[35] A. Fundurulic, I. Valenti, A. Celant, B. Barbaro, M. Costa, A. Manhita, E. Severi, C.B. Dias, D. Magri, Millets and cereal meals from the early iron age underwater settlement of "Gran Carro" (Bolsena Lake, Central Italy), Sustainability 14 (2022) 3941, doi:10.3390/su14073941.
[36] M. Kottek, J. Grieser, C. Beck, B. Rudolf, F. Rubel, World map of the Köppen-Geiger climate classification updated, Meteorol. Z. 15 (2006) 259–263, doi:10.1127/0941-2948/2006/0130.
[37] C. Blasi, Fitoclimatologia del Lazio, 1994.
[38] Acqua - SIRA - ARPALazio, (n.d.). https://sira.arpalazio.it/web/guest/rapporti/acqua#/ (accessed June 3, 2025).
[39] R. Burt, Kellogg Soil Survey Laboratory Methods Manual, United States Department of Agriculture, Natural Resources Conservation Service, National Soil Survey Center, Kellogg Soil Survey Laboratory, 2014.
[40] L. van Reeuwijk, Procedures for Soil Analysis, 6th ed., ISRIC, FAO, Wageningen, 2002.
[41] N.E. Keon, C.H. Swartz, D.J. Brabander, C. Harvey, H.F. Hemond, Validation of an arsenic sequential extraction method for evaluating mobility in sediments, Environ. Sci. Technol. 35 (2001) 2778–2784, doi:10.1021/es001511o.
[42] C. Gleyzes, S. Tellier, M. Astruc, Fractionation studies of trace elements in contaminated soils and sediments: a review of sequential extraction procedures, TrAC - Trends Anal. Chem. 21 (2002) 451–467, doi:10.1016/S0165-9936(02)00603-9.
[43] M.C. Marx, M. Wood, S.C. Jarvis, A microplate fluorimetric assay for the study of enzyme diversity in soils, Soil. Biol. Biochem. 33 (2001) 1633–1640, doi:10.1016/S0038-0717(01)00079-7.
[44] P. Nannipieri, L. Giagnoni, G. Renella, E. Puglisi, B. Ceccanti, G. Masciandaro, F. Fornasier, M.C. Moscatelli, S. Marinari, Soil enzymology: classical and molecular approaches, Biol. Fertil. Soils 48 (2012) 743–762, doi:10.1007/s00374-012-0723-0.
[45] S. Marinari, E. Bonifacio, M.C. Moscatelli, G. Falsone, L. Vittori Antisari, G. Vianello, Soil development and microbial functional diversity: proposal for a methodological approach, Geoderma 192 (2013) 437–445, doi:10.1016/j.geoderma.2012.08.023.
[46] M.E. Schutter, R.P. Dick, Comparison of fatty acid methyl ester (FAME) methods for characterizing microbial communities, Soil. Sci. Soc. Am. J. 64 (2000) 1659–1668, doi:10.2136/sssaj2000.6451659x.
[47] F. García-Orenes, A. Morugán-Coronado, R. Zornoza, K. Scow, Changes in soil microbial community structure influenced by agricultural management practices in a Mediterranean agro-ecosystem, PLoS One 8 (2013) e80522, doi:10.1371/journal.pone.0080522.
[48] A. Frostegård, E. Bååth, The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil, Biol. Fertil. Soils 22 (1996) 59–65, doi:10.1007/bf00384433.
[49] J.L. Mentzer, R.M. Goodman, T.C. Balser, Microbial response over time to hydrologic and fertilization treatments in a simulated wet prairie, Plant Soil. 284 (2006) 85–100, doi:10.1007/s11104-006-0032-1.
[50] L. Ludvigsen, H.J. Albrechtsen, D.B. Ringelberg, F. Ekelund, T.H. Christensen, Distribution and composition of microbial populations in a landfill leachate contaminated aquifer (Grindsted, Denmark), Microb. Ecol. 37 (1999) 197–207, doi:10.1007/s002489900143.
[51] N. Rajendran, O. Matsuda, R. Rajendran, Y. Urushigawa, Comparative description of microbial community structure in surface sediments of eutrophic

bays, Mar. Pollut. Bull. 34 (1997) 26–33, doi:10.1016/S0025-326X(96)00057-4.
