Contrasting Effects of an Alien Worm on Benthic N Cycling in Muddy and Sandy Sediments

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Abstract: The North American oligochaete *Sparganophilus tamesis* is widespread in European freshwaters. Its ecological effects on benthic nitrogen (N) biogeochemistry were studied in two contrasting environments: the organic-rich muddy sediments of the eutrophic Mincio River (Italy) and the organic-poor sandy sediments of the oligotrophic Cazaux-Sanguinet Lake (France). Oxygen and inorganic N fluxes and denitrification rates (IPT) were measured by dark incubation of intact cores with different worm biomass. Sediment oxygen demand and denitrification were higher in muddy than in sandy sediments; however, at the two sites, bioturbation by the oligochaetes stimulated differing microbial O_2 and NO_3^- respiration and NH_4^+ production. In particular, the relative effect of *S. tamesis* on sediment metabolism was greater in Cazaux-Sanguinet Lake than in the Mincio River. As a result, *S. tamesis* favored net N loss in the Mincio River, whereas it increased NH_4^+ recycling and lowered denitrification efficiency in the Cazaux-Sanguinet Lake. Our results suggest that the effects of *S. tamesis* on N biogeochemistry might differ depending on local trophic settings. These results have implications for the conservation of isoetids in the French Lake, whose persistence can be menaced by oligochaete-induced nutrient mobilization.

Keywords: alien species; *Sparganophilus tamesis*; sediments; nitrogen; fluxes; denitrification

1. Introduction

In aquatic ecosystems invasive species have by default a negative connotation, with very limited exceptions in the literature [1]. Invasion by alien species may affect the interactions of species within communities and the cycling of energy and matter within ecosystems and produce a cascade of consequences and sequential shifts from pristine conditions [2]. This might be the case for bioturbating fauna, which may support both bacteria and primary producers by mobilizing refractory or scarcely bioavailable (e.g., deep and buried) organic matter, adding new nutrient input to the system [3]. If this can be true in well-preserved ecosystems, the invasion of heavily impacted ecosystems by alien species can paradoxically produce unexpected trajectories [1].

Invasive macrofauna, (able to colonize and spread, even under oligotrophic conditions), may determine a large nutrient mobilization within the sediments and from the sediments to the water column [4]. It is difficult to predict long-term net effects of alien species on biogeochemical functioning and on communities [5]. Generally, the highest impact takes place within the early invasion phase, when alien species spread and reach peak density as a result of a strong competition capacity or the abundance of resources [6]. Later on, pristine communities tend to reorganize and contrast

the invasion, resulting in a decrease of density of the invader and a trajectory back to the original condition. This may require different time spans, depending on a large number of factors. In aquatic ecosystems, one of the most studied invasive species is *Dreissena polymorpha*, due to the impressive densities of this reef-forming filter feeder and its capacity to invade a wide range of environments, from plankton-rich eutrophic to oligotrophic environments [7–10]. *D. polymorpha* was studied due to its supposed capacity to control and reverse eutrophication, an apparent paradox for an alien species [11]. Specific hypotheses postulated the reduction in plankton biomass due to high filtration rates, increase of water transparency, increase of benthic production, and a regime shift that reverses a turbid status to a transparent one [12]. This trajectory was only partially verified, as multiple analyzed systems revealed that oligotrophication might be short-term or invalid in relatively deep systems where *Dreissena* has limited access to phytoplankton or where excreted nutrients stimulate new algal growth [13,14]. Another interesting example of the biogeochemical effects produced by an alien species in a marine environment is that of *Marenzelleria* spp. in the Baltic Sea. This invasion was studied due to the critical conditions (e.g., large suboxic areas) of the Baltic area and to the contrasting effects of this species' production of nutrients. It was demonstrated that *Marenzelleria* spp. inhibited denitrification and stimulated the dissimilative nitrate reduction to ammonium (DNRA). The latter process is negative for a eutrophic ecosystem as it recycles ammonium. However, *Marenzelleria* spp. enhanced the oxidation of sediments and the long-term retention of phosphorus, which may favor the recolonization of pristine macrofauna. These effects may contribute to the recovery of impacted, anoxic, and poorly biodiverse sea bottom [4,15–18]. Macrofauna may produce contrasting effects on nutrient cycling, as it may stimulate permanent or temporal retention of nutrients, or loss or favor their mobilization [1]. In eutrophic environments, retention and losses are preferred over recycling (to contrast excess carbon fixation, infilling, and so on) [19]. In oligotrophic settings, macrofauna communities contribute to the slow cycling of elements and support the activity of primary producers [20].

In this study, we analyzed the effect of an alien worm in two freshwater environments: the eutrophic Mincio River (northern Italy) and the oligotrophic Cazaux-Sanguinet Lake (Atlantic coast of France). *Sparganophilus tamesis* is native in North America. It is now spreading all over Europe in a wide range of shallow environments generally colonized by macrophytes, but with variable trophic levels and sedimentary features [21]. We analyzed the effect of *S. tamesis* on rates of benthic respiration and nitrogen dynamics, hypothesizing a net stimulation of N_2 production in the eutrophic site and N recycling in the oligotrophic site. The latter hypothesis is supported by the fact that this invasive worm may exploit refractory fragments of macrophytes as nutrient source, scattered within the upper sediment horizon. As such, it may mobilize N, otherwise associated and buried as particulate nitrogen (PN), and favor the displacement of N–PN (from the sediment to the water column) through burrow ventilation in the ammonium (NH_4^+) form. Furthermore, we hypothesized that there would be a limited stimulation of denitrification at the oligotrophic site due to low organic pools in sediment and low nitrate (NO_3^-) in the water column, but with a high excretion and release of NH_4^+. Whereas ammonium mobilization by *S. tamesis* can produce negative consequences in nutrient-poor environments, the stimulation of processes such as denitrification might be positive in nitrate-rich environments. Therefore, the main aim was to compare the role of *S. tamesis* in two different freshwater environments in order to understand if a system could benefit from the invasion by an alien species.

