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Effects of the native-invasive-alien substitution of ecosystem engineers on sediment reworking and nutrient cycling

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Introduction: Ria de Aveiro, a coastal lagoon on the Atlantic coast of Portugal, was invaded by *Arenicola* spp. 15 years ago, with the new species successfully spreading throughout the system and replacing the native *Diopatra* species. With opposite bioturbation traits (*Diopatra* as sediment stabilizers *vs Arenicola* as sediment reworkers), the impacts of this replacement can spread across the entire ecosystem.

Methods: In a 21 days microcosm study, we evaluated the effects of the incremental substitution of *Diopatra* by *Arenicola* species on relevant proxies of ecosystem functioning, such as sediment reworking depths and nutrient dynamics.

Results: The results show a strong directional influence on most of the analyzed parameters as a response to higher densities of *Arenicola*. Specifically, *Arenicola*-dominated communities were characterized by deeper reworking depths and higher concentrations of ammonium and phosphate in the water column.

Discussion: These results are discussed in the context of the available knowledge on the accompanying biological communities, which are typically fostered by these distinct functional groups. Therefore, there is strong evidence that the introduction of a novel species' trait will have major consequences across several levels of the invaded system.

KEYWORDS

species replacement, biological invasions, ecological processes, microcosm, bioturbation, Ria de Aveiro

Introduction

Coastal lagoons are complex and dynamic socio-ecological systems, supporting highly productive biological communities (Barbier et al., 2011; Daborn and Redden, 2017; Rodrigues-Filho et al., 2023). Several ecosystem services provided by coastal systems are related to sediment reworking or bioturbation (the mixing of sediments by living organisms) (Volkenborn et al., 2007; Mermillod-Blondin, 2011; Kristensen et al., 2012).

In fact, bioturbation - including bio-mixing (sediment particle mixing) and bioirrigation (water transport induced by fauna) – is an ecological processes that affect other processes such as decomposition and organic matter remineralization, nutrient balance, or sediment and pollutant resuspension (Mortimer et al., 1999; Michaud et al., 2010; Kristensen et al., 2012; Aller and Cochran, 2019; Bugnot et al., 2022). Also, bioirrigation, by transporting solutes from, to, and within the sediment matrix, alters several biogeochemical parameters and disrupts diffusional gradients within sediments (Kristensen et al., 2012). The rates of these processes can be affected directly, either by the mechanical remobilization of sediment or by effects on the physicochemical environment and on micro-, meio- and macrofaunal endobenthic communities. However, the impact of bioturbation depends on the species and on the context where they occur (i.e., influenced by biotic and abiotic variables), and, therefore, is fundamental to quantify the amount of sediment reworking mediated by species with different traits. In aquatic systems, the way that species interact with the sediment could be very diverse: while some species are sediment stabilizers, acting as ecosystem engineers that create semi-permanent structures that benefit other species (Berke, 2022; Bugnot et al., 2022), other species can have high rates of sediment remobilization, disturbing and raising unconsolidated sediments (Volkenborn et al., 2007; Kauppi et al., 2018; Lacoste et al., 2018).

Due to human use, coastal areas are under several stressors (Dolbeth et al., 2016; Rodrigues-Filho et al., 2023). Among these, the invasion by Invasive Alien Species (IAS) threatens the ecological functions of these ecosystems, compromising their provision of ecosystem services and incurring in the risk of being irreversibly compromised (Queirós et al., 2011; Gallardo et al., 2016; Roy et al., 2024). The introduction of IAS in an ecosystem might result in changes in the overall functioning if, for instance, the novel species also introduces a new trait to the system. This is the case of Ria de Aveiro, a shallow coastal lagoon located on the western Atlantic coast of Portugal (Sousa et al., 2016; Bueno-Pardo et al., 2018; Costa et al., 2022), and the replacement of annelid polychaetes from the genus *Diopatra* spp. by non-indigenous *Arenicola* spp. The events related with this replacement and the recent status of the species are included in Figure 1. *Arenicola marina* and *A. defodiens* (lugworms) were likely introduced via oyster seeds from France for oyster cultures at Mira Channel (Pires et al., 2015). Although no long-term quantitative data are available, information gathered through structured questionnaires with key stakeholders - particularly those who rely directly on the lagoon for their livelihoods - indicates that *Diopatra neapolitana* is perceived as having declined in the past and is expected to continue declining. In contrast, the IAS *Arenicola* spp. is perceived to be expanding its distribution in the future (Luís et al., 2025). Even though no additional quantitative data is currently available, *in situ* observation in the last five years confirm the increase of the *Arenicola* spp. density and distribution in other areas of the lagoon (authors' personal observation).

The species from the genus *Diopatra* (hereinafter referred to as *Diopatra*) are considered omnivorous grazers (Jumars et al., 2015; Berke, 2022), and inhabit intertidal mud sediments or mixed mud-sand areas, living inside buried and emergent tubes made of a secreted layer and externally covered by sand particles and other fragments, like shells and algae (Fauvel, 1923; Dağli et al., 2005; Rodrigues et al., 2009). *Arenicola* species (hereinafter referred to as *Arenicola*) are deposit feeders, inhabiting U- or J-shaped burrows in subtidal and intertidal habitats (Reise, 1985; Andresen and Kristensen, 2002; Volkenborn and Reise, 2006). They ingest surface sediment through a feeding funnel and expel it in the form of fecal casts (Reise, 1985; Zebe and Schiedek, 1996; Wendelboe et al., 2013), with pervasive impacts on sediment stabilization and infaunal communities (Volkenborn and Reise, 2006; Wendelboe et al., 2013). *Diopatra* and *Arenicola* co-exist in many coastal areas worldwide (Berke et al., 2010). Both taxa are commonly used as bait (Cunha et al., 2005; Dağli et al., 2005; Escobar-Ortega et al., 2024), and can reach high densities in many habitats: *e.g. Diopatra*, 144 ind.m^{-2} in Ria de Vigo, Spain (Escobar-Ortega et al., 2024), or 198 ind.m^{-2}, in Izmir Bay, Turkey (Dağli et al., 2005); *Arenicola*, 66 ind.m^{-2} in Oosterschelde, The Netherlands (Montserrat et al., 2011), or 70 ind.m^{-2} in Odense Fjord, Denmark (Delefosse et al., 2012). In Ria de Aveiro, *Diopatra* specimens are mainly found in the Mira channel, with densities reaching 48 ind.m^{-2} in 2020 (unpublished data - BioPradaRia project), while *Arenicola* specimens are widely spread all over the lagoon with higher abundances also found in the Mira channel (as high as 170 ind.m^{-2} in 2020) (unpublished data - BioPradaRia project). Amongst the most expensive marine bait sold on the global

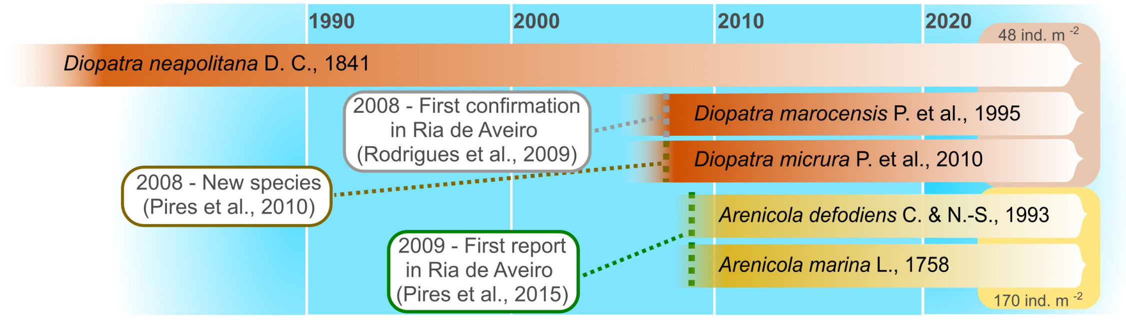

FIGURE 1
Timeline for the status of the species of the genus *Diopatra* and *Arenicola* in Ria de Aveiro, Portugal. Density values from BioPradaRia project (unpublished data).

fisheries market (Watson et al., 2017), both taxa are highly sought after by bait diggers.

