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Research article

Biochar-based concrete as biocompatible building material for marine artificial structures

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ABSTRACT

The growing exploitation of marine space for human activities is leading to the proliferation of artificial structures in coastal and offshore areas. Hence, improving building materials to minimize the impact of their introduction in the marine environment and to enhance their overall environmental sustainability is essential. The addition of biochar, a common carbon-rich by-product of thermochemical treatments of discarded biomass, in concrete mixtures for marine uses may lead to progresses in this perspective, combining waste recycling with carbon storage and sequestration potential. However, comprehensive evaluations of possible effects of biochar-based concrete on the marine biota are lacking to date. Here, laboratory tests and manipulative field experiments were carried out in three different sites (NE Adriatic, Mediterranean Sea) to assess whether biochar-based concrete could affect marine organisms and the colonization of micro- and macrobenthic assemblages on artificial hard substrata in shallow subtidal habitats. No relevant ecotoxicological responses to biochar exposure for several target marine organisms from different trophic levels were found. With respect to conventional concrete, adding up to 10 %wt. biochar to the mixture did not determine significant changes neither in microfouling assemblages at early stages of colonization, nor in the structure of macrobenthic assemblages and their total biomass on submerged substrata during the six-month field experiment. Although further investigations in other habitats and with different types of biochar are needed to draw generalizations, such findings highlight that biochar-based concrete can be biologically suitable for marine constructions and could contribute to reduce the carbon footprint of marine artificial structures.

1. Introduction

Coastal and marine areas worldwide are disseminated with artificial structures mostly made by concrete (e.g., ports, coastal defenses, energy production or extraction infrastructures) which in some areas, such as the Mediterranean and European seas, cover up to 50–60 % of the coastline (Mammides et al., 2024), and will probably extend in the next future due to human population growth and the ensuing coastal artificialization. Implementing novel solutions to increase the sustainability of marine artificial hard structures remains therefore a critical challenge to manage ocean sprawl and mitigate its potential environmental impact (Paxton et al., 2025).

Approaches to confer an ecological value to man-made marine structures have focused on modifying the macro- and microtopography of substrata to act as habitat enhancers by providing shelter, nursery grounds and suitable space for settlement of marine species (Sella and Perkol-Finkel, 2015; Ben-Natan and Shashar, 2025; Paxton et al., 2025). Complementary strategies also explored the potential of marine artificial structures to help waste recycling, for example substituting traditional aggregates (e.g., natural sand and gravel) with biogenic debris (e.

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g., coral rubbles, oyster shells) or discarded materials from human activities (e.g., construction waste) in concrete mixtures (Dias et al., 2024; Tam et al., 2018; Zhang et al., 2025). The addition of oyster shells to concrete, for instance, has been found to enhance mussel (*Mytilus galloprovincialis*) recruitment (Rupasinghe et al., 2024), whereas including whelk shells in the mixture could support higher biological coverage on substrata with respect to traditional concrete (Dennis et al., 2018). However, incorporating shell debris in concrete may favor only specific taxonomic groups (e.g., ascidians) resulting in completely distinct community structures between traditional and modified concrete mixtures (da Costa Queiroz et al., 2024). Moreover, in some cases the localized and limited availability of these alternative aggregates, or decontamination constraints, prevent a systematic application of these materials, while the eco-friendly shape of artifacts may have only a relatively minor effect in reducing the environmental costs associated to marine constructions.

A major issue related to the ongoing expansion of the built environment, both on land and sea, concerns the carbon footprint of building materials, and especially of concrete production which alone is responsible for ~8 % of global anthropogenic CO_2 emissions (Miller et al., 2018), and of the overall construction sector that accounts for ~34 % of emissions (UNEP, 2025). This has stimulated intense research efforts to develop low carbon concrete mixtures through the optimization of production processes, energy supply from non-fossil sources, the use of alternative supplementary cementitious materials (e.g., fly ashes), alkali-activated binders, and recycled concrete aggregates, among others (Chen et al., 2023).

In this perspective, the addition of residues from biomass treatment to concrete, such as biochar, is particularly promising (Barbhuiya et al., 2024). Biochar is a solid, porous, carbon-rich material typically produced from pyrolysis of biomass (Yaashikaa et al., 2020), a process of thermochemical decomposition of dry organic matter at high temperature in anaerobic conditions. The different production settings (e.g., temperature, heating rate, residence time) and biomass feedstock result into varying physical-chemical properties of biochar, making this material suitable for a wide range of applications, from soil amendments to feed additive to improve livestock health (Windeatt et al., 2014; Chen et al., 2024), for soil remediation, air purification and wastewater treatment (Campion et al., 2023), or even as raw material for energy production alternative to the exploitation of fossil fuels (Mishra et al., 2023). The high recalcitrance and carbon content make biochar a privileged material to enhance the carbon storage and sequestration potential of concrete (Barbhuiya et al., 2024; Osman et al., 2024; Zhang et al., 2022). For example, the addition of relatively small amounts (2–3 wt.%) of biochar to cement mortar could reduce the equivalent carbon associated with cementitious building materials by 2–6 % (Gupta and Kashani, 2021). More comprehensive evaluations of concrete carbon budgets, encompassing the whole process from biochar production to biochar-based concrete manufacturing, estimated a net reduction of global warming potential (GWP) of 576.76 $kgCO_2$-eq per ton of biochar used (He et al., 2022), or even 1351–1505 $kgCO_2$-eq when replacing one ton of cement with biochar in brick production (Osman et al., 2024).

A number of factors, including the type of feedstock, biochar features, and structural limits to biochar incorporation in concrete matrices may affect the magnitude and effectiveness of reductions in CO_2 emissions associated to concrete, though mounting evidence is consolidating the potential of biochar to improve the strength, durability and sustainability of concrete (Kushwah et al., 2024; Mishra et al., 2023; Senadheera et al., 2023; Zhang et al., 2022). Most studies have focused on the properties of biochar-based concrete for terrestrial applications, and recent evidence highlighted the advantages for the mechanical performance and durability of biochar-based concrete in the marine environment (e.g., Xu et al., 2024), thus supporting the extension of its use and the potential environmental benefits to marine and coastal constructions. However, if land uses of biochar-based concrete are of relatively minor concern for terrestrial organisms, marine applications could be more problematic as the introduction of novel substrata could pose several issues for the marine biota (Firth et al., 2016). Indeed, composition and physical properties of the substratum may strongly affect the structure of epibenthic communities colonizing artificial hard structures, possibly leading to changes in adjacent communities on natural rocky habitats, modifying connectivity patterns between habitat patches, introducing pollutants, or favoring the spread of pathogens and invasive species (Bishop et al., 2017; Bulleri and Airoldi, 2005; Lemasson et al., 2024).