2. Materials and Methods

2.1. Sampling Procedure and Sediment Characterization

Water, sediments, and oligochaetes were collected from two sites: a branch of the Mincio River in proximity of Goito (MN, northern Italy) and a littoral zone of the Cazaux-Sanguinet Lake (Atlantic coast of southern France). Both the sampling sites were shallow (~50 cm) with transparent water. The Italian site was characterized by a high nutrient concentration in the water column and muddy

sediment, whereas Cazaux-Sanguinet Lake was a nutrient-poor and soft water environment with predominantly sandy sediments [22].

Intact sediment cores (n = 12 for Mincio and n = 16 for Cazaux-Sanguinet) were collected by means of plexiglass liners (inner diameter = 4 cm, height = 20 cm) vertically inserted into the sediment in order to have nearly 12 cm of sediment and nearly 8 cm of a water phase. Individuals of *S. tamesis* collected from the same sites were then added to the cores in order to have variable biomass: 0 g_{dw} m^{-2} (n = 3), 35 ± 7 g_{dw} m^{-2} (n = 3), 55 ± 11 g_{dw} m^{-2} (n = 3), 65 ± 3 g_{dw} m^{-2} (n = 3) for the Mincio River and 0 g_{dw} m^{-2} (n = 4), 45 ± 13 g_{dw} m^{-2} (n = 4), 65 ± 8 g_{dw} m^{-2} (n = 4), 85 ± 4 g_{dw} m^{-2} (n = 4) for the French site [21]. Nearly 50 L of in situ water was collected for preincubation and incubation procedures.

Four additional plexiglass liners (inner diameter = 4 cm, height = 20 cm) were collected from the sites for sediment characterization. The upper sediment layer (0–10 cm) was extruded with a piston and homogenized with a spatula. A sub-sample of 5 mL of fresh sediment was dried at 70 °C for 48 h for density, porosity, and organic matter analyses. The homogenized sediment was collected by means of a cut-off 5 mL syringe. Bulk density was measured as the weight of a volume of 5 mL fresh material, and porosity was calculated after drying at 70 °C until reaching a constant weight. Organic matter content (OM) was measured as a percentage of weight loss on ignition (450 °C, 2 h) from dried, powdered sediment.

2.2. Incubation Setup and Measurement of Benthic Fluxes

Once collected, water and intact sediment cores added with oligochaetes were transferred to the laboratory within two hours. Cores were submersed with the top open in a large incubation tank that contained in situ water that was well-mixed and aerated and maintained at ambient temperature (24 ± 0.5 °C). One week after the sampling, all cores were closed, and dissolved gas and nutrient fluxes were measured in the darkness. The incubation procedure was standard, with initial and final samplings from each core water phase as detailed in [23]. Incubations lasted 2 h for Mincio sediment cores and 3 h for the French sediment cores, and incubations started when a gas-tight lid with a sampling port and a compensation valve was positioned on the top of the liners. A Teflon-coated stirring bar gently mixed the water inside each liner to avoid stagnation and to guarantee homogeneous conditions within the cores. At the beginning and at the end of the incubations an aliquot of water was transferred to a 12 mL glass vial (Exetainer®, Labco Limited, High Wycombe, UK) and fixed with 100 μL of 7 M $ZnCl_2$ for O_2 analysis by means of Membrane Inlet Mass Spectrometer (MIMS, Bay Instrument, sensitivity 0.2 μM). In addition, an aliquot of 20 mL was filtered (Whatman GF/F glass fiber filters) and transferred to a plastic vial for NH_4^+, NO_3^-, and nitrite (NO_2^-) analyses performed with standard spectrophotometric techniques [24,25]. Fluxes were calculated according to the equation below:

$$Flux\ x = \frac{([x]_f - [x]_i) \times V}{A \times t},\qquad(1)$$

where x_f and x_i, expressed in μM or mM, are the concentrations of the solute x at the end and at the start of the incubation, respectively, V (L) is the volume of the core water phase, A (m^2) is the area of the sediment, and t (h) is the incubation time.

The top lids were thereafter removed, and the water in the tank was replaced with fresh in situ water. In the afternoon, a sequential incubation was performed that aimed at measuring the denitrification rates with the isotope pairing technique (IPT) [26]. Briefly, 0.1 mL (Cazaux-Sanguinet) and 0.5 mL (Mincio) of a 20 mM $^{15}NO_3^-$ stock solution was added to the water phase of each liner to reach 10 and 50 μM final concentrations of labelled nitrate at the oligotrophic and eutrophic sites, respectively. The top lids were then positioned and the cores were incubated in the dark for 3 h. At the end of the incubation the lids were removed, and the whole sediment and water phase was gently mixed to create a slurry, which was subsampled and transferred to the Exetainers, then poisoned with 200 μL of 7 M $ZnCl_2$ for labelled N_2 analysis by means of MIMS. At the end of the procedure the cores were sieved in order to check for the occurrence of other macrofauna and to retrieve the oligochaetes.