Introduced species can often constitute new functional components in the recipient community and produce shifts in the structure and functioning of ecosystems (Thomsen et al., 2014; Gallardo et al., 2016). Contrary to the sediment-stabilizer *Diopatra* (Berke, 2022), *Arenicola* species are important ecosystem engineers with high sediment reworking capacity (Volkenborn and Reise, 2006; Montserrat et al., 2011). In fact, Berke et al (2010) highlighted the opposite engineering abilities of both taxa, with different effects on the ecosystem functioning.

The replacement of a sediment stabilizer by a highly active bioturbator will increase sediment reworking and bioirrigation. This will be reflected on nutrients and gas fluxes across the sediment-water interface (Kauppi et al., 2018), sediment properties (Volkenborn et al., 2007), organic matter remobilization and mineralization (Michaud et al., 2010), displacement and/or bioaccumulation of chemical and organic contaminants and metals (Remaili et al., 2017), and benthic fauna community structure and diversity (Whitton et al., 2016). Benthic fauna behavior (Moyo et al., 2017), microbial community (Lei et al., 2010; Lacoste et al., 2018) and vegetation (*e.g.*, seagrass) fragmentation and establishment (Philippart, 1994; Blackburn and Orth, 2013; Costa et al., 2022) will also be affected by this species replacement. Therefore, it is important to understand how the replacement of a native species, with strong ecosystem engineering capabilities, by an IAS, with opposite traits, affects a) sediment reworking and b) the flux of nutrients across the water-sediment interface. Microcosms are highly useful to assess ecosystem processes under controlled conditions (Benton et al., 2007): by manipulating the ratios of *Diopatra* and *Arenicola* under controlled microcosm conditions for 21 days, we evaluated the impact of this replacement, to test the null hypothesis that the native species substitution by IAS with opposed ecosystem engineering traits have no effect on sediment reworking and nutrient cycling. Hence, the objective of this work is to: a) quantify the sediment reworking effect of the different species ratios, traced with luminophores; b) quantify the sediment-water column nutrient flux promoted by the different species ratios; and c) discuss and conclude on the potential impact of native/invasive replacement among contrasting ecosystem engineers' on overall ecosystem functions (sediment reworking) and processes (nutrient dynamics).

Materials and methods

Fauna and sediment preparation

Specimens of *Diopatra* and *Arenicola* complexes, as well as sediment, were collected in Ria de Aveiro, a shallow, mesotidal costal lagoon located on the northwestern coast of Portugal, between 40° 30`N and 40° 51`N (Vaz et al., 2005), with a longitudinal gradient of salinity from about 0 to 36. Extensive intertidal mud and sand flats, salt marshes and islands can be found along its main channels (Costa et al., 2022). Given the large number of specimens needed and the effort associated with their capture, *Diopatra* and *Arenicola* were obtained from a fishing store that receives the specimens collected by local fishermen in Ria de Aveiro. Sediment was collected in Mira Channel, one of the southern channels, where the presence of *Arenicola* has been reported (Pires et al., 2015; Costa et al., 2022). Sediment was homogenized and macrofauna removed by hand-piking for use in housing and microcosms experiments. Specimens were transported to the laboratory and placed in containers (370 x 270 x 230 mm, L x W x H) with 120 mm of sediment overlain with 100 mm of artificial seawater (salinity 28, prepared by mixing Red Sea Coral Pro Salt (Germany) and reverse osmosis water). The salinity level was established based on the distribution areas of both species in Ria de Aveiro (Rodrigues et al., 2011; Vargas et al., 2017). The specimens passed by a housing period of 12 days to ensure the complete regeneration of the posterior part and to ensure that all the individuals were healthy and feeding (Pires et al., 2012). During the housing period, continuous aeration was provided using air-stones and the containers were supplied with fish food (Supreme 50, Skretting) every day by placing a small grain next to the entrance of each tube. If the food was not dragged into the tube within 5 minutes, it was removed. Food was not provided on the day before the polychaete specimens were transferred to the microcosms. During the housing period, the average water temperature in the containers was of 17.9 ± 1.0°C, pH 8.2 ± 0.3, oxygen concentration 7.3 ± 0.8 mg L−1, and salinity 28.0 ± 0.1 (mean ± s.d.).

Microcosm setting

A microcosm set-up was designed to simulate different degrees of *Diopatra* (Dp) habitat invasion by *Arenicola* (Ar). Sediment was distributed along 30 vertical glass aquaria (135 × 135 × 500 mm, internal dimensions), creating a 150 mm column (~ 1/3 of the height of the aquarium, 2.7 L of sediment) and filled with artificial seawater (~ 2/3 of the height of the aquarium, 5.5 L of water). The detailed timeline of the microcosms' experiment, related to water replacement, housing period, acclimation, luminophores introduction, and monitoring of bioturbation and water column nutrients' concentration is provided in Supplementary Table S1. Each aquarium was gently aerated by releasing air bubbles through two capillary tubes (ø = 0.84 mm), connected by a silicon tube (ø = 4.00 mm) to an air pump (RESUN LP-100, 140 L min^{-1}). Aquaria were randomly distributed across the bench and exposed to natural light conditions with a 10 h light:14 h dark photoperiod. Five different ratio of *Diopatra*: *Arenicola*, with an equivalent constant density of ~180 $ind.m^{-2}$, plus Control (no organisms), were placed in the aquaria to be tested: 4 *Diopatra* (coded as 4Dp:0Ar), 3 *Diopatra* and 1 *Arenicola* (3Dp:1Ar), 2 *Diopatra* and 2 *Arenicola* (2Dp:2Ar), 1 *Diopatra* and 3 *Arenicola* (1Dp:3Ar), and 4 *Arenicola* (0Dp:4Ar) (5 replicates each, totaling 30 aquaria). Adult *Diopatra* individuals were introduced into the experimental units without their original tubes. However, by Day 0, prior to the introduction of luminophores and the start of the experiment, all individuals had already built new tubes (confirmed by direct observation). These newly constructed tubes were functional and appeared structurally stable throughout the experimental period. To minimize evaporation and prevent pronounced salinity shifts, all aquaria were covered with Parafilm® Sealing Film. The water in each aquarium was not renewed and neither sediment nor food were added during the acclimation and experimental period.