Understanding whether biochar-based concretes can be biologically compatible with marine uses is therefore decisive, but still underexplored, for future developments and applications of this material to the construction of coastal and marine infrastructures. In this study, through a multidisciplinary approach involving ecotoxicological bioassays, DNA metabarcoding techniques and underwater field experiments, it was carried out a comprehensive assessment of the putative effects of the addition of biochar to concrete on marine organisms and on colonization processes of artificial hard substrata by micro- and macrobenthic assemblages (Fig. S1 in supplementary material).

2. Materials and methods

2.1. Biochar

Since this is one of the first field studies assessing the biological compatibility of biochar-based concrete in the marine environment, it was decided to focus on biochar with a minor content of high-priority contaminants, in light of the precautionary principle, and derived from pyrolysis of plant biomass, due to its common production and widespread application (Mishra et al., 2023; Yaashikaa et al., 2020). Based on these criteria, a high-quality commercial biochar was selected, with a total fixed carbon content >75 %, obtained through pyrolysis of feedstocks composed by woody biomass from wood processing wastes (wood chips) and woodland cleanings. The overall grain size of the biochar was <5 mm, with ~75 % of particles sizing less than 2 mm. Chemical contamination of the biochar was contained, with very low concentrations of benzo [a]pyrene (1 $\mu g\ kg^{-1}$) and total polycyclic aromatic hydrocarbons ($\Sigma PAHs = 0.5\ mg\ kg^{-1}$), and heavy metals far below the limits imposed by the European Law for the use in agriculture (Table S1 in supplementary material).

2.2. Laboratory bioassays

Prior to the field experiment, the potential of the tested biochar to cause toxic effects on the marine biota was examined implementing a battery of bioassays on target organisms. Bioassays were conducted in a liquid phase, thus requiring a suitable water extract. In the absence of specific guidelines for biochar, two different approaches were followed, both based on regulatory references applied in similar contexts. In the first case, hereinafter referred to as ecotoxicological approach 1 (EA1), biochar was treated as a waste material, considering its granular nature and origin as a by-product. In the second approach (EA2), given the possible application of biochar in the marine environment, specifically within the benthic compartment, biochar was treated as a sediment. Full details on both approaches were reported in Appendix S1 (supplementary material).

2.2.1. EA1

For EA1, the aqueous extracts were prepared following the UNI EN 12457 procedure (suitable for the characterization of granular waste <4 mm) at a liquid/solid ratio of 10 (i.e., 100 $g\ L^{-1}$), using artificial seawater (ASW, 35 PSU) as the leachant.

A dilution sequence, similar to those conventionally performed for effluents (100 %, 50 %, 25 %, 12.5 %, and 6.25 %), was prepared by diluting each previous concentration by half with ASW (US EPA, 1985). These different solutions were then used for the bioassays with *Artemia* *franciscana* (acute test, 24 h, mortality), *Brachionus plicatilis* (acute test, 48 h, mortality), and *Phaeodactylum tricornutum* (chronic test, 72 h, growth inhibition). Briefly, a 24 h LC_{50} bioassay was performed in a 24-well multiplate using instar II-III larvae of *A. franciscana* using the ARTOXKIT M kit purchased from MicroBioTest Inc. (https://www.microbiotests.com). A 48 h LC_{50} bioassay was performed in a multi-well test plate using larvae of the planktonic rotifer *B. plicatilis* (L-strain) using the ROTOXKIT M kit purchased from MicroBioTest Inc., according to ISO 19820:2016. A 72 h algal growth inhibition test was performed in long cell vials with the marine diatom *P. tricornutum* (strain CCAP 1052/1 A) using the ALGALTOXKIT M kit purchased from MicroBioTest Inc., according to UNI EN ISO 10253:2017.

Results obtained from the battery of bioassays were synthetized through the Toxicity test Battery Integrated index (TBI; ISPRA, 2011), suitable to classify the ecotoxicological risk assessment of leachates originating from a wide range of anthropogenic materials (Piccardo et al., 2022), which expresses the overall effects as a percentage with respect to the control (%E).

2.2.2. EA2

For water extracts in EA2, it was applied the ISPRA 16/2021 protocol (ISPRA, 2021) concerning the production of elutriates intended for the application of ecotoxicological test batteries as required by the Italian Law laying down methods and technical criteria for the analysis of dredged marine sediments. Only 100 % of the water extract was tested in a multispecies battery composed of *P. tricornutum* (chronic test, 72 h, growth inhibition), *Aliivibrio fischeri* (acute test, 15 and 30 min, bioluminescence), and *Paracentrotus lividus* (chronic test, 72 h, development). As for EA1, a 72 h algal growth inhibition test was performed with the marine diatom *P. tricornutum* (internal propagation of strain ref. n. 05-01-24_PT_08–09) according to UNI EN ISO 10253:2017. Biological responses on bacteria were checked on the species *A. fischeri* (strain BL11800724) according to UNI EN ISO 11348–3:2019 using freeze-dried bacteria purchased by Microtox® (Ecotox) and a photometer purchased by Ecotox®. Finally, a 72 h embryotoxicity test was set up in 6-well plates using wild mature *P. lividus* specimens following the methodology described in the ISPRA Ecotoxicology Notebooks (ISPRA, 2017). Full details on bioassays are reported in Appendix S1 (supplementary material).

The hazard quotient ($HQ_{Battery}$) combining the results from the different bioassays was calculated using the Sediqualsoft® software, an operational tool to integrate chemical and ecotoxicological features of marine sediments (Regoli et al., 2019), which determines a quantitative index that classify the ecotoxicological hazard level associated with the sample.

2.3. Field experiment

2.3.1. Experimental tiles

The experiment aimed to assess whether the development of marine benthic assemblages on artificial hard substrata made with concrete used in the construction of marine artificial structures (e.g., jetties, breakwaters), could be affected by the addition of biochar to the composite. The null hypothesis is that colonization processes, including microfouling (i.e., bacterial biofilms and micro-eukaryotic assemblages) formation and temporal changes in macrobenthic assemblage structure, would not differ between concrete and biochar-based concrete. To test for this hypothesis, three different types of experimental tiles were investigated in the field: tiles entirely made with (i) conventional concrete for marine uses that served as control substratum, tiles of concrete containing (ii) 5 wt% and (iii) 10 wt% biochar, respectively. These percentages by weight were chosen to represent realistic biochar contents not compromising, or even improving, the mechanical properties of concrete (e.g., compressive strength, water permeability) (Barbhuiya et al., 2024; Kushwah et al., 2024; Senadheera et al., 2023 and references therein).

The experimental tiles were produced in the laboratory using a commercial concrete for marine uses (class of exposure: XS1-3, specific resistance: Rck 45 N mm^{-2}, EN 206 UNI 11104), which was a ready-to-use mixture of siliceous aggregates (0/10 mm), Portland cement, polymer fibers (30 mm) and additives. Biochar-based tiles were obtained by adding the biochar to the concrete mixture through a process of dry mixing, in which biochar was directly incorporated into the dry mix of aggregates and cement, thus ensuring the uniform dispersion of biochar particles throughout the mixture (Barbhuiya et al., 2024). The three different mixtures were prepared separately and moisturized by adding water (0.06 L kg^{-1}) and remixing repeatedly. Finally, the wet mixtures were poured into 15 × 10 × 2 cm plastic molds and left in dim light conditions at ambient temperature (∼20 °C) until complete solidification. Tiles were then removed from the molds and drilled in the center to help fixing. A total of 135 tiles (45 tiles per type) were produced.