The revised version of the IPT was not used at the sampling sites, as sediment slurries demonstrated the absence of anammox (Benelli unpublished). The rates of denitrification were calculated according to the equations and assumptions of [26]: $D_{15} = p(^{15}N^{14}N) + 2p\ (^{15}N^{15}N)$ and $D_{14} = p(^{15}N^{14}N) + 2p(^{14}N^{14}N)$, where D_{15} and D_{14} were equal to the rates of denitrification based on $^{15}NO_3{}^-$ and $^{14}NO_3{}^-$, respectively; and $p(^{14}N^{14}N)$, $p(^{15}N^{14}N)$, and $p(^{15}N^{15}N)$ were equal to the rates of production of labelled and unlabeled N_2 species. Because the $p(^{14}N^{14}N)$ cannot be readily measured, estimation of D_{14} was obtained from: $D_{14} = D_{15} \times p(^{15}N^{14}N)/2p(^{15}N^{15}N)$. The proportion of D_{14} supported by unlabeled $NO_3{}^-$ from the water column (D_W) was calculated from: $D_W = D_{15} \times f/(1 - f)$, where f is a mole fraction of $^{14}NO_3{}^-$ in the water column. The coupled nitrification–denitrification (D_N) was calculated as the difference: $D_N = D_{14} - D_W$.

Individuals of *S. tamesis* retrieved from the cores were analyzed for the wet (g_{ww}) and dry weights (g_{dw}, after drying the soft tissue at 70 °C to a constant weight).

The sum of the fluxes of inorganic N forms was calculated in order to estimate the denitrification efficiency (DE) that is calculated as:

$$DE = \frac{D_{tot}}{DIN_{fluxes} + D_{tot}} \times 100, \tag{2}$$

where D_{tot} is the sum of D_W and D_N, and DIN_{fluxes} is the sum of $NH_4{}^+$, $NO_3{}^-$, and $NO_2{}^-$ net fluxes (only values >0 are considered; when DIN_{fluxes} are negative DE is 100%). Denitrification efficiency represents the fraction of mineralized N that is released to the water column as N_2. When DE is 100% it suggests tightly coupled ammonification, nitrification, and denitrification and no inorganic N efflux.

2.3. Statistical Analyses

Differences between sedimentary features and fluxes at the two sites were tested by one-way ANOVA. As process rates at the two sites were markedly different, in order to compare the site-specific effect produced by increasing worm biomass on benthic processes, an enhancement factor was calculated by dividing the rates measured in the bioturbated sediments by the rates measured in control sediments. The enhancement factor, representing the relative increase of different processes along with variable *S. tamesis* biomass, was analyzed via linear regression; the obtained slopes were compared with a *t*-test.

3. Results

3.1. Sedimentary Features

At the two sites, inorganic nitrogen concentration (DIN), sediment density, porosity, and organic matter content were significantly different (one-way ANOVA, $p < 0.001$). In the Mincio River, sediments were soft, muddy, and very organic, whereas the sandy sediment of Cazaux-Sanguinet Lake displayed a high density, low porosity, and low organic matter content (Table 1). The Mincio River sediments appeared dark and chemically reduced, with a sharp redox discontinuity a few mm below the interface, whereas those of the Cazaux-Sanguinet Lake were light brown and homogeneously oxidized along the upper 10–15 cm depth profile.

Table 1. Inorganic nitrogen concentrations measured in replicated water column samples ($n = 3$) collected at the two investigated sites and sediment features ($n = 4$) obtained by pooling the upper 10 cm sediment horizon. The reported values correspond to the average ± standard error.

	Mincio	**Cazaux-Sanguinet**
NH_4^+ (µM)	2.0 ± 0.2	3.6 ± 0.1
NO_3^- (µM)	160.7 ± 5.6	1.5 ± 0.1
NO_2^- (µM)	3.7 ± 0.3	0.4 ± 0.1
Sediment typology	Muddy	Sandy
Density (g cm^{-3})	1.34 ± 0.08	1.83 ± 0.09
Porosity	0.77 ± 0.04	0.36 ± 0.05
Organic matter content (%)	9.20 ± 0.70	0.11 ± 0.03