To avoid additional manipulative stress before implementing the experiment, the polychaetes were measured (total length) and weighted only at the end of the experiment. Organisms passed by a depuration period of 24 h to clear their gut before weighing. The average length and weight were 74.08 ± 10.63 mm and 2.12 ± 0.46 g for *Diopatra*, respectively; and 86.62 ± 22.23 mm and 3.78 ± 0.75 g for *Arenicola* (mean ± s.d.), respectively.

Bioturbation

Particle reworking was measured non-invasively using fluorescent sediment profile imaging (f-SPI; Solan et al., 2004; Lopes et al., 2018; Crespo et al., 2021), based on the visual detection of fluorescent luminophores on the sides of the aquaria. This profile imaging provides descriptors for faunal-mediated sediment reworking, which are surrogate measures for different aspects of infaunal organisms' behavior (Teal et al., 2010; Hale et al., 2014). To avoid the strong initial activity associated with polychaetes establishment and burrowing, luminophores (40 g, 125 - 250 μm diameter, yellow color) were added on the top of the sediment, near the side of each aquarium which would be photographed later, at least 24 h after the introduction of organisms (D_0). This method allows the deposition of a 1–2 cm layer of luminophores on the side that will be photographed and ensures the supply of luminophores throughout the entire experimental period.

The aquaria were photographed within a dark box, under Ultraviolet light, using a GoPro HERO6 camera (12 megapixels sensor, set for 1/8 seconds exposure, diaphragm aperture of f/2.8, and film speed (light sensitivity) equivalent to ISO 3199). Each image obtained was subsequently cropped according to the internal width of the aquarium (135 mm = 2050 pixels, effective resolution = 63.4 μm px^{-1}), converted to a red-green-blue (RGB) stack and saved with JPEG compression (Joint Photographic Experts Group). Images were then analyzed using a custom-made plugin that runs within ImageJ (Version 1.48c), a java-based public domain program developed at the US National Institutes of Health (available at http://rsb.info.nih.gov/ij/index.html). The plugin output provides details on the mean (${}^{f\text{-}SPI}L_{mean}$, time-dependent indication of mixing), median (${}^{f\text{-}SPI}L_{med}$, typical short-term depth of mixing), and maximum (${}^{f\text{-}SPI}L_{max}$, maximum extent of mixing over the long-term) mixed depth of particle redistribution (Hale et al., 2014), for each aquarium, after the semi-automatic conversion of the images to a binary data matrix of the distribution luminophore pixels (0 = background sediment, 1 = luminophore). For each image, the vertical distance between the highest and lowest points of the water sediment boundary (surface boundary roughness – SBR) was manually assessed. SBR is a proxy for surficial activity and is particularly useful in the case of species with high accretion abilities, such as *Arenicola*, which creates noticeable faecal casts on the surface of the sediment (Volkenborn et al., 2007). As bioturbation processes are cumulative, only the final day (day 21) image data were considered, and the values found for the tested species ratio were considered against those found for the procedural control.

Sediment and water characteristics; dissolved nutrient content

Before the experiment inception, five sub-samples were analyzed to determine grain-size and organic matter content on the homogenized sediment. Sediment grain-size was analyzed by dry sieving standard method (Sutherland, 1998). Median and percentage of fines were used to classify the sediment, according to the Wentworth scale (Blott and Pye, 2001): very fine sand (median from 3 - 4 Φ; 63 - 125 μm); fine sand (2 - 3 Φ; 125 - 250 μm); medium sand (1 - 2 Φ; 250 - 500 μm); coarse sand (0 - 1 Φ; 500 - 1000 μm); very coarse sand (-1 - 0 Φ; 1000 - 2000 μm). The final classification adopted the description 'clean', 'silty' or 'very silty' when the % fines fraction of the total sediment, by dry weight, ranged from 0% to 5%, 5% to 25% and 25% to 50%, respectively (Blott and Pye, 2001). Samples with >50% fines content were classified as mud. Total organic matter content was obtained by loss on ignition as described by Kristensen and Andersen (1987).

At days 0, 2, 5, 8, 12, 16, and 21, 10 ml water aliquots (filtered through Whatman GF/C glass-fiber filters) were sampled on the top of the water column (0–5 cm), from each aquarium: this low volume ensured a loss lower than 10% on the microcosm total volume. Samples were stored at −20°C until analysis of dissolved inorganic nutrients (ammonium, NH_4-N; oxidized form of dissolved inorganic nitrogen, NO_x-N; phosphate, PO_4-P). The determination of the concentrations of NH_4-N and PO_4-P was performed following standard methods described in Limnologisk Metodik (Københavns Universitet, 1992). The concentrations of NO_x-N were determined using a flow injection system (FIAstar 5000 Analyzer, Höganäs, Sweden), following Strickland and Parsons (1972). Analytical quality control was ensured by calibration curves derived from standard solutions, run at the beginning of the analysis and in parallel with blanks and samples. Water temperature, pH, concentration of dissolved oxygen and salinity were measured in the mesocosm on every sampling day using a WTW – pH 330i/set equipped with SenTix® 41; a WTW – cond 3110/set 1 equipped with TetraCon® 325 and a WTW – Oxi 3210/set 2 equipped with CellOx® 325-3.

Statistical analyses

The dependent variables related to sediment reworking descriptors (SBR, ${}^{f\text{-}SPI}L_{mean}$, ${}^{f\text{-}SPI}L_{med}$, and ${}^{f\text{-}SPI}L_{max}$) and dissolved nutrient concentrations ([NH_4-N], [NO_x-N], and [PO_4-P]), on day 21, were analyzed using independent regression models with fauna ratios as the fixed term. The dependent variables were analyzed under the null hypothesis of no significant differences among the levels of the response variables across the different ratios of

Diopatra (Dp) and *Arenicola* (Ar), and the Control (defaunated microcosms), and the statistical significance of their terms ($p < 0.05$) were tested. To deal with heteroscedasticity and avoid data transformation, the models were extended to include the appropriate variance covariate structure using a generalized least squares (GLS) estimation procedure (Zuur et al., 2009; Pinheiro et al., 2014). The variance covariate structure was determined using a restricted maximum likelihood (REML) estimation, supported by the Akaike Information Criteria (AIC) and visual comparisons of residuals plots, in contrast to the initial model. Pairwise comparisons of the treatments with different ratios of Dp and Ar were derived from each model's t-table, re-expressed successively against each level as baseline.

Additionally, water nutrient concentrations of NH_4-N, NO_x-N and PO_4-P were represented in ordination analyses, using a Principal Component Analysis (PCA) (Anderson et al., 2008). All statistical analyses were performed using R statistical and programming environment (R Development Core Team, 2020) and the packages *nlme* (Pinheiro et al., 2014) for the regression analysis, and *vegan* (Oksanen et al., 2020) with *functions metaMDS* and *envfit*, to overlay vectors in the ordination analysis. Graphical outputs were produced with R's package *ggplot2* (Wickham, 2016).

Results

Physical-chemical parameters and sediment characterization

The sediment sampled in Mira channel was classified as medium sand (125- 250 µm; φ = 2) with 8.00 ± 0.86% of fines (< 63 µm) and the total organic matter content was of 3.0 ± 0.5% (mean ± s.d.).