2.3.2. Study area and experimental set-up

The experiment was carried out along a stretch of coast (45°43′03″ N, 13°42′00″ E) in the Gulf of Trieste, North Adriatic Sea (Mediterranean Sea), one of the most anthropized areas of the basin where maritime transport, coastal industries, fisheries, and tourism activities densely concentrate (Furlan et al., 2019). The seabed is primarily composed of sand, clay and mud, whereas the coastline is mainly characterized by artificial reefs, due to the high coastal urbanization.

Three small marinas, 1000 s m apart along the shore with comparable exposure to dominant winds and depth range, were randomly selected as experimental sites and hereafter referred to as S1, S2 and S3. At each site, 45 tiles (15 tiles of each substratum) were deployed at approximately 2 m depth in the subtidal zone. To facilitate underwater deployment, tiles were fixed with ties to five semi-rigid plastic grids (each grid hosting nine haphazardly selected tiles, three of each substratum), which were then secured with ropes and screws to the vertical wall of a pier inside the marinas. This allowed placing all grids contiguously on the wall, and ensuring vertical orientation, steadiness and uniform environmental conditions (e.g., light incidence, hydrodynamism) for all tiles during the study. To avoid possible effects of different roughness of tiles on colonization processes, tiles were fixed to grids in order to expose the smoothed side to open water. The experiment started in July 2024 and examined the first six months of the colonization process on experimental substrata, ending in January 2025.

2.3.3. Sampling

Microfouling on tiles was sampled after 7 and 45 days since immersion. For each site and sampling time, three tiles of each substratum were randomly collected across the five panels by SCUBA divers, carefully deployed into plastic bags and brought onboard. Microfouling was then sampled directly onboard by rubbing a standard unit surface (15 cm^2) on each tile with sterile cotton swabs. Swabs were placed into sterile Eppendorf safe-lock tubes kept at +4 °C, dark, and then transported to the laboratory where they were stored at −20 °C until molecular analysis. For sessile macrobenthos, three replicate tiles for each type were collected at each site after 60, 120, and 180 days since immersion. Sampled tiles were stored in tanks with seawater at field temperature and transported to the laboratory for subsequent analysis.

2.4. Sample analysis

2.4.1. Microfouling

Environmental DNA (eDNA) was extracted from swab samples using the E.Z.N.A.® Mag-Bind® Environmental DNA 96 Kit (Omega Bio-tek, Norcross, GA, USA), following the manufacturer's protocol. A negative control, consisting of PCR-grade water, was processed in parallel with all eDNA extractions under the same conditions. To detect target organisms, two primer sets were used: 16 S rRNA (V3-V4 region) [Pro341 F (5′-CCTACGGGNBGCASCAG-3′) and Pro805 R (5′-GACTACNVGGGTATCTAATCC-3′)] (Takahashi et al., 2014), 18 S rRNA (V4 region)

[TAReukFWD1 (5′-CCAGCASCYGCGGTAATTCC-3′) and TAReukREV3 (5′-ACTTTCGTTCTTGATYRA-3′)] (Stoeck et al., 2010). The DNA markers amplification was performed as by Schroeder et al. (2021). Briefly, a two-step polymerase chain reaction (PCR) was performed, amplifying the target regions in the first PCR and bounding the sample specific indexes and illumina p5/p7 tails in the secondary PCR. Details on amplification mixtures and settings of the thermal cycling profiles were reported in Appendix S2 (supplementary material). All reactions included a non-template control (NTC) to verify the absence of contamination, as well as extraction negative controls (NCs). PCR products were visualized on a 2 % agarose gel. PCRs were purified using the Mag-Bind® TotalPure NGS (Omega Bio-tek). Afterwards, the library was purified, quantified, and prepared for high-throughput sequencing by pooling an equimolar amount of amplicon on an Illumina Novaseq6000 2 × 250.

Raw amplicon reads were inspected with FastQC (0.12.1) before and after preprocessing. Sequencing adapters, low-quality bases and universal 16 S and 18 S PCR primers were excised with fastp (Chen et al., 2018). High-quality reads were denoised with DADA2 (1.26.0; Callahan et al., 2016) trained on 10^7 reads, yielding amplicon-sequence-variant (ASV) tables. Taxonomic assignment was performed in QIIME 2 (2024.10.1; Bolyen et al., 2019) with its naïve-Bayes classifier trained on SILVA (138.2; Quast et al., 2012) or PR2 (5.1.0; Guillou et al., 2012) reference databases for 16 S and 18 S data, respectively. The 16 S ASV table (for bacterial biofilm) was analyzed as obtained, whereas the 18 S ASV table was filtered excluding metazoans and macroalgae, thus retaining only micro-eukaryotic taxa.

2.4.2. *Sessile macrobenthic assemblages*

Sessile macrobenthos were analyzed on tiles immediately after sampling. In the laboratory, each tile was photographed in high-resolution while immersed in seawater. Pictures were then analyzed to identify sessile organisms and estimate their abundance as surface cover. To help estimate, a grid of 6 × 4 sub-quadrats was superimposed on images and the relative abundances of taxa were assessed on a scale from 0 (absence) to 4 (fully covered) on each sub-quadrat. Values from sub-quadrats were then summed and the sum expressed as percentage cover on the total surface (Guarnieri et al., 2009). Tiles were also visually inspected under magnification to help the identification of organisms and to integrate the visual census from images. Taxa that were not easily identifiable at species level were grouped into higher taxonomic groups or into morphological groups. Vagile fauna (e.g., gastropods, free-living polychaetes) were not considered in the analysis.

At the end of the experiment (180 days), sessile assemblages on tiles were removed after visual census to determine their total biomass. All sessile organisms on each tile were carefully scratched using scalpels and dried at +50 °C to constant weight (~72 h). Samples were then burnt in a furnace at +550 °C for 4 h, the ashes were weighed, and the total biomass estimated by subtraction and expressed as ash-free dry weight per unit area (mg cm^{-2}).

2.5. *Statistical analysis*

Differences between treatments and controls in ecotoxicological bioassays were tested using the Student *t*-test. Data were first tested for normality with the Shapiro-Wilk test and for homogeneity of variance with the Cochran's *C*-test. The Welch *t*-test or the Mann-Whitney test were used instead of the Student *t*-test in case of heteroscedasticity or non-normal distribution, respectively.