3.2. Benthic Fluxes Along Increasing Sparganophilus tamesis Biomass

Microbial respiration rates measured in sediments without macrofauna were significantly higher in the Mincio River, likely due to higher organic content (one-way ANOVA, $p < 0.001$). At both sites, the addition of increasing *S. tamesis* biomass resulted in increased sediment O_2 demand (SOD) (Figure 1).
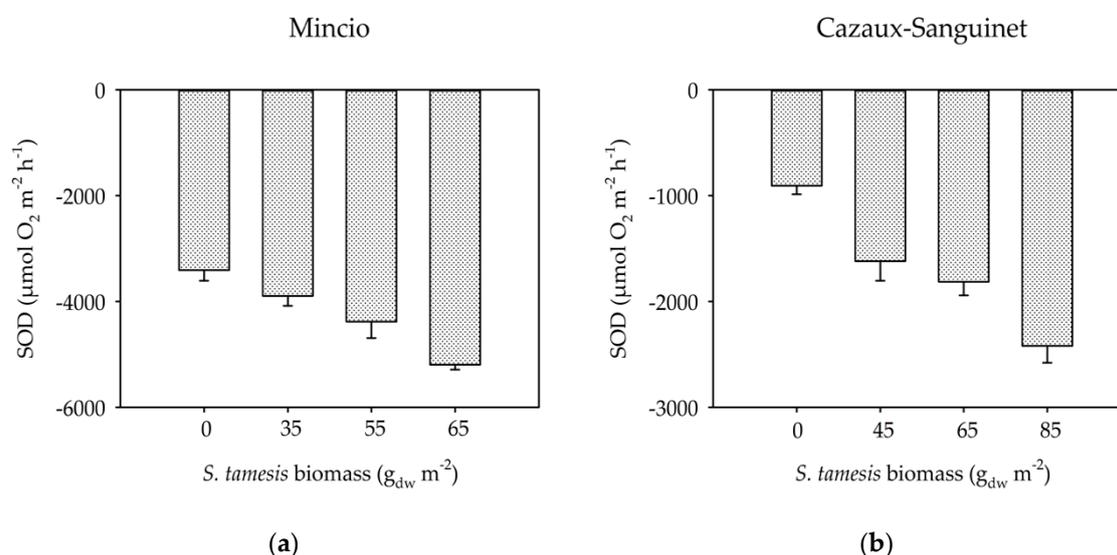

Figure 1. Sediment oxygen demand in the four treatments in the Mincio River (**a**) and in the Cazaux-Sanguinet Lake (**b**). Averages ± standard errors are reported. Note different scales on *y*-axis.

Denitrification rates measured with the IPT are reported in Figure 2. N_2 production increased in the four treatments along with increasing *S. tamesis* biomass at both sites. Compared to the aerobic respiration, denitrification rates were high in the Mincio sediments (Figure 2a), whereas they were very low in the sandy sediments of Cazaux-Sanguinet Lake (Figure 2b), even with the highest biomass of oligochaetes. In the four treatments, denitrification supported by nitrification averaged 82 ± 22 and 0.38 ± 0.15 µmol m^{-2} h^{-1} in Mincio sediments and Cazaux-Sanguinet sediments, respectively, without significant differences between control and bioturbated sediments. In Cazaux-Sanguinet Lake, D_N represented nearly 50% of total denitrification in control sediments, whereas this share was reduced to 6% in the highest biomass treatment.

Comparing the effect produced by the worm on the processes measured at the two sites, different enhancement factors were calculated for SOD and total denitrification ($D_W + D_N$) (Figure 3). At both sites, increasing worm biomass produced a higher relative effect on the nitrate-based than on the aerobic respiration. Furthermore, the stimulatory effect of *S. tamesis* on both processes was relatively higher in the Cazaux-Sanguinet Lake than in the Mincio River (*t*-test, SOD $p < 0.001$, Dtot $p < 0.05$).
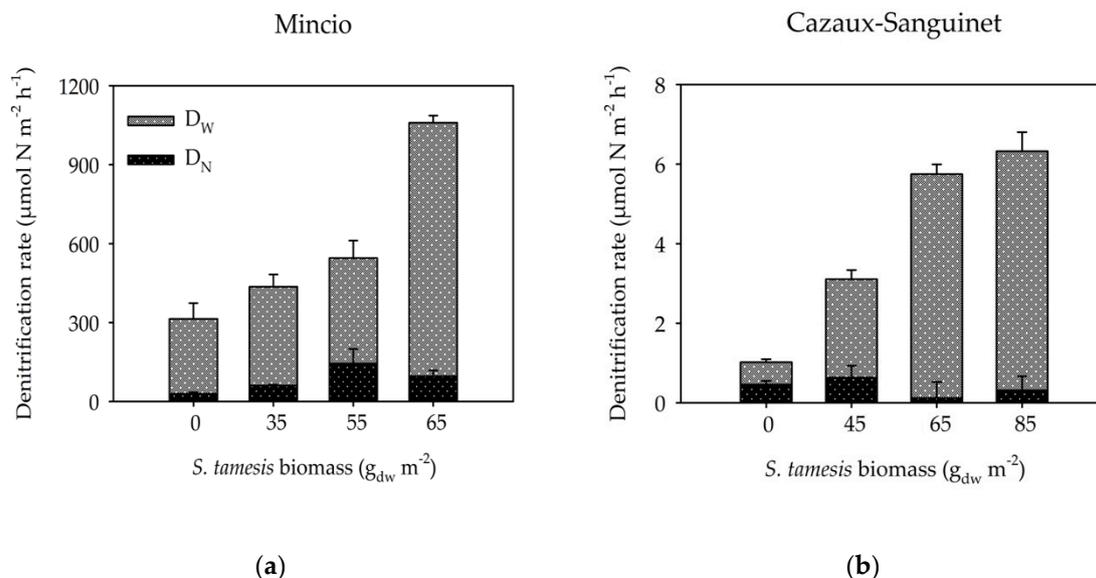
Figure 2. Denitrification rates measured via isotope pairing technique (IPT) in the four treatments in the Mincio River (**a**) and in the Cazaux-Sanguinet Lake (**b**). D_W represents the denitrification of NO_3^- diffusing to anoxic sediments from the water column, whereas D_N is the denitrification of NO_3^- produced by nitrification in the sediment. Averages ± standard errors are reported. Note different scales on *y*-axis.
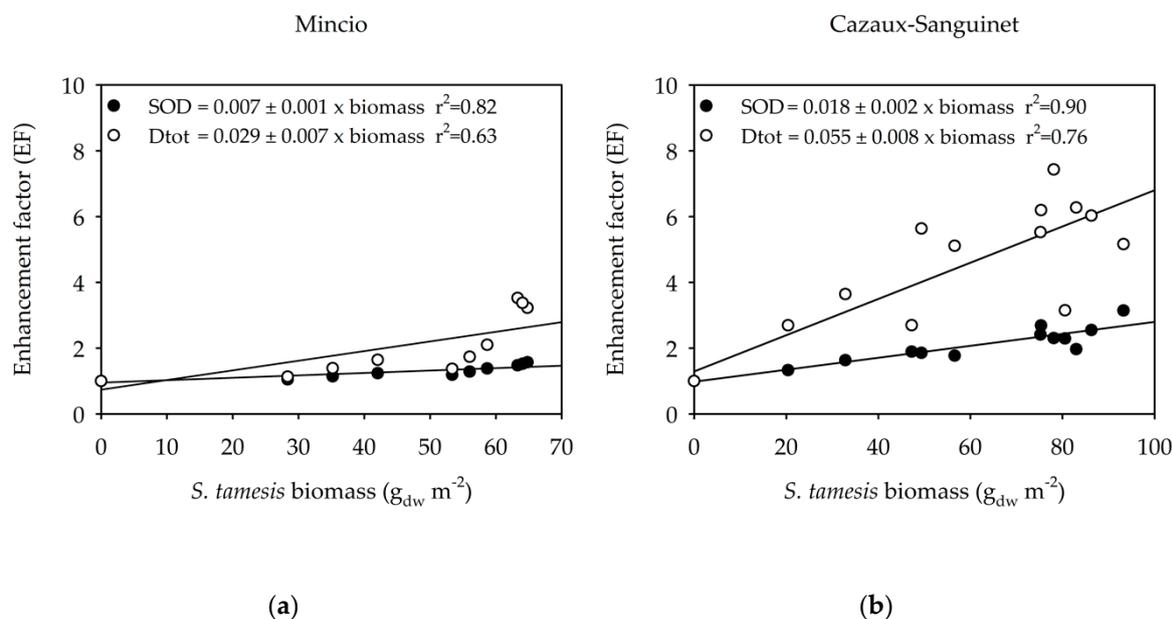