[52] N. Reuss, L.K. Poulsen, Evaluation of fatty acids as biomarkers for a natural plankton community. A field study of a spring bloom and a post-bloom period off West Greenland, Mar. Biol. 141 (2002) 423–434, doi:10.1007/s00227-002-0841-6.
[53] J.K. Volkman, S.W. Jeffrey, P.D. Nichols, G.I. Rogers, C.D. Garland, Fatty acid and lipid composition of 10 species of microalgae used in mariculture, J. Exp. Mar. Biol. Ecol. 128 (1989) 219–240, doi:10.1016/0022-0981(89)90029-4.
[54] J.R. Vestal, D.C. White, Lipid analysis in microbial ecology - quantitative approaches to the study of microbial communities, Bioscience 39 (1989) 535–541, doi:10.2307/1310976.
[55] J. Williams, Thirty years of monitoring in England — what have we learnt? Conserv. Manag. Archaeol. Sites 14 (2012) 442–457, doi:10.1179/1350503312Z.00000000038.
[56] C.G. Ingersoll, D.D. Mac Donald, A guidance manual to support the assessment of contaminated sediments in freshwater ecosystems, Volume III – Interpretation of the Results of Sediment Quality Investigations, US Environmental Protection Agency, Great Lakes National Program Office, Chicago, 2002.
[57] D. Parrone, S. Ghergo, E. Preziosi, B. Casentini, Water-rock interaction processes: a local scale study on arsenic sources and release mechanisms from a volcanic rock matrix, Toxics 10 (2022) 288, doi:10.3390/toxics10060288.
[58] D. Parrone, S. Ghergo, E. Frollini, D. Rossi, E. Preziosi, Arsenic-fluoride co-contamination in groundwater: background and anomalies in a volcanic-sedimentary aquifer in central Italy, J. Geochem. Explor. 217 (2020) 106590, doi:10.1016/j.gexplo.2020.106590.
[59] A. Baiocchi, F. Lotti, V. Piscopo, Influence of hydrogeological setting on the arsenic occurrence in ground-water of the volcanic areas of central and southern Italy, AQUA Mundi 4035 (2011) 131–142, doi:10.4409/Am-035-11-0035.
[60] V. Rimondi, P. Costagliola, P. Lattanzi, T. Catelani, S. Fornasaro, D. Medas, G. Morelli, M. Paolieri, Bioaccessible arsenic in soil of thermal areas of Viterbo, Central Italy: implications for human health risk, Environ. Geochem. Health 44 (2022) 465–485, doi:10.1007/s10653-021-00914-1.
[61] G. Sappa, S. Ergul, F. Ferranti, Geochemical modeling and multivariate statistical evaluation of trace elements in arsenic contaminated groundwater systems of Viterbo Area, (Central Italy), Springerplus 3 (2014) 237, doi:10.1186/2193-1801-3-237.
[62] D.D. MacDonald, C.G. Ingersoll, T.A. Berger, Development and evaluation of consensus-based sediment quality guidelines for freshwater ecosystems, Arch. Environ. Contam. Toxicol. 39 (2000) 20–31, doi:10.1007/s002440010075.
[63] National Recommended Water Quality Criteria - Aquatic Life Criteria Table | US EPA, (n.d.). https://www.epa.gov/wqc/national-recommended-water-quality-criteria-aquatic-life-criteria-table (accessed January 13, 2025).
[64] L. Yan, X. Xie, Y. Wang, K. Qian, Z. Chi, J. Li, Y. Deng, Y. Gan, Organic-matter composition and microbial communities as key indicators for arsenic mobility in groundwater aquifers: evidence from PLFA and 3D fluorescence, J. Hydrol. 591 (2020) 125308, doi:10.1016/j.jhydrol.2020.125308.
[65] M.R. Samadzadeh Yazdi, A. Khodadadi Darban, Heavy metals in sediments and remediation technologies, in: Ż. Bargańska (Ed.), The Proceedings of 15th International Conference on Heavy Metals in the Environment, Department of Analitical Chemistry. Chemical Faculty. Gdańsk University of Technology, Gdańsk, 2010.