For microfouling molecular data, analysis of variance (ANOVA) was performed to test for differences among substrata in α-diversity quantified as the rarefied number of observed ASVs, after verifying normality with the Shapiro–Wilk test and homogeneity of variance with Cochran's *C*-test. The Student-Newman-Keuls (SNK) test was employed for post-hoc multiple comparisons for terms involving the fixed factors, when appropriate (Underwood, 1997). The design for the analysis consisted of three factors: Time [Ti, two levels (7 and 45 days since immersion), fixed], Site [Si, three levels (S1, S2 and S3), random and orthogonal], Substratum [Su, three levels (concrete only, concrete +5 wt% and +10 wt% biochar, hereafter referred to as C, B and BB, respectively), fixed and orthogonal], with $n = 3$ per each combination of factors. A distance-based permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001) was used to test the hypothesis of no effect of substratum on microfouling assemblages colonizing the tiles. The analysis was based on the full design, as for ANOVA, Bray–Curtis dissimilarities on the cumulative sum scaled (CSS) normalized ASV counts, with 5000 permutations. Post-hoc pair-wise comparisons for terms involving fixed factors were tested using the PERMANOVA *t*-test and 999 permutations, when appropriate. Multivariate patterns were visualized by Principal Coordinates Analysis (PCoA) based on the Bray–Curtis dissimilarity matrix. Multi-rank bar plots were used to show the composition of microfouling assemblage among sites, times and substrata.

For sessile macrobenthic assemblages, ANOVA was performed to test for differences among substrata in α-diversity expressed as taxon richness. In this case, the design for the analysis was the same as for microfouling assemblages, but factor Time included three levels (60, 120 and 180 days since immersion). ANOVA was also carried out to test for differences in total biomass among substrata in the last time of sampling. In this case, the design for the analysis involved only factors Site and Substratum. PERMANOVA was done to test the hypothesis of no effect of the substratum on sessile macrobenthic assemblages colonizing the tiles. The analysis was based on the full design as for ANOVA, Bray–Curtis dissimilarities on untransformed data, with 5000 permutations. Temporal patterns of colonization among substrata in the three experimental sites were depicted by a canonical analysis of principal coordinates (CAP; Anderson and Willis, 2003) for factor Ti × Si based on the distance matrix of Ti × Si × Su centroids. The individual taxa mostly responsible for group differences were investigated by calculating product–moment correlations of original variables (taxa) with the two canonical axes (r_1 and r_2). Taxa were considered only if $\sqrt{{r_1}^2 + {r_2}^2} \geq 0.35$.

All analyses were performed using the software PRIMER v7 (Clarke and Gorley, 2015), including the add-on package PERMANOVA+ (Anderson, 2014), and R 4.4.2 (R Development Core Team, 2024) using the packages 'GAD' (Sandrini-Neto and Camargo, 2023), 'vegan' (Dixon, 2003) and 'microeco' (Liu et al., 2021).

3. Results

3.1. *Ecotoxicological effects*

The ecotoxicological responses obtained following EA1 and EA2 are summarized in Fig. 1, reporting the maximum observed effect (%) with respect to the control for each bioassay. For EA1, tests on *B. plicatilis* and *A. franciscana* did not detect statistically different responses with respect to controls (Figure S2 in supplementary material), with consistent low mortality levels (≤13.30 % and ≤4.20 %, respectively). Tests on *P. tricornutum* did not reveal growth inhibition, but rather a slight stimulatory effect that, although statistically significant ($p < 0.05$) for the 25 %, 50 % and 100 % water extract (Figure S2), was always ≤4.10 % thus remaining far below the threshold generally considered as biologically relevant (i.e., 20 %; Persoone et al., 2003). Data integration showed a TBI<5 % (−1.36 %) indicating absent toxicity.

Similar results, with all effects <20 %, were also observed following EA2. Specifically, *A. fischeri* showed a bioluminescence stimulation of 14.50 % and 0.60 % (at 30 and 15 min, respectively), the test on *P. lividus* revealed an effect of 12.33 % and finally, a minor biostimulation (0.16 %) was recorded in the *P. tricornutum* test (Fig. 1). Data integration led to an $HQ_{\text{battery}} = 0$, underlining no ecotoxicological hazard.

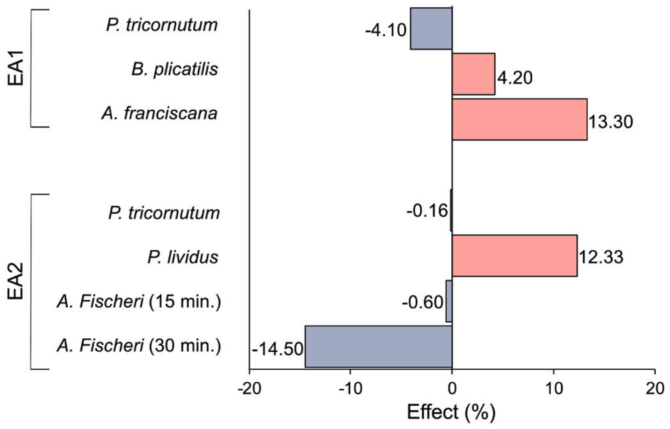

Fig. 1. Maximum ecotoxicological effect (%) of biochar based on the different bioassays from the two approaches used in the study (EA1 and EA2). Negative and positive values indicate, respectively, stimulation or inhibition/mortality compared to control.

3.2. Microfouling assemblages

There was no effect of biochar addition to concrete on α-diversity of the two investigated components of microfouling, but only a significant Ti × Si interaction (Table 1), indicating different temporal changes at varying sites for both bacterial biofilm and micro-eukaryotes. In S2 and S3, post-hoc SNK tests detected a significant ($p < 0.05$) increase through time of the number of ASVs for bacterial biofilm, while micro-eukaryotes diversity remained comparable between 7 and 45 days (Fig. 2a and b). Instead, in S1, no temporal changes were detected for biofilm but micro-eukaryotes diversity decreased in time (Fig. 2a and b).

No significant differences among substrata were found in the multivariate structure of bacterial biofilm (Table 2). Again, a significant Ti × Si interaction was detected, indicating different temporal patterns of biofilm formation among sites. Specifically, different biofilm structures characterized the three sites at 7 days (Fig. 3a) due to among-sites variations in most abundant prokaryote taxa (i.e., Proteobacteria, Bacteroidota, Actinobacteriota, Verrucomicrobiota and Cyanobacteria, Fig. S3 in supplementary material), which then converged at 45 days between S1 and S3 but not S2 (Fig. 3a).