Figure 3. Enhancement factors of *Sparganophilus tamesis* biomass on sediment O_2 demand (SOD) and denitrification rates. Results of linear regressions are reported in the graphs. The relative stimulations were calculated by dividing the rates measured in the bioturbated sediments by the rates measured in control sediments, in the Mincio River (**a**) and in the Cazaux-Sanguinet Lake (**b**). Averages ± standard errors are reported.

Measured fluxes of the dissolved inorganic N forms had different trends, depending on the *S. tamesis* biomass (Figure 4). In the Mincio River, bare sediments acted as a source of NH_4^+ and NO_3^-. Ammonium fluxes were augmented along increasing oligochaete biomass, whereas the presence of the worms reversed NO_3^- fluxes, which turned highly negative (Figure 4a). In Cazaux-Sanguinet Lake, in the absence and presence of the low oligochaete biomass, sediments acted as a sink for all N forms, whereas in sediments with the worm biomass equal or higher than 65 g_{dw} m^{-2} the fluxes

were reversed and NH_4^+ and NO_3^- were regenerated to the water column ($NH_4^+ > NO_3^-$) (Figure 4b). Nitrite fluxes were low and negative in all treatments.

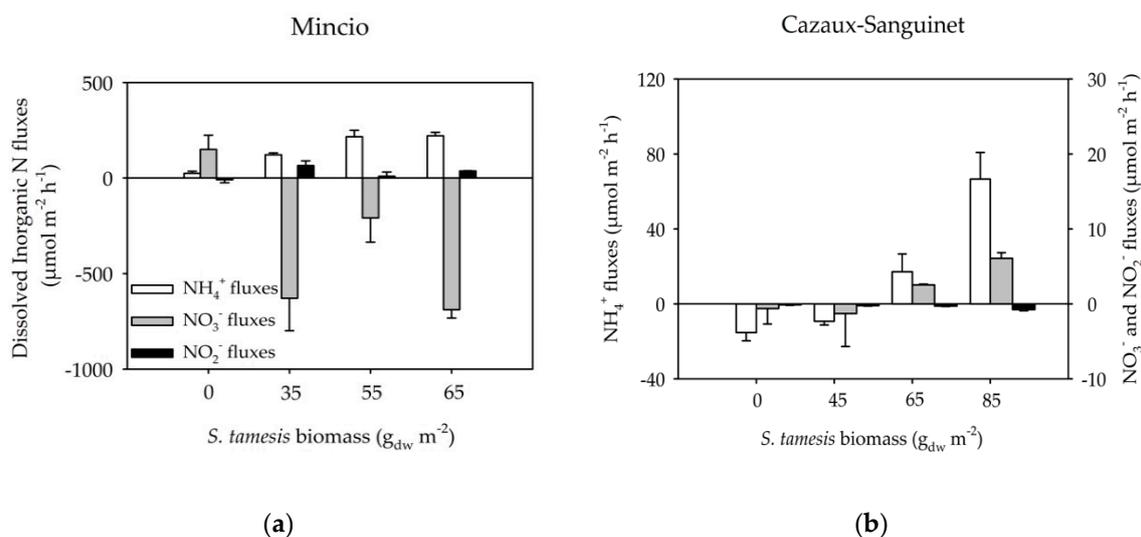(**a**)　　　　　　　　　　　　　　　　　　　　　　　　　　(**b**)

Figure 4. Benthic fluxes of N forms (NH_4^+, NO_3^-, and NO_2^-) expressed in $\mu mol\ m^{-2}\ h^{-1}$ measured during dark incubation in the sediment from the Mincio River (**a**) and from the Cazaux-Sanguinet Lake (**b**). Averages ± standard errors are reported. Note different scales on *y*-axes.