During the experimental period, the average water temperature in the aquaria was of 18.4 ± 1.2°C, pH 8.1 ± 0.2, oxygen concentration was 7.8 ± 0.7 mg L^{-1} and salinity was 28.0 ± 0.2 (mean ± s.d.).

Bioturbation

In the Control microcosms, luminophores were concentrated in the superficial layer (<2.0 cm) (Figure 2A), corresponding to the initial layer deposited on the top of the sediment. In the different treatments, the number of luminophores in the superficial layer tend to decrease, increasing in deeper layers with the increasing number of *Arenicola* (Figures 2A–F). All bioturbation parameters responded to the increasing density of *Arenicola* (Figures 3A–D; Table 1).

Surface boundary roughness (SBR) ranged between 0.26 cm (Control) and 6.48 cm (1Dp:3Ar). The highest mean SBR value was recorded in the 1Dp:3Ar treatment (4.18 ± 1.93 cm, mean ± s.d., Figure 3A), and no significant differences in the mean SBR were found whenever *Arenicola* was present. Nevertheless, the monospecific treatment 4Dp:0Ar was significantly different from all the other treatments (Figure 3A; Table 1; Supplementary Table S2).

The mean mixed depth of particle redistribution ($^{f\text{-}SPI}L_{mean}$) for each aquarium ranged between 0.81 cm (4Dp:0Ar) and 5.86 cm (1Dp:3Ar), with the highest value of 3.87 ± 0.97 cm (mean ± s.d.), for the 0Dp:4Ar treatment (Figure 3B). There were no statistical differences between the Control and the 4Dp:0Ar ratio, between the 3Dp:1Ar, 2Dp:2Ar and 1Dp:3Ar ratios, and finally when comparing 2Dp:2Ar, 1Dp:3Ar and 0Dp:4Ar ratios (Figure 3B; Table 1; Supplementary Table S3).

The median mixed depth of particle redistribution ($^{f\text{-}SPI}L_{med}$) had its lowest value in the 4Dp:0Ar treatment, 0.83 cm, while the highest value was recorded in the 0Dp:4Ar treatment, with a $^{f\text{-}SPI}L_{med}$ of 6.01 cm. Collectively, the ratio 0Dp:4Ar had the highest mean $^{f\text{-}SPI}L_{med}$, with 4.99 ± 1.18 cm (mean ± s.d.) (Figure 3C). Pairwise comparisons revealed no significant differences among Control, 4Dp:0Ar, and 3Dp:1Ar, among 3Dp:1Ar and 2Dp:2Ar, and among 2Dp:2Ar, 1Dp:3Ar and 0Dp:4Ar (Figure 3C; Table 1; Supplementary Table S4).

Finally, the maximum mixed depth of particle redistribution ($^{f\text{-}SPI}L_{max}$) ranged from 1.36 cm (4Dp:0Ar) to 11.26 cm (0Dp:4Ar). The lowest mean $^{f\text{-}SPI}L_{max}$ was found in the Control (1.59 ± 0.15 cm, mean ± s.d.), followed by the 4Dp:0Ar treatment (2.65 ± 1.18 cm, mean ± s.d.) while the highest mean of 8.03 ± 2.66 cm (mean ± s.d.), was recorded in the 1Dp:3Ar treatment (Figure 3D). When comparing the different species ratios, no significant differences were detected between the Control and the 4Dp:0Ar ratio, between the 4Dp:0Ar, 3Dp:1Ar and 2Dp:2Ar, and between 2Dp:2Ar, 1Dp:3Ar 0Dp:4Ar ratios (Figure 3D; Table 1; Supplementary Table S5).

Water nutrients' concentration

The statistical model indicates that the concentration of all analyzed nutrients in the water column in the end of the experiment (21 days later) was influenced by species ratio (Table 1). During the experimental period, the concentration of NH_4-N increased with the increasing number of *Arenicola* in the aquaria (2Dp:2Ar < 1Dp:3Ar < 0Dp:4Ar) (Figure 4A). However, the concentration of NH_4-N in the lower *Arenicola* densities (Control, 4Dp:0Ar, 3Dp:1Ar) have peaked around day 5, while in the intermediate levels (2Dp:2Ar and 1Dp:3Ar) the concentration of NH_4-N has peaked around day 16. The monospecific *Arenicola* treatment (0Dp:4Ar) showed an increase in the concentration of NH_4-N until day 21 (Figure 4A). At the end of the experiment, in the presence of fauna, the highest and lowest mean concentrations of NH4-N were registered in the monospecific scenarios ($[NH4\text{-}N]_{0Dp:4Ar}$ = 8.07 ± 0.6, $[NH4\text{-}N]_{4Dp:0Ar}$ = 1.49 ± 0.04 mg L^{-1}, mean ± s.d.). In the Control, the mean concentration of NH_4-N was 1.42 ± 0.03 mg L^{-1} (mean ± s.d.). The statistical analysis revealed significant differences between all comparisons, except between 2Dp:2Ar and 1Dp:3Ar (Figure 4A; Table 1; Supplementary Table S6).