[66] W. Shi, W. Song, Y. Luo, G. Qile, J. Zheng, C. Lü, J. He, Transformation pathways of arsenic species: SRB mediated mechanism and seasonal patterns, Chemosphere 263 (2021) 128255, doi:10.1016/j.chemosphere.2020.128255.
[67] Z. Niu, J. Yan, X. Guo, M. Xu, Y. Sun, F. Tou, G. Yin, L. Hou, M. Liu, Y. Yang, Human activities can drive sulfate-reducing bacteria community in Chinese intertidal sediments by affecting metal distribution, Sci. Total. Environ. 786 (2021) 147490, doi:10.1016/j.scitotenv.2021.147490.
[68] Y. Fors, F. Jalilehvand, E. Damian Risberg, C. Björdal, E. Phillips, M. Sandström, Sulfur and iron analyses of marine archaeological wood in shipwrecks from the Baltic Sea and Scandinavian waters, J. Archaeol. Sci. 39 (2012) 2521–2532, doi:10.1016/J.JAS.2012.03.006.
[69] J. Sun, A.N. Quicksall, S.N. Chillrud, B.J. Mailloux, B.C. Bostick, Arsenic mobilization from sediments in microcosms under sulfate reduction, Chemosphere 153 (2016) 254–261, doi:10.1016/j.chemosphere.2016.02.117.
[70] R. Alam, K. McPhedran, Applications of biological sulfate reduction for remediation of arsenic – a review, Chemosphere 222 (2019) 932–944, doi:10.1016/j.chemosphere.2019.01.194.
[71] M. Sandström, Y. Fors, I. Persson, The Vasa's new battle: Sulphur, Acid and Iron, Swedish National Maritime Museums, Stockholm, 2003.
[72] M. Yu, M. Wu, F. Secundo, Z. Liu, Detection, production, modification, and application of arylsulfatases, Biotechnol. Adv. 67 (2023) 108207, doi:10.1016/j.biotechadv.2023.108207.
[73] A. Mrozik, A. Nowak, Z. Piotrowska-Seget, Microbial diversity in waters, sediments and microbial mats evaluated using fatty acid-based methods, Int. J. Environ. Sci. Technol. 11 (2014) 1487–1496, doi:10.1007/s13762-013-0449-z.
[74] R.A. White, S.A. Soles, A.L. Brady, G. Southam, D.S.S. Lim, G.F. Slater, Biosignatures associated with freshwater microbialites, Life 10 (2020) 66, doi:10.3390/life10050066.
[75] N.A. Dijkman, H.T.S. Boschker, L.J. Stal, J.C. Kromkamp, Composition and heterogeneity of the microbial community in a coastal microbial mat as revealed by the analysis of pigments and phospholipid-derived fatty acids, J. Sea Res. 63 (2010) 62–70, doi:10.1016/j.seares.2009.10.002.
[76] C.T. Green, K.M. Scow, Analysis of phospholipid fatty acids (PLFA) to characterize microbial communities in aquifers, Hydrogeol. J. 8 (2000) 126–141, doi:10.1007/s100400050013.
[77] Z. Ma, M. Zhang, R. Xiao, Y. Cui, F. Yu, Changes in soil microbial biomass and community composition in coastal wetlands affected by restoration projects in a Chinese delta, Geoderma 289 (2017) 124–134, doi:10.1016/j.geoderma.2016.11.037.
[78] Å. Frostegård, A. Tunlid, E. Bååth, Use and misuse of PLFA measurements in soils, Soil. Biol. Biochem. 43 (2011) 1621–1625, doi:10.1016/j.soilbio.2010.11.021.
[79] G.T. Hill, N.A. Mitkowski, L. Aldrich-Wolfe, L.R. Emele, D.D. Jurkonie, A. Ficke, S. Maldonado-Ramirez, S.T. Lynch, E.B. Nelson, Methods for assessing the composition and diversity of soil microbial communities, Appl. Soil. Ecol. 15 (2000) 25–36, doi:10.1016/S0929-1393(00)00069-X.
[80] K. Steger, K. Premke, C. Gudasz, I. Sundh, L.J. Tranvik, Microbial biomass and community composition in boreal lake sediments, Limnol. Oceanogr. 56 (2011) 725–733, doi:10.4319/lo.2011.56.2.0725.