The mean value of DE in the four treatments increased along with the increase in the biomass of oligochaetes in Mincio sediments (Figure 5a), whereas the DE value decreased along with the increase in the biomass of oligochaetes in Cazaux-Sanguinet Lake, as a result of increasing and positive NH_4^+ fluxes (Figure 5b).
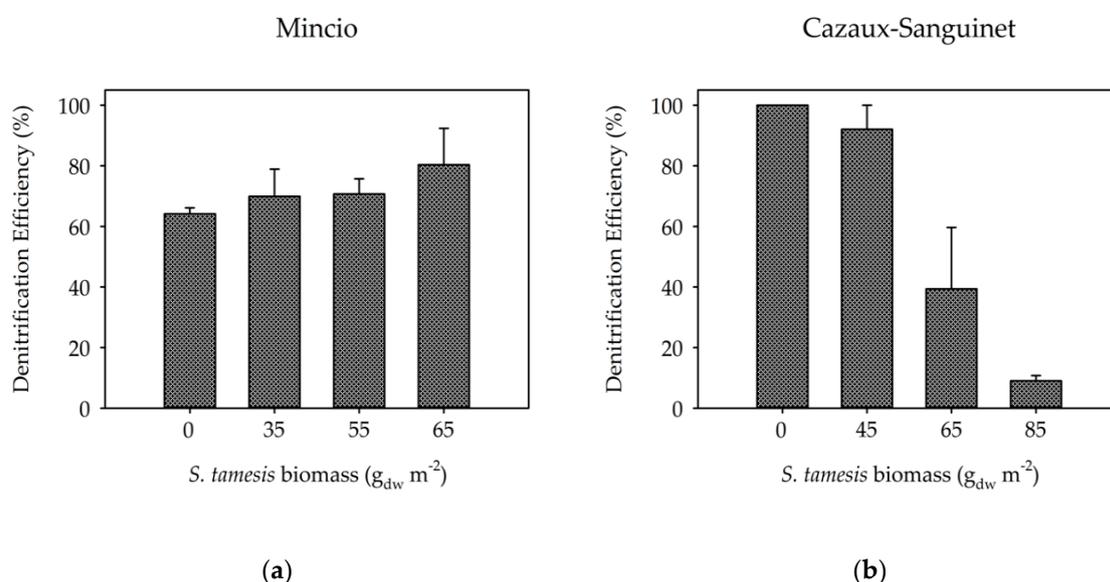

(**a**)　　　　　　　　　　　　　　　　　　　　　　　　　　(**b**)

Figure 5. Denitrification efficiency (%) calculated in the 4 treatments of (**a**) the Mincio River and of (**b**) the Cazaux-Sanguinet Lake. Averages ± standard errors are reported.

4. Discussion

We have investigated the biogeochemical effects of an alien, plastic species that is abundant in the contrasting environments of the eutrophic Mincio River and the oligotrophic Cazaux-Sanguinet Lake [21]. Our results suggest that *S. tamesis* produced opposite effects at the two sites on benthic N cycling. Such effects might be considered as positive (e.g., increased N removal from nutrient-enriched

Mincio) or negative (e.g., increased N recycling in the oligotrophic Cazaux-Sanguinet) for the invaded ecosystem. Analogous results are reported after the invasion of the bivalve *D. polymorpha*, whose ecosystem consequences can be opposite and site-specific (e.g., spanning from the control of algal blooms to their enhancement) [14,27]. Also, for the invasive worm *Marenzelleria* spp., different authors report contrasting ecosystem level effects, depending upon the invaded site and the considered temporal scale [1,15,16].

S. tamesis was found at both sites in proximity to native macrophytes: *Vallisneria spiralis* in Italy [28] and *Lobelia dortmanna* in the Cazaux-Sanguinet Lake [21]. The close association with macrophytes might depend on the favorable chemical conditions promoted by roots via radial oxygen loss [29] that may increase the survival of worm cocoons. A large plasticity is suggested for this species [21] that allows successful invasion in dramatically different sedimentary environments compared to those from the study sites. Different food availabilities or qualities locally affect the worms' growth, as adults retrieved from the two areas differ in size: they were larger in muddy sediments and smaller in sandy sediments. The two sites displayed a very different sediment oxygen demand, which was higher in Mincio, as muddy sediments were organic-rich and chemically reduced, and was lower in the organic-poor and oxidized sandy sediments at Cazaux-Sanguinet. The presence of oligochaetes increased O_2 consumption at both sites because of the worms' direct metabolic contribution and indirect stimulation of microbial or chemical processes [30,31]. However, the degree of stimulation was different and larger at the sandy site. We calculated that a worm biomass of 100 g_{dw} m^{-2} might stimulate sediment oxygen demand by a factor of 1.8 in sandy sediments, compared to an enhancement factor of 0.7 calculated for muddy sediments. Such a difference could be due to different porosities and solute transports in sandy VS muddy sediments.