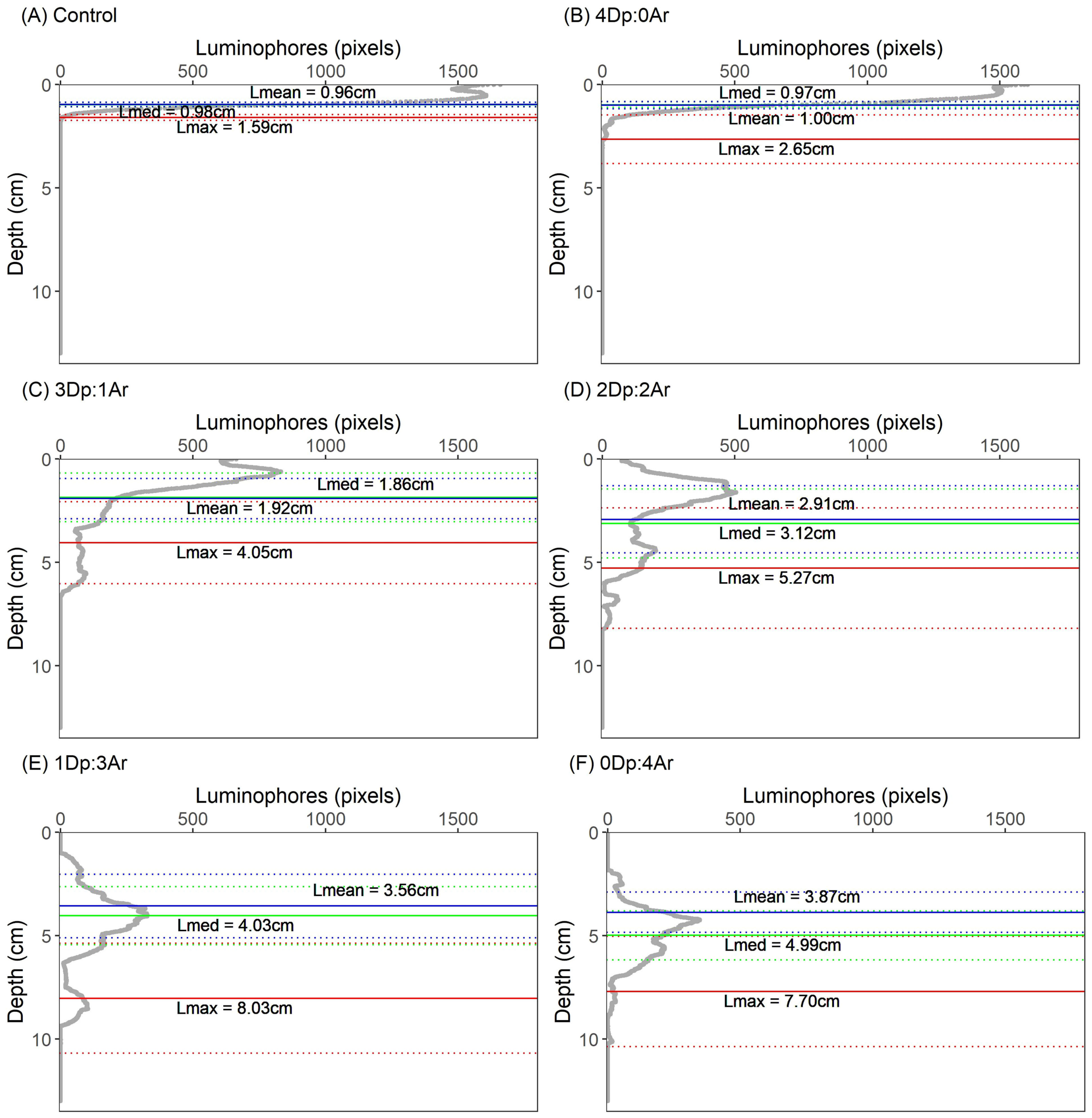

FIGURE 2

Averaged (n=5) vertical profile of luminophores in the sediment after 21 days of bioturbation associated to different ratios of *Arenicola* (Ar): *Diopatra* (Dp): **(A)** Control (no fauna); **(B)** 4Dp:0Ar; **(C)** 3Dp:1Ar; **(D)** 2Dp:2Ar; **(E)** 1Dp:3Ar; **(F)** 0Dp:4Ar. Solid horizontal lines represent mean ${}^{f\text{-}SPI}L_{mean}$ (blue), ${}^{f\text{-}SPI}L_{med}$ (green) and ${}^{f\text{-}SPI}L_{max}$ (red) mixed depths of luminophores' redistribution, and dashed lines their respective standard deviation limits.

During the experimental period, the average concentration of NO_x-N showed an opposite trend comparatively with NH4-N, *i.e.*, it increased in the aquaria with higher densities of *Diopatra*. The maximum concentration of NO_x-N was 4.44 mg L^{-1}, measured at day 16, in the monospecific treatment 4Dp:0Ar. In most treatments, NO_x-N concentrations started to increase after the 5th day. At the end of the experiment, the lowest concentration of NO_x-N was 0.61 ± 0.15 mg L^{-1} (mean ± s.d.), found in the 0Dp:4Ar treatment, while the highest, 4.26 ± 0.22 mg L^{-1} (mean ± s.d.), was found in the 4Dp:0Ar treatment. For day 21, the concentration of NO_x-N in the Control was 1.64 ± 0.15 mg L^{-1} (mean ± s.d., Figure 4B). Contrarily to what was observed for NH_4-N, significant differences were observed in the mean concentration of NO_x-N at day 21, between the Control and the aquaria with higher ratios of *Diopatra* (4Dp:0Ar, 3Dp:1Ar) and also between the aquaria with 0Dp:4Ar. No differences were found the concentration of NO_x-N between the Control, 2Dp:2Ar and 1Dp:3Ar treatments (Figure 4B; Table 1; Supplementary Table S7).

For PO_4-P, the Control showed a reduction from day 0 to day 21, while the monospecific 4Dp:0Ar treatment had only a marginal decrease. The remaining treatments showed an increase in the concentration of PO_4-P from day 0 to day 21 (Figure 4C). The lowest concentration of PO4-P was 0.02 mg L^{-1} (day 12, Control), while the highest was 0.24 mg L^{-1} (day 21, 1Dp:3Ar). The mean concentration of PO_4-P tended to increase with the increasing ratio of *Arenicola*, achieving a maximum concentration of 0.20 ± 0.02 mg L^{-1} (mean ± s.d.) for the monospecific *Arenicola* treatment (0Dp:4Ar) in day 12 (Figure 4C). The statistical analysis for the concentration of PO_4-P at day 21 showed no differences between 3Dp:1Ar, 2Dp:2Ar, and 1Dp:3Ar ratios (respectively 0.14 ± 0.03,

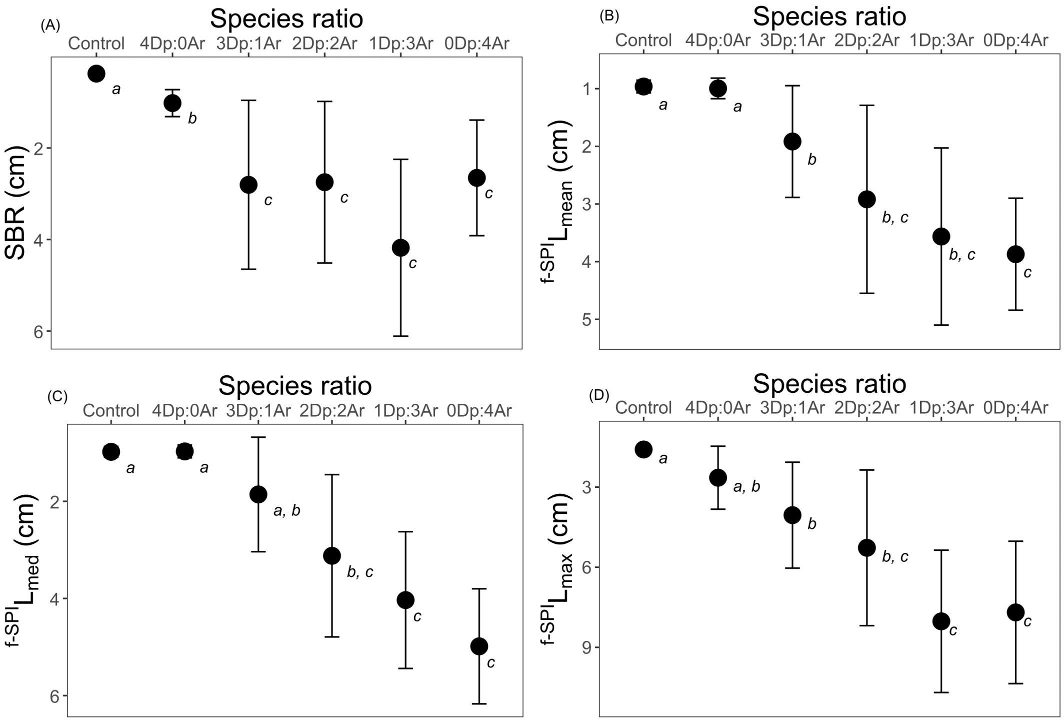

FIGURE 3
The effects of different species ratio [*Arenicola* (Ar): *Diopatra* (Dp)] and Control (no organisms) on the sediment reworking (cm, mean ± s.d.; n=5) in the final day (day 21): **(A)** Surface boundary roughness (SBR); **(B)** mean mixed depth of luminophores' redistribution ($^{f\text{-}SPI}L_{mean}$); **(C)** median mixed depth of luminophores' redistribution ($^{f\text{-}SPI}L_{med}$); **(D)** maximum mixed depth of luminophores' redistribution ($^{f\text{-}SPI}L_{maxn}$). Italicized letters identify statistical similarities.