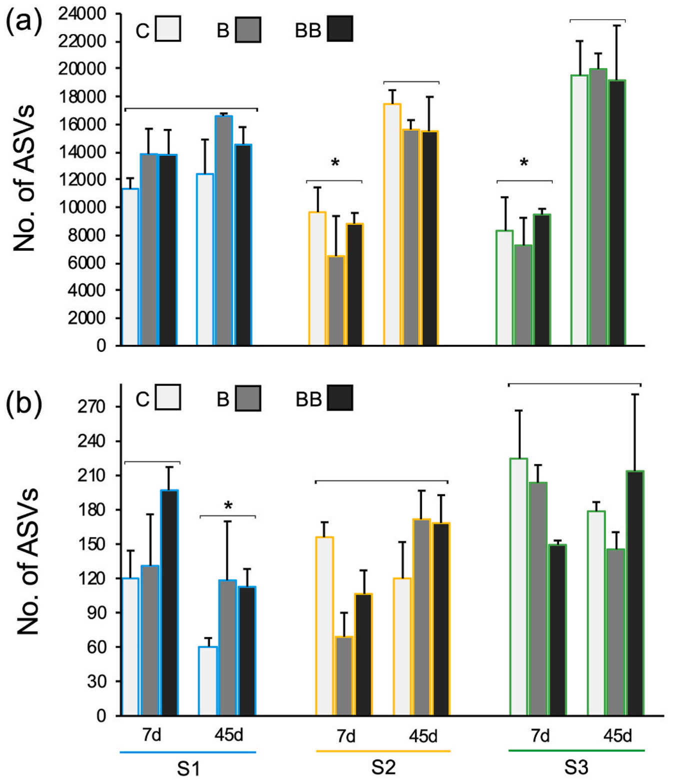

Fig. 2. Mean ASVs (±SE, n=3) of (a) bacterial biofilm and (b) micro-eukaryotes on the different substrata (C, B, BB) at the three sites (S1, S2, S3) in each time of sampling (7 and 45 days).

For micro-eukaryote assemblages, PERMANOVA detected a significant Ti × Si × Su interaction (Table 2), highlighting different temporal changes at varying sites and substrata. Analyses carried out separately for each site detected significant differences in assemblages between 7 and 45 days with no effect of substratum for S1 and S3. At S2, instead, conventional concrete (C) differed from BB at 7 days; however, no effect of substratum was detected at 45 days for this site. These patterns were clearly depicted in the PCoA ordination showing a clear segregation among sites in both times, though S1 and S2 placed closer at 45 days, and a separation of BB from C centroids at 7 days in S2 (Fig. 3b). Accordingly, the overall assemblage composition was quite different among sites in both times of sampling as far as the main taxa, even though generally consistent among substrata (Fig. S4 in supplementary material). Assemblages on control tiles from S1 also showed a quite different average composition at 45 day, with a high proliferation of Thraustochytriaceae with respect to B and BB tiles (Figure S4), although no statistically significant difference was detected among substrata in both times for S1.

Table 1

Summary of ANOVAs testing for differences among substrata in α-diversity of microfouling components (bacterial biofilm and micro-eukaryotes) and macrobenthic assemblages, quantified respectively as number of amplicon-sequence-variants (ASVs) and number of taxa. For macrobenthos, results of ANOVA on total biomass at 180 days was also reported.

Source of variation[a]	No. of ASVs (bacterial biofilm)			No. of ASVs (micro-eukaryotes)			No. of taxa (macrobenthic assemblages)			Biomass macrobenthos (180 days)		
	d.f.	MS	*F*	d.f.	MS	*F*	d.f.	MS	*F*	d.f.	MS	*F*
Time = Ti	1	637,797,220.0		1	778.0		2	132.2				
Site = Si	2	15,707,327.0		2	20,692.0		2	168.4		2	16.9	139.534***
Substratum = Su	2	891,808.0	0.079^{NS}	2	1658.0	0.653^{NS}	2	2.5	1.056^{NS}	2	0.3	1.056^{NS}
Ti × Si	2	109,493,031.0	9.699***	2	10,264.0	4.041*	4	10.5	5.572***			
Ti × Su	2	7,154,304.0	0.634^{NS}	2	5313.0	2.092^{NS}	4	1.3	0.347^{NS}			
Si × Su	4	13,528,685.0	1.198^{NS}	4	3180.0	1.252^{NS}	4	2.3	1.240^{NS}	4	0.1	1.240^{NS}
Ti × Si × Su	4	159,189.0	0.014^{NS}	4	5565.0	2.191^{NS}	8	3.9	2.069^{NS}			
Residual	36	11,289,426.0		36	2540.0		54	1.9		18	0.1	
Shapiro-Wilk test		$W = 0.977^{NS}$			$W = 0.967^{NS}$		$W = 0.982^{NS}$				$W = 0.976^{NS}$	
Cochran's C-test		$C = 0.231^{NS}$			$C = 0.295^{NS}$		$C = 0.433^{NS}$				$C = 0.204^{NS}$	

^{NS} not significant at $\alpha = 0.05$; *$p < 0.05$; ***$p < 0.001$.

[a] Terms already involved in significant higher-order interactions were not tested.

Table 2

Summary of PERMANOVAs testing for differences among substrata in assemblage structure of microfouling components (bacterial biofilm and micro-eukaryotes) and macrobenthic assemblage structure.

Source of variation[a]	Bacterial biofilm			Micro-eukaryotes			Macrobenthic assemblages		
	d.f.	MS	Pseudo-*F*	d.f.	MS	Pseudo-*F*	d.f.	MS	Pseudo-*F*
Time = Ti	1	25,827.0		1	25,377.0		2	14,996.0	
Site = Si	2	14,651.0		2	16,587.0		2	9196.4	
Substratum = Su	2	791.3	0.948^{NS}	2	2564.5		2	370.9	0.667^{NS}
Ti × Si	2	7840.0	8.613***	2	12,173.0		4	2968.0	9.141***
Ti × Su	2	786.2	1.123^{NS}	2	2740.1		4	393.8	1.254^{NS}
Si × Su	4	834.0	0.917^{NS}	4	2813.9		4	428.0	1.352^{NS}
Ti × Si × Su	4	700.4	0.769^{NS}	4	2924.5	1.225*	8	314.0	0.992^{NS}
Residual	36	910.2		36	2386.3		54	316.6	

^{NS} not significant at $\alpha = 0.05$; *p(perm) < 0.05; ***p(perm) < 0.001.

[a] Terms already involved in significant higher-order interactions were not tested.