Similarly, rates of total denitrification were different and two orders of magnitude higher at the Mincio site. Such a difference is likely a combination of higher organic carbon and higher NO_3^- concentrations in the water column of the Mincio site, resulting in elevated N_2 production mainly from D_W [32]. The presence of *S. tamesis* increased the rates of denitrification at both sites, but, as for oxygen demand, the degree of stimulation was different and higher in Cazaux-Sanguinet Lake. With a worm biomass of 100 g_{dw} m^{-2}, the enhancement factor of N_2 production in the sandy sediments was 5.5, whereas that calculated for the Mincio sediments was 2.9. Both denitrification enhancement factors were higher than those calculated for oxygen respiration. A higher relative stimulation of denitrification, as compared to O_2 consumption, was also reported by other authors in similar bioturbation experiments [33]. In the Mincio River, denitrification rates support a substantial fraction of CO_2 production (see next sections), whereas in the Cazaux-Sanguinet Lake, denitrification is quantitatively irrelevant as compared to the aerobic respiration.

In the Mincio sediments, the presence of the oligochaete reversed the role of the system from a source to a sink for NO_3^-, whereas sediments acted always as an NH_4^+ source. The presence of *S. tamesis* augmented NH_4^+ production, suggesting an important contribution to N regeneration by the reworking activities and the excretion by the oligochaetes [30,31]. However, in the Mincio sediments *S. tamesis* stimulated NO_3^- consumption to an extent that always exceeded NH_4^+ production, resulting in negative DIN fluxes. In Cazaux-Sanguinet Lake, a worm biomass equal to or higher than 65 g_{dw} m^{-2} reversed the role of sediment from N sink to N source, with NH_4^+ efflux as the main driver of the net DIN regeneration. The comparison between the two sites suggests that the effect of *S. tamesis* on net DIN fluxes depends on the environmental conditions, specifically the background nitrate level and the organic content.

Burrow ventilation and bioirrigation, with a few exceptions, always result in a higher consumption of O_2 and NO_3^- [34,35]. This is due to increased sediment–water interfaces and to thinner oxic layers within burrows along a vertical sediment profile [36–38]. A few species of burrowers (e.g., *Marenzelleria* spp.) do not stimulate denitrification, but rather NO_3^- ammonification due to specific pore water movement [15]. Some studies on N cycling in faunated sediments support the major contribution of NO_3^- from the overlying water to the total N_2 production [33,39–41]. This is likely true at the eutrophic sites with elevated

NO_3^- concentrations in the water column [38]. Other studies demonstrated that nitrification and, hence, coupled nitrification–denitrification had the highest contribution to N_2 production [42]. The latter result likely depends on factors such as the sediment redox or the NH_4^+ availability in the upper sediment layer. In sulfidic sediments, nitrification can be suppressed and the same can be true in oligotrophic sandy sites with elevated benthic microalgal activity and little exchangeable pools of NH_4^+. In the case of Cazaux-Sanguinet Lake, we believe that the low activity of the population of nitrifiers and the limited stimulation of nitrification by the oligochaete can be explained by NH_4^+ limitation, high competition with plants and benthic algae, and by general inhibition of these bacteria by primary producers [43]. On the other hand, the limited relevance of denitrification of water column NO_3^- in Cazaux-Sanguinet Lake strongly depended on NO_3^- concentration in the overlying water, which was very low (<6 μM). In the Mincio sediments, the high NH_4^+ availability enhanced the process of nitrification and, therefore, its share in the total N_2 production.

From NO_3^- reduction, it was also estimated that the denitrification in bare sediments contributed differently in the two sites: 11.5% and 0.1% of the total mineralization rate in Mincio and Cazaux-Sanguinet sites, respectively. O_2 fluxes were converted into CO_2 production, assuming an O_2:CO_2 with a 1:1 stoichiometry, whereas denitrification was converted into CO_2 production assuming a NO_3^-:CO_2 stoichiometry of 1.25 [44]. In the presence of the oligochaetes with the highest biomass, denitrification contributed 25.5% and 0.3% of the total CO_2 production. Hence, in both sediments the highest biomass of *S. tamesis* enhanced the contribution of the anaerobic denitrification to total mineralization rates, but such contribution was completely different at the two sites. The contrasting effects of *S. tamesis* on benthic N cycling at the two sites were confirmed by the calculation of denitrification efficiency, which increased in the Mincio River along with an increasing worm biomass. *S. tamesis* stimulated the process of denitrification and reduced the recycling of inorganic N forms to the water column. The opposite result was found in the Cazaux-Sanguinet Lake. Here, the denitrification efficiency measured in bare sediment was 100%, which suggested a tightly coupled ammonification, nitrification and denitrification, and no inorganic N efflux. Along with an increasing oligochaete biomass, denitrification efficiency decreased in the Cazaux-Sanguinet Lake due to increased NH_4^+ recycling.