[81] C.G. Björdal, T. Nilsson, G. Daniel, Microbial decay of waterlogged archaeological wood found in Sweden. Applicable to archaeology and conservation, Int. Biodeterior. Biodegrad. 43 (1999) 63–73, doi:10.1016/S0964-8305(98)00070-5.
[82] R.A. Blanchette, A review of microbial deterioration found in archaeological wood from different environments, Int. Biodeterior. Biodegrad. 46 (2000) 189–204, doi:10.1016/S0964-8305(00)00077-9.
[83] J. Akai, A. Kanekiyo, N. Hishida, M. Ogawa, T. Naganuma, H. Fukuhara, H.N. Anawar, Biogeochemical characterization of bacterial assemblages in relation to release of arsenic from South East Asia (Bangladesh) sediments, Appl. Geochem. 23 (2008) 3177–3186, doi:10.1016/j.apgeochem.2008.06.018.
[84] H. Azarbad, N.M. van Straalen, R. Laskowski, K. Nikiel, W.F.M. Röling, M. Niklińska, Susceptibility to additional stressors in metal-tolerant soil microbial communities from two pollution gradients, Appl. Soil. Ecol. 98 (2016) 233–242, doi:10.1016/j.apsoil.2015.10.020.
[85] D. Ghosh, P. Bhadury, J. Routh, Coping with arsenic stress: adaptations of arsenite-oxidizing bacterial membrane lipids to increasing arsenic levels, Microbiologyopen 7 (2018), doi:10.1002/mbo3.594.
[86] S. Honschopp, N. Brunken, A. Nehrkorn, H.J. Breunig, Isolation and characterization of a new arsenic methylating bacterium from soil, Microbiol. Res. 151 (1996) 37–41, doi:10.1016/S0944-5013(96)80053-X.
[87] R. Bentley, T.G. Chasteen, Microbial methylation of metalloids: arsenic, antimony, and bismuth, Microbiol. Mol. Biol. Rev. 66 (2002) 250–271, doi:10.1128/mmbr.66.2.250-271.2002.
[88] H. Kim, A review of factors that regulate extracellular enzyme activity in wetland soils, Korean J. Microbiol. 51 (2015) 97–107, doi:10.7845/kjm.2015.4087.
[89] N. Mahmoudi, S.M. Hagen, T.C. Hazen, A.D. Steen, Patterns in extracellular enzyme activity and microbial diversity in deep-sea Mediterranean sediments, Deep Sea Res. Part I: Oceanogr. Res. Pap. 158 (2020) 103231, doi:10.1016/j.dsr.2020.103231.
[90] P. Perliński, Z.J. Mudryk, Enzymatic biodegradation of high molecular weight polymers in the sediment–water interface in the coastal river estuary, River. Res. Appl. 34 (2018) 745–754, doi:10.1002/rra.3308.
[91] A. Zoppini, S. Amalfitano, S. Fazi, A. Puddu, Dynamics of a benthic microbial community in a riverine environment subject to hydrological fluctuations (Mulargia River, Italy), Hydrobiologia 657 (2010) 37–51, doi:10.1007/s10750-010-0199-6.
[92] A.C. Souza, T.K. Pease, W.S. Gardner, Vertical profiles of major organic geochemical constituents and extracellular enzymatic activities in sandy sediments of Aransas and Copano Bays, TX, Estuaries Coasts 35 (2012) 308–323, doi:10.1007/s12237-011-9438-y.
[93] R. Singh, S. Singh, P. Parihar, V.P. Singh, S.M. Prasad, Arsenic contamination, consequences and remediation techniques: a review, Ecotoxicol. Environ. Saf. 112 (2015) 247–270, doi:10.1016/j.ecoenv.2014.10.009.
[94] P. Bhattacharyya, S. Tripathy, K. Kim, S.H. Kim, Arsenic fractions and enzyme activities in arsenic-contaminated soils by groundwater irrigation in West Bengal, Ecotoxicol. Environ. Saf. 71 (2008) 149–156, doi:10.1016/j.ecoenv.2007.08.015.