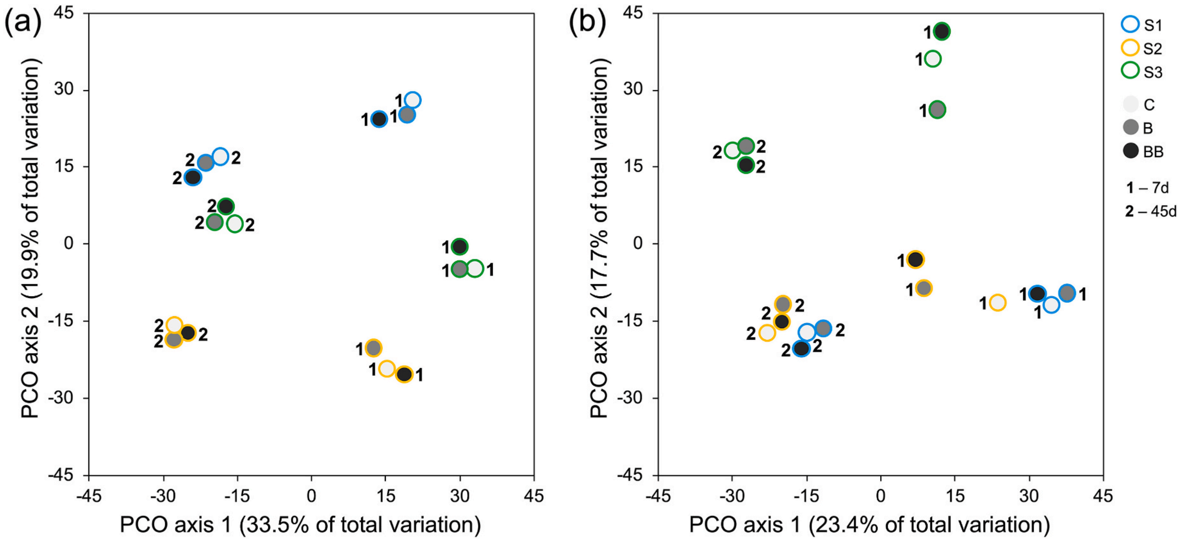

Fig. 3. PCoA plots of (a) bacterial biofilm and (b) micro-eukaryotes at each site (S1, S2, S3), time (7 and 45 days) and substrata (C, B, BB).

3.3. *Patterns of colonization of sessile macrobenthos*

A total of 39 sessile macrobenthic taxa were identified from photographic samples during the experiment and grouped into 28 taxa or morphological groups for the analysis (Table S2 in supplementary material). ANOVA on the mean number of taxa did not detect significant differences among substrata, consistently for all sites and times of sampling (Table 1, Fig. 4a), indicating no significant effects of biochar addition on taxon richness. The analysis detected a significant Ti × Si interaction (Table 1), revealing that changes in taxon richness through time were not consistent among sites. Specifically, for S1 and S3, SNK tests highlighted that the mean number of taxa at 120 and 180 days was comparable and significantly higher ($p < 0.001$) than at 60 days, while for S2 the number of taxa significantly ($p < 0.001$) increased at each time of sampling (Fig. 4a). The total biomass of assemblages at the end of the experiment significantly varied among sites, although no significant differences were found among substrata (Table 1, Fig. 4b).

PERMANOVA did not detect significant differences among substrata in the structure of macrobenthic assemblages, irrespective of site and colonization stage (Table 2). The overall temporal patterns of colonization significantly varied among sites, as indicated by the significant Ti × Si interaction term in the analysis (Table 2). For each site, pairwise tests among times of sampling detected significant ($p < 0.001$) changes in assemblage structure between 60, 120 and 180 days since immersion, with site-specific temporal trajectories and magnitude of changes. This was clearly depicted in the CAP plot (96 % allocation success with $m = 6$ PCO, 99 % explained variance) showing a separation among assemblages of all times for S1 and S2 but not for S3, where the major changes occurred after 120 days (Fig. 5a). Moreover, while S2 clearly differed from the other sites through time, assemblage structure at S1 and S3 appeared to converge in the late stages of colonization. Several taxa were highly correlated with the canonical axes, thus mostly contributing to the observed patterns (Fig. 5b). At all sites, after 60 days (and for S3 also until 120 days) assemblages were dominated by cyanobacteria mats, encrusting bryozoans, and *Spirorbis* spp., though other taxa such as *Bugulina* sp. were also abundant at S2. At a later colonization stage (180 days), several invertebrates (e.g., barnacles, *Bugula neritina*, *Botryllus schlosseri*, *Styela plicata*) and encrusting macroalgae (e.g., ECR, *Peyssonnelia rubra*) characterized S1 and S3, while a larger erect macroalgal component (*Jania rubens*, *Dictyota* spp., *Colpomenia* sp.) and different invertebrate taxa (e.g., *Spirobranchus triqueter* and other serpulids) discriminated S2.

4. Discussion

This study provided the first, extensive assessment of the potential effects of biochar addition to concrete on micro- and macrobenthic marine organisms colonizing submerged artificial hard substrata. Adding up to 10 %wt. biochar to concrete did not affect the structure of prokaryote, micro-eukaryote and macrobenthic assemblages on the substratum, supporting the hypothesis that biochar-based concrete is as biologically suitable as conventional concrete for applications in the marine environment.

Changes in bacterial biofilms and their overall composition were

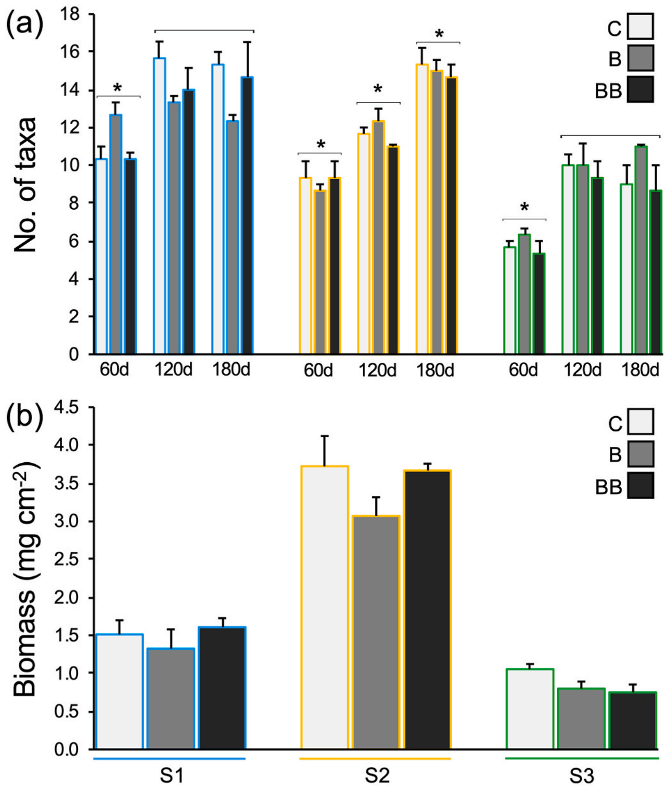

Fig. 4. Mean number of taxa (±SE, *n*=3) of (a) macrobenthic assemblages on the different substrata (C, B, BB) at the three sites (S1, S2, S3) in each time of sampling (60, 120, 180 days) and (b) mean biomass (±SE) at 180 days for each site and substratum.

indistinguishable among the three concrete mixtures and representative of biofilm on artificial hard substrata of different types, showing the typical trend of higher abundance of pioneer and tolerant colonizers such as Rhodobacteraceae and Alteromonadaceae in the early stages of biofilm formation, followed by an increase in other Proteobacteria, Bacteroidota, Cyanobacteria and Actinobacteriota (Quian et al., 2022; Yu et al., 2025). As found in other studies investigating marine microfouling on concrete (e.g., Margapuram et al., 2024), micro-eukaryote assemblages on the three substrata were dominated by dinoflagellates at the early stage of colonization, whereas diatoms and other taxa increased in abundance later (after 45 days since immersion). Again, the effect of biochar addition was substantially neutral, except at S2, where assemblages on BB (10 %wt. biochar) at 7 days were distinct from those on C (control). This was mostly due to a higher relative abundance of diatoms and a lower presence of Chlorophyta and Prasinodermophyta on BB tiles; however, such differences disappeared at the second time of sampling, indicating only a transient putative effect of biochar addition.