Oxygen and DIN fluxes were related to the dry biomass of the incubated organisms with a linear regression. Assuming a 1:1 ratio of O_2 and CO_2 fluxes, we calculated a C:N molar ratio of excreted nutrients (8 for the Mincio River and 19 for Cazaux-Sanguinet Lake) from the slopes of the regression. The C:N ratio calculated for Cazaux-Sanguinet was within the range reported by [45] for the detritus of *L. dortmanna* (C:N = 19–26). This result suggests that *S. tamesis* in Cazaux-Sanguinet Lake likely feeds on *L. dortmanna* detritus, which is characterized by a low N content. The C:N calculated for Mincio reflected the C:N molar ratio of more labile organic matter.

Overall results from the present study demonstrated that the alien worm *S. tamesis* could have both positive and negative effects on the invaded ecosystem, depending on the trophic conditions of the environment. At the eutrophic site the high density of burrowers that dig and rework the sediment, and flush oxic and nitrate-enriched water into a reduced sediment, stimulate the process of denitrification, which could alleviate the high nutrient load of the system. At the oligotrophic site, bare sediment and sediment with a low biomass of oligochaetes per m^2 acted as buffers for inorganic N, whereas the increase of *S. tamesis* biomass enhanced the stimulation of benthic organic matter mineralization and nutrient cycling. Consequently, if the density of *S. tamesis* dramatically increased, the benthic–pelagic coupling of this shallow-water system would change, favoring the growth of pelagic primary producers and, hence, modify the overall function of the lake. The effects of alien species may vary with time, and a rapid expansion of *S. tamesis* combined with changes in environmental conditions (e.g., higher temperature regimes) may result in higher mobilization of nutrients buried within sediments. This may produce undesired effects such as the growth of epiphytic algae on the native isoetid *L. dortmanna* or of phytoplankton in the water column. In fact, previous studies demonstrated that >80% of the inorganic N requirements of *L. dortmanna* is absorbed by the roots, in the forms of NO_3^- rather than NH_4^+ [46]. It is, therefore, likely that higher ammonium availability would favor microalgal primary production. Higher phytoplanktonic or epiphytic

productivity may in turn enrich sediments with labile organic matter and create other positive feedbacks on benthic respiration and nutrient recycling [45]. All these cascade effects may hamper protected isoetid vegetation and ultimately result in a regime shift of the lake.

5. Conclusions

Results from this study support the hypothesis that the biogeochemical effects of an invasive worm on sedimentary processes are not univocal, but site-specific. In particular, they demonstrated that bioturbation of muddy sediments in contact with nitrate-rich water resulted in net removal of inorganic N and elevated denitrification efficiency. These biogeochemical effects can be considered positive, as they counteract eutrophication. On the contrary, bioturbation of sandy sediments in contact with a nutrient-poor water column produced a net effect of increased ammonium recycling and decreased denitrification efficiency. These biogeochemical effects have a negative connotation, as they might stimulate algal growth and produce positive feedback. The ecosystemic effects of invasions by alien species needs careful and comprehensive evaluations along large temporal scales.

References

1. Norkko, J.; Reed, D.C.; Timmermann, K.; Norkko, A.; Gustafsson, B.G.; Bonsdorff, E.; Slomp, C.P.; Carstensen, J.; Conley, D.J. A welcome can of worms? Hypoxia mitigation by an invasive species. *Glob. Chang. Biol.* **2012**, *18*, 422–434. [CrossRef]

2. Strayer, D.L. Alien species in fresh waters: Ecological effects, interactions with other stressors, and prospects for the future. *Freshw. Biol.* **2010**, *55*, 152–174. [CrossRef]

3. Kristensen, E. Benthic fauna and biogeochemical processes in marine sediments: Microbial activities and fluxes. In *Nitrogen Cycling in Coastal Marine Environments*; Blackburn, T.H., Sorensen, J., Eds.; John Wiley & Sons Ltd.: Hoboken, NI, USA, 1988; pp. 275–299.

4. Hietanen, S.; Laine, A.O.; Lukkari, K. The complex effects of the invasive polychaetes *Marenzelleria* spp. on benthic nutrient dynamics. *J. Exp. Mar. Biol. Ecol.* **2007**, *352*, 89–102. [CrossRef]

5. Strayer, D.L. Effects of alien species on freshwater mollusks in North America. *Freshw. Sci.* **1999**, *18*, 74–98. [CrossRef]

6. Crooks, J.A. Characterizing ecosystem-level consequences of biological invasions: The role of ecosystem engineers. *Oikos* **2002**, *97*, 153–166. [CrossRef]

7. Strayer, D.L.; Caraco, N.F.; Cole, J.J.; Findlay, S.; Pace, M.L. Transformation of freshwater ecosystems by bivalves: A case study of zebra mussels in the Hudson River. *Bioscience* **1999**, *49*, 19–27. [CrossRef]

8. Strayer, D.L. Twenty years of zebra mussels: Lessons from the mollusk that made headlines. *Front. Ecol. Environ.* **2009**, *7*, 135–141. [CrossRef]

9. Ruginis, T.; Bartoli, M.; Petkuviene, J.; Zilius, M.; Lubiene, I.; Laini, A.; Razinkovas-Baziukas, A. Benthic respiration and stoichiometry of regenerated nutrients in lake sediments with *Dreissena polymorpha*. *Aquat. Sci.* **2014**, *76*, 405–417. [CrossRef]

10. Sousa, R.; Novais, A.; Costa, R.; Strayer, D.L. Invasive bivalves in fresh waters: Impacts from individuals to ecosystems