0.13 ± 0.10. and 0.19 ± 0.04 mg L^{-1}, mean ± s.d.), and between 1Dp:3Ar and 0Dp:4Ar (0.19 ± 0.01 mg L^{-1}, mean ± s.d.) ratios, (Figure 4C; Table 1; Supplementary Table S8).

The principal components ordination clearly shows the evolution of the mesocosms water concentration along time, clustering the samples from days 0, 2 and 5 along Axis 1, which explains almost 60% of the total variance (Figure 5). The nutrients concentration vectors are spread also along Axis 2 and showed that the Control, the monospecific scenario 4Dp:0Ar and the ratio 3Dp:1Ar were characterized by higher concentrations of NO_x-N, while the other treatments (2Dp:2Ar, 1Dp:3Ar, 0D:4Ar) were defined by higher concentrations of NH_4-N and PO_4-P, particularly for the dates after day 8 (Figure 5).

TABLE 1 Summary of significant terms for the generalized least squares (GLS) models analysis of the sediment particle redistribution parameters and nutrient concentrations, against species ratio as explanatory variable and as variance co-variate.

Dependent variable	Num. d.f.	F-value	P-value	Residuals S.E.
Sediment reworking variables				
SBR	5	14.654	<.0001	1.845
$^{f\text{-}SPI}L_{mean}$	5	13.857	<.0001	0.969
$^{f\text{-}SPI}L_{med}$	5	18.128	<.0001	1.179
$^{f\text{-}SPI}L_{max}$	5	14.723	<.0001	1.983
Nutrients				
[NH_4-N]	5	145.105	<.0001	0.077
[NO_x-N]	5	136.971	<.0001	0.251
[PO_4-P]	5	110.530	<.0001	0.026

(Denom. d.f.: 24).

Discussion

In the context of the worldwide spread of species across different habitats and ecosystems, it is fundamental to understand the outcomes of species substitution by functionally distinct counterparts. Measuring the amount of change that these substitutions will produce can help predict the ecological consequences in invaded habitats, as different species might also show different traits and non-linear responses (Strayer and Hillebrand, 2012; Strayer, 2020). The present study confirms that the replacement of native sediment stabilizer species of the *Diopatra* (solitary tube worm) genus by highly active bioturbator *Arenicola* (lugworm) species will have consequences on important ecological processes, which can resonate across the functioning of the overall ecosystem. The results of the experiment showed a strong response to the increasing density of *Arenicola* individuals in the microcosms: higher densities on *Arenicola* exhibited higher values in almost all analyzed parameters, both for bioturbation and nutrients concentration.

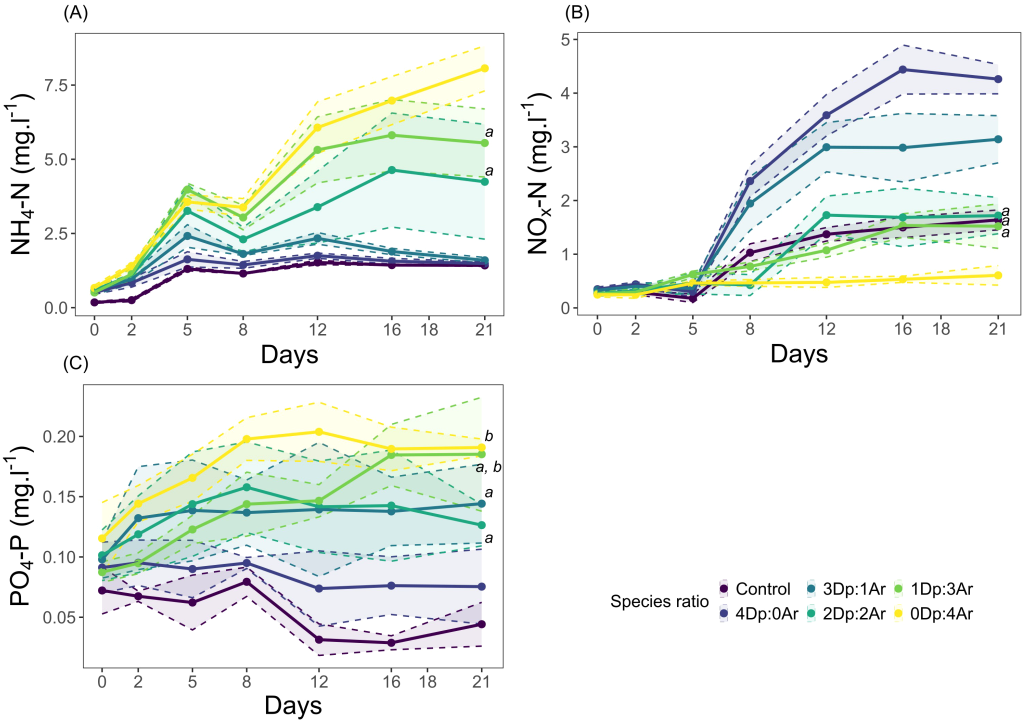

FIGURE 4
The effects of different species ratio [*Arenicola* (Ar): *Diopatra* (Dp)] and Control (no organisms) on the inorganic dissolved nutrient content of the water column along time (mg L^{-1}, mean; n=5; shaded bands represent the 95% confidence interval): **(A)** ammonium (NH_4-N); **(B)** oxidized form of dissolved inorganic nitrogen (NO_x-N); **(C)** phosphate (PO_4-P). Italicized letters identify statistical similarities (p-value > 0.05) in the final day (day 21).