The structure of sessile macrobenthic assemblages developing on experimental tiles was characterized at the first sampling time (60 days after immersion) by the dominance of cyanobacteria mats, *Spirorbis* spp., encrusting bryozoans and ascidians, while macroalgae, bivalves, barnacles and solitary ascidians increased in abundance as the colonization progressed, in analogy with macrofouling development typical of the study area (Fortič et al., 2021). Patterns of colonization of sessile assemblages, their diversity, total biomass and the abundance of alien (e.g., *Balanus trigonus*) or potentially invasive (e.g., *Botryllus schlosseri*) species did not differ among substrata, showing that biochar-based concrete neither interfered with the development of native macrobenthic communities nor seemed to facilitate the colonization of non-native species with respect to conventional concrete. A recurring pattern, observed in both micro- and macrofouling assemblages, was the significant spatial variability among sites during colonization. Such spatial heterogeneity in colonization processes is quite common since local-scale environmental factors, the structure of neighboring benthic communities, and spatial variations in propagule availability reflect on the new assemblages determining site-specific successional trajectories (Fraschetti et al., 2002; Guarnieri et al., 2009). This further reinforce the generality of results, underlining the neutral effect of biochar beyond the natural variability among sites.

Biochar addition to concrete could alter the physical-chemical properties of substrata, such as microtopography, roughness, composition and color, among others, which play a pivotal role for settlement and recruitment of marine organisms and ultimately shape fouling assemblages (Bone et al., 2022; Faimali and Terlizzi, 2010; Quian et al., 2022). For example, increased substratum porosity could negatively affect settlement of some macrobenthic species (e.g., barnacles; Berntsson et al., 2000) while increasing the biomass of the bacterial biofilm (O'Reilly and Willerth, 2024). Also, hazardous compounds present in biochar may inhibit less tolerant organisms and increased organic carbon and residual nutrients in the substratum can modify bacterial biofilm (Guo et al., 2021), potentially affecting micro-eukaryote and macrobenthic assemblages (Salta et al., 2013). In

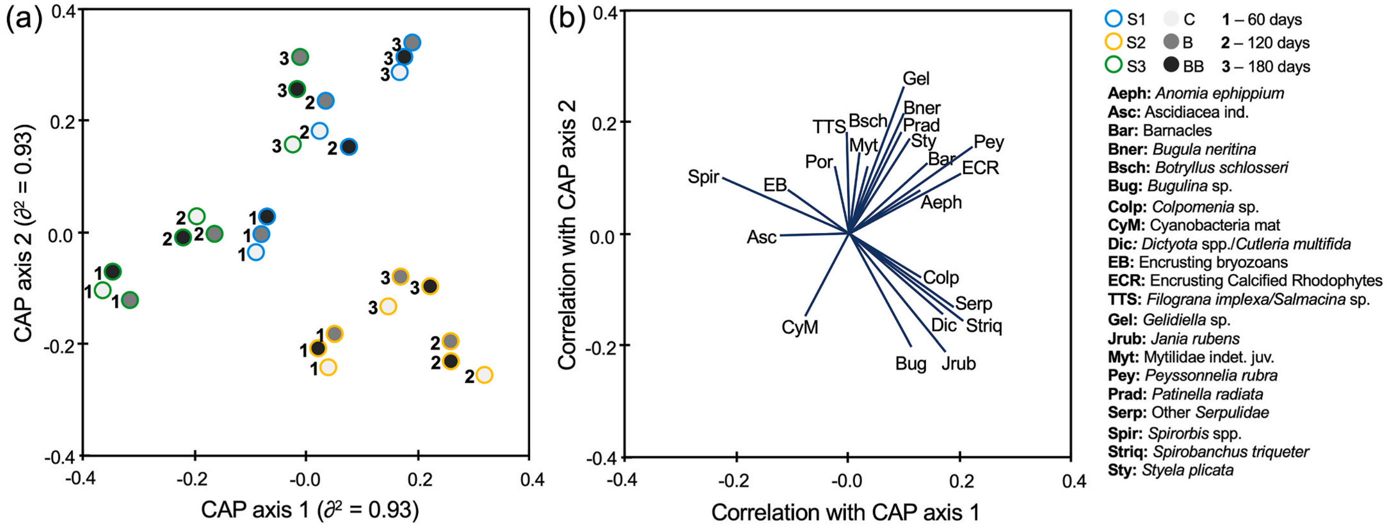

Fig. 5. (A) CAP plot based on the similarity matrix of macrobenthic assemblages at each site (S1, S2, S3), time (60, 120, 180 days) and substrata (C, B, BB) and (b) vectors of correlation for individual taxa highly correlated with canonical axes in the CAP plot.

our case, the relatively low level of contamination in the biochar likely had irrelevant toxic effects on settling organisms, as confirmed by bioassays on test species, and the incorporation of biochar particles into the cementitious matrix could have further reduced the bioavailability of contaminants, as well as the leakage of the labile carbon fraction and nutrients, despite weak signals of growth stimulation for bacteria and microalgae emerged from bioassays. Our findings support the potential application of biochar-based concrete as biocompatible building material for marine artificial structures. Analogous results were obtained in previous studies assessing the effects of biochar-based concrete on single components of benthic communities (Bracho-Villavicencio et al., 2024; Teong et al., 2024). However, further research is needed to assess the long-term responses of benthic communities to the introduction of biochar-based materials across a wider range of marine habitats (Bone et al., 2022).