Ecological effects of functionally distinct species

Sediment reworking

Sediments and infaunal organisms in aquatic systems have severe limitations in accessing oxygen (Jansen et al., 2009; Kristensen et al., 2012). Bioturbation in aquatic systems is a process that facilitates the transport of fluids within the sediment matrix and across the sediment-water interface through ventilation, including oxygen from the overlying water into sediment interstices (Maire et al., 2010; Kristensen et al., 2012). Bioturbation also includes particle reworking (*i.e.*, the transport of particles within the sediment matrix), which enables the relocation of several forms of organic matter from and into the sediment (Kristensen et al., 2012; Aller and Cochran, 2019). This means that bioturbation is fundamental to the proper ecological functioning of benthic communities (Mermillod-Blondin, 2011; Aller and Cochran, 2019). However, species differ widely in the way they bioturbate (Volkenborn et al., 2009; Mermillod-Blondin, 2011; Kristensen et al., 2012) and, therefore, different bioturbation mechanisms will mediate different ecological responses. *Diopatra* species are known as proficient ecosystem engineers, that create robust and permanent tubes that extends deep into the sediment (Berke, 2022; Arias et al., 2023). These tubes provide structural complexity and heterogeneity, benefiting other endo- or epibenthonic species (Bailey-Brock, 1984; Thomsen et al., 2011; Santos and Aviz, 2019). Additionally, the stabilizing effect of *Diopatra* tubes is important in highly hydrodynamic systems, by reducing the resuspension of sediments (Bailey-Brock, 1984; Luckenbach, 1986; Berke, 2022). On the other hand, *Arenicola* species are known to be upward conveyors, with a highly intense reworking activity (Riisgård and Banta, 1998; Wethey and Woodin, 2022), reducing the sediment heterogeneity, and with the potential to increase sediment erosion under strong currents (Wendelboe et al., 2013). As evidenced by our results, shifting from the monospecific *Diopatra* to *Arenicola* treatments led to a 3 to 4-fold increase in the reworking depths, reflecting the different mechanisms on which each genera uses to rework the sediment. Although *Diopatra* tubes can reach high depths within the sediment, the sediment matrix is kept relatively undisturbed, which is supported by the low particle redistribution depth observed in *Diopatra* dominated treatments. Particularly, the monospecific *Diopatra* treatment was statistically indistinct from the controls in all bioturbation parameters, with luminophores concentrated on the uppermost 2 cm. By contrast, in *Arenicola* dominated microcosms there was an effective homogenization of the sediment along the uppermost 6 cm. Luminophores' particles redistribution could have been evaluated on all sides of the aquaria, increasing the probabilities to detect movement and providing more robust data. This is particularly true for *Diopatra* which presented very low mobility, and which random distribution along the sediment could have impaired the detection of their eventual sediment reworking. However, the results were consistent across replicates, even when using data from only one side of the aquaria as a proxy for bioturbation potential for the full sediment column.

We could not statistically differentiate the reworking depths when there were two or more *Arenicola* individuals in each microcosm, suggesting that even if communities show some equitability, there is a disproportionate effect of *Arenicola* on sediment reworking: even lower densities show strong responses on the sediment reworking depths. Our *Arenicola* specimens were able to bury as deep as 11 cm in the highest ratio treatment, perhaps as an intraspecific avoidance behavior. De Cubber (2020) mentions that these species avoid intra-specific competition by migrating down the shore, reflecting a behavioral tendency for avoidance. Confined within the volume of our microcosms, the only way to avoid each other is to go deeper. However, the average maximum luminophores mixing depth, of ~8 cm, was very similar in 1Dp:3Ar and 0Dp:4Ar ratios, which could indicate an asymptotic threshold dependent on the specimens' size. The depths of reworking in *Arenicola* species have been reported to vary with organisms' size/age: while the species could live in burrows up to 30 cm in depth, Wethey and Woodin (2022) (after Beukema and De Vlas, 1979) mention that this depth can be reduced as function of body size. Based on De Cubber et al. (2020), for the average size of our *Arenicola* specimens (~8.7 cm length) the burrows could extend as deep as ~22 cm. This means that our specimens did not attain the full reworking potential that was expected for their size, which could be related with the size of the aquaria. However, in no circumstance during our experiment the sediment reworking reached the maximum sediment depth of our microcosms, which means that some other drivers have limited these movements. It seems that under starvation or other stress sources the species reduce their reworking activity and burrow size (Wethey and Woodin, 2022), which can explain the difference between our maximum reworking depths and the predicted size of the burrows. Additionally, as an intertidal species, low tide might trigger deeper burrowing. However, tides were not considered in our experimental setup which held a constant water level throughout the all period. The Surface Boundary Roughness (SBR) values were statistically indistinguishable across treatments containing *Arenicola*, regardless of the number of individuals. These values were a consequence of the faecal cast deposition on the top of the sediment, due to the highly intensive feeding activity, based on the non-selective, conveyer-belt, ingestion of large amounts of sediment (Riisgård and Banta, 1998; Volkenborn and Reise, 2006). In this case, SBR offers information on the height of the faecal casts (the distance between the highest and the lowest points on the water-sediment interface), which could be an asymptoticly limited, *i.e.* there should be a limit on the amount of sediment that can be accumulated vertically on these casts, before they start to collapse. Even if it seems contra-intuitive, it was expected that this measure would reach an asymptotic threshold, and, in this case, this limit was reached in the treatment with the lowest densities of *Arenicola*.

Sediment-water column nutrient dynamics

The concentrations of water nutrients can have different sources: 1) the feeding and metabolic activity of the polychaetes; and 2) the microbiome and meiofauna (considering that the sediment was defaunated, and no other macroinvertebrates are present, besides the introduced polychaetes). Regarding the first source, the feeding and metabolic activity of the worms, it is possible that the highly active *Arenicola* is responsible for the larger production of ammonium, which is accumulated in the water column. The initial values (day 0) were very similar between treatments, but soon the concentration of NH_4-N started to become distinct in the different treatments. However, in the final day, the *Diopatra* dominated treatments (4Dp:0Ar; 3Dp:1Ar) showed a reduction in the NH_4-N values, similar to those found in the control, after peaking on day 5. This suggests that the microbial community fostered by *Diopatra* could be intercepting this nutrient, even if is being generated by the polychaetes in the microcosm. The trends observed for NO_x-N were different, with the highest values recorded in the monospecific *Diopatra* treatment, while the lowest values occurred in the monospecific *Arenicola* treatment, which showed lower values than the control, after day 8. This indicates that, under the influence of *Arenicola*, there is a consumption of NO_x-N. However, the prominent increase in the *Diopatra* dominated treatments, after day 5 could indicate that *Diopatra* might be stimulating the nitrifying community (Voss et al., 2013), responsible for the oxidation of NH_4-N to NO_x-N. This is reinforced by the fact that the NO_x-N values in the *Diopatra* dominated treatments are significantly higher than those in the Control. If, indeed, there is an increase in photosynthetic organisms biomass promoted by *Diopatra*, as described in the literature (Berke et al., 2010; Fuirst et al., 2021; Bugnot et al., 2023), the oxygen present in the water and in the interstitial water of the top layers of the sediment can alter the redox conditions, influencing the nitrification-denitrification processes (Voss et al., 2013; Høgslund et al., 2023). This higher oxygen availability also affects the phosphate dynamics, which is consumed by the microphytobenthos to build biomass, while also causing the oxidation of iron compounds in the sediment which improves P adsorption and sequestration in the sediment (Høgslund et al., 2023). The phosphate concentrations were very fast to reach the equilibrium, with most treatments reaching the peak after day 2. This fast response could indicate that the concentration of PO_4-P was chemically controlled, more than biologically mediated, *i.e.*, independent from the microbiome community establishment. The microcosms dominated by *Arenicola* were characterized by high levels of NH_4-N and PO_4-P, while the Control and *Diopatra* microcosms were dominated by high levels of NO_x-N. However, this distinction was only evident in samples collected after day 8. Without following the microbiome dynamics, it is only possible to speculate regarding the detailed mechanisms involving the nutrient generation and consumption, as they depend on the relative equilibrium between the elements C, N, and P (Voss et al., 2013) and on the biological interactions that take place (Mallin and Cahoon, 2020). Also, the *Arenicola* species are not only strong biomixers, but also highly active advective bioirrigators, causing a dramatic effect on the porewater flow, and able to flush significant levels of solutes (Kristensen et al., 2012) which can include NH_4-N and PO_4-P. Nevertheless, without assessing the rates of critical biogeochemical processes such as remineralization, nitrification, and denitrification, or bioirrigation, it is unclear if the detected nutrient differences are a consequence of increased production, intense flushing, or decreased removal, or due to a combination of the these processes. Yet, our results demonstrate that species occupying similar niches may not share similar traits and their impacts on the ecosystem will be profoundly distinct.