Even though the mechanical performances of biochar-based concrete under marine conditions lay outside the aims of this study, it is nevertheless crucial for its applications in the marine environment, requiring additional investigations especially considering key durability indicators relevant to the seawater exposure and to the effects of biological colonization on the erosion and structural integrity of artificial structures (Mohammadi Ghahsareh et al., 2025; Perdanawati et al., 2025). Several studies demonstrated that biochar, when used as a component of cement mixtures (1–7 wt.%), enhances the mechanical strength, durability, and water resistance of concrete (Barbhuiya et al., 2024; Kiran et al., 2025; Thapsamut et al., 2025; Yang and Wang, 2021). A recent review of the scientific literature on the topic identified the optimal biochar dosage as a binder replacement to range between 2 and 5 wt% (with a minimum biochar content for conventional mixtures between 0.5 and 1 wt.% and a maximum biochar content equal to 10 wt%), while for fine aggregates the recommended replacement is 10–15 wt% (Usha Rani et al., 2025). Such percentages by weight are aligned with those tested in the present study, which considered two representative dosages (i.e., 5 % and 10 % by weight) compatible with the preservation, or enhancement, of the structural properties of concrete. For example, biochar-based concrete showed higher compressive strength with respect to standard concrete, up to 32.9 % higher in a 7-day test, when up to 6.5 wt% cement was replaced by biochar (Qin et al., 2021). Dosages of biochar around 2 wt% have been found to increase the compressive strength, splitting tensile strength, and flexural load at fracture by 18.95 %, 19.64 %, and 12 % respectively (Patel et al., 2025). This can be a result of improved pore structure and hydration behavior, since biochar addition may act as a filler making the concrete more compact (Qin et al., 2021) and/or increasing calcium-silicate-hydrate hydration products (Qing et al., 2023; Patel et al., 2025). Sirico et al. (2022) found that the compressive strength of biochar-based concrete with 2 wt% replacement of cement with biochar increased by 7 % and 9 % after 1 year and 2 years, respectively, suggesting that biochar addition may enhance the structural properties of concrete even in the long term. Biochar addition (5–10 wt%) could also improve the split tensile strength of concrete by reducing porosity and improving the bonding within the material (Kiran et al., 2025), whereas lower dosages, usually <1 wt%, may enhance concrete flexural strength (Maljaee et al., 2021). These structural properties of biochar-based concrete can be further improved using accelerated carbonation curing, which facilitates the carbonation of biochar resulting in the formation of calcium carbonate, thus increasing compressive and flexural strength, along with durability (Yang and Wang, 2021). As far as specific structural properties of concrete involved in the resistance of the material to marine conditions, evidence strongly supported the suitability of biochar-based concrete for marine uses. Water permeability of biochar-based concrete samples was improved with dosages of biochar ranging 0.1–2 wt% (Wu et al., 2025), whereas concrete with higher dosages (0.65–13.5 wt%) showed similar permeability to biochar-free ones (Qin et al., 2021). Research on the resistance to chloride and sulphate attacks, which could mine the integrity of concrete structures in the marine environment, highlighted that incorporating supplementary cementitious materials such as biochar, could improve the chloride resistance of concrete in seawater (Gupta et al., 2021; Qu et al., 2024; Xu et al., 2024). Also, biochar exposed to simulated marine conditions exhibited an increase in Young's modulus and hardness by ∼40 % compared to virgin biochar, with a long-term rebound ratio reached of 96.6–99.2 % (Xu et al., 2024), or promoting the formation of more nucleation sites for self-healing processes (Lin et al., 2025), implying the potential advantage of biochar-cement composites for long-term durability in the marine environment (Xu et al., 2024).

Undoubtedly, the suitability of biochar-based concrete for applications to marine and coastal man-made structures still need further research efforts to shed light on potential issues related to the exposure to harsh conditions like those characterizing the marine environment. Different dosages and cement mixtures should be evaluated on a case-by-case basis, and especially through long-term field trials, to ensure an effective implementation of marine artificial structures made with biochar-based concrete. Future research would also benefit from testing different types of biochar in order to maximize the exploitation of different waste feedstocks. Critical environmental concerns may arise when using biochar from sludge or manure waste (Schlederer et al., 2024), due to higher contaminant levels if compared to high-quality biochar like the one tested in this study, possibly limiting the array of applicable residues. A practicable solution to this issue could involve context-specific biochar selections depending on the final utilization and deployment of building materials: while 'cleaner' biochar could be added to concrete for the construction of habitat enhancers or trawling defenses in marine reserves or fishing closures, moorings for aquaculture facilities, or for marine restoration purposes (e.g., Bracho-Villavicencio et al., 2024), biochar with non-negligible contaminant levels could find a better application in contexts where ambient concentrations of pollutants allow less stringent standards for building materials (e.g., industrial infrastructures, commercial harbors). Finally, exploring the carbon storage and sequestration performance of biochar-based concrete in the marine environment can significantly increase the benefits of this approach in reducing CO_2 emissions from the construction industry. Considering an average embodied carbon of $\sim$362 kgCO_2 m^{-3} across high-grade concrete (>C40; Dobrucali, 2024; Fantilli et al., 2019), and an average weight of 2300 kg m^{-3} of concrete, the addition of 1 %wt. biochar (>75 % carbon content as in our case) will lead to a potential storage of 63.3 kgCO_2-eq m^{-3}, which corresponds to $\sim$17 % m^{-3} decrease in embodied carbon, taking into account an average net negative GWP of 2.5 kgCO^2-eq per kg of biochar (Jeswani et al., 2022). As for terrestrial constructions (Gupta and Kashani, 2021; Osman et al., 2024; Senadheera et al., 2023; Zhang et al., 2022), the application of biochar-based concrete may exploit marine man-made structures as carbon sinks in the absence of relevant consequences for the marine biota, representing a more realistic and less hazardous alternative for long-term storage of carbon in the ocean than, for instance, seaweed or other organic matter dumping in the deep-sea (Chopin et al., 2024).

5. Conclusion

The global physical footprint of marine artificial structures has increased in the last decades achieving 32,000 km^2 in 2018 and is projected to raise up to 39,400 km^2 by 2028 (Bugnot et al., 2021). Under this scenario, implementing new technologies and alternative production processes combining waste recycling and carbon storage potential, and leveraging artificial substrata to mimic or enhance natural habitats could bring built-in ecological benefits essential for a sustainable coastal and offshore infrastructure development (Chen et al., 2023; Sella and Perkol-Finkel, 2015; Paxton et al., 2025). In the present study, laboratory and field experiments demonstrated for the first time that the addition of biochar in concrete mixtures, with percentages by weight compatible with the maintenance of concrete structural properties, may have no significant biological effects on marine organisms and benthic assemblages. Such findings open the way for future research on the application of biochar-based concrete for marine uses in order to exploit artificial structures as a potential sink of CO_2, in the absence of relevant ecological consequences for marine ecosystems. In this view, the use of biochar-based concrete may integrate the existing strategies by improving waste valorization across land and sea and representing a biocompatible approach to reduce the carbon footprint of marine artificial structures.

CRediT authorship contribution statement

Manuela Piccardo: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Monia Renzi:** Writing – review & editing, Resources, Methodology, Investigation. **Alberto Pallavicini:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Claudio Gennaro Ametrano:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Camilla Spoto:** Writing – review & editing, Methodology, Data curation. **Marta Faggion:** Writing – review & editing, Investigation. **Serena Anselmi:** Methodology, Investigation. **Annalisa Falace:** Writing – review & editing, Validation. **Saul Ciriaco:** Writing – review & editing, Investigation. **Antonio Terlizzi:** Writing – review & editing, Resources. **Stanislao Bevilacqua:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of generative AI in scientific writing

No generative AI was used for preparation of text, graphics or any other part of the manuscript.

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Declaration of competing interest

There is no conflict of interest between the authors - pls proceed.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2025.127518.

Data availability

All data will be made publicly available at https://bit.ly/4jkmLul

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