While other factors may influence the composition of aquatic communities (Menegotto et al., 2019; Kennish, 2021; Guan et al., 2024),

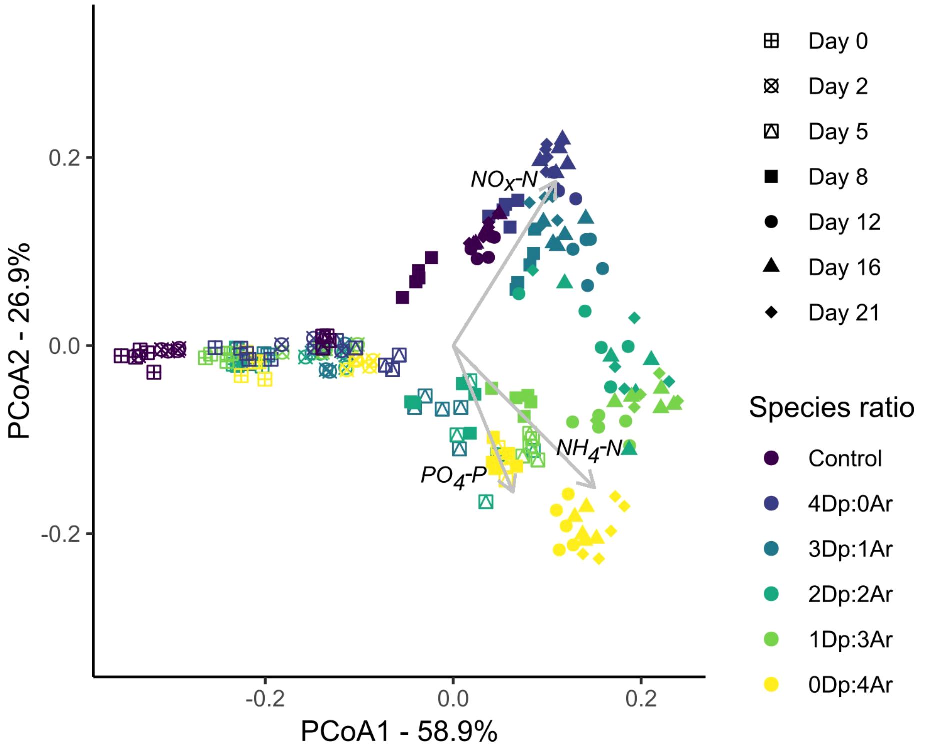

FIGURE 5
Principal component analysis based on the concentrations (mg L^{-1}) of ammonium (NH_4-N), oxidized form of dissolved inorganic nitrogen (NO_x-N) and phosphate (PO_4-P) in the Control (C) and different treatments of *Diopatra* (Dp) and *Arenicola* (Ar) considering seven sampling times (Days 0, 2, 5, 8, 12 and 21) during the experimental period.

primary production, which is highly dependent on nutrient availability, is the main responsible for the biomass build-up (Philippart et al., 2003; Beukema and Dekker, 2020). However, the effects of the increase in the nutrient loads can lead to more eutrophic aquatic systems and more frequent anoxic events, magnified by global climate warming and the raise in the average water temperature. Consequently, widespread mortalities may occur more frequently in these systems (Rabalais et al., 2009; Antón et al., 2011) (Rabalais et al., 2009; Antón et al., 2011) when dominated by *Arenicola*, and associated with low water circulation and long retention periods.

Other potential effects of species substitution

Beside the ecological effects shown by our results, the replacement of *Diopatra* by *Arenicola* in Ria de Aveiro will have consequences on the accompanying biota. Several studies have reported that *Arenicola* species exclude other macroinvertebrates from their habitats (Pires et al., 2015) leading to communities with lower macroinvertebrates diversity, particularly among tube building amphipods and polychaetes, and small bivalves (Riisgård and Banta, 1998; Volkenborn et al., 2009). Arenicolidae species are also responsible for the uprooting of macrophytes (Philippart, 1994; Costa et al., 2022). Among the meiofauna, nematodes suffer negative effects (Riisgård and Banta, 1998). Nevertheless, some species can benefit from the presence of *Arenicola*, such as amphipods, copepods, or subsurface deposit feeding worms such as *Scoloplos* sp (Volkenborn and Reise, 2007), along with various groups of microorganisms (Lei et al., 2010; Chennu et al., 2015). *Diopatra* tubes provide substantial benefits to meiofaunal communities, which explore the tube caps and capitalize food availability (Bell and Coen, 1982). These tubes also serve as hotspots for microbiome diversity (Fuirst et al., 2021). Bugnot et al (2022) reported an increase in species richness following the introduction of *Diopatra aciculata* in a restoration experiment. Even under small densities, *Diopatra cuprea* seems to enhance the community diversity in macrotidal sandy beaches along the Brazilian Amazon Coast (Santos and Aviz, 2019). The replacement of *Diopatra* species by *Arenicola* will likely drive profound changes in the accompanying community (Berke, 2022), with predominantly negative consequences outweighing potential benefits.

Finally, it should be noted that while both genera are economically relevant as bait species (Pires et al., 2012; Watson et al., 2017; Cabral et al., 2019; Berke, 2022; Arias et al., 2023), *Diopatra* has historically been the most sought species in the Ria de Aveiro (Cunha et al., 2005; Cabral et al., 2019). Understanding whether bait diggers and fishermen are willing to adapt their long acquired habits is crucial, as bait digging represents an important supplementary income for local communities.

Conclusion

Prominent shifts in species range are expected to become more frequent, as consequence of human-mediated dispersion with changes in environmental parameters of receiving habitats due to modification and climate change impacts. Therefore, it is crucial to clarify the context that frames these events, as consequences that arise from those are often difficult to predict if based only on generalized assumptions. Yet, our results demonstrate a directional forcing on relevant ecological proxies, such as bioturbation and nutrient concentrations, elicited by the shift in equitability towards *Arenicola* dominated communities. The present work demonstrates that the substitution of native polychaete species by IAS can result in significant impacts in fundamental ecological processes and functions (bioturbation and nutrient availability), particularly if the new species has contrasting traits.

Moving forward, it will be important to disclose which are the accompanying microbial communities and simulate scenarios which include the natural environmental variability in transitional aquatic systems, such as tidal effects on salinity, temperature, and oxygen availability.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The manuscript presents research on animals that do not require ethical approval for their study.

Author contributions

ML: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Methodology, Writing – original draft, Writing – review & editing. DC: Data curation, Formal Analysis, Visualization, Writing – original draft, Writing – review & editing. VC: Methodology, Writing – review & editing. PR: Methodology, Writing – review & editing. AS: Conceptualization, Funding acquisition, Methodology, Resources, Supervision, Writing – review & editing. AL: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2025.1641983/full#supplementary-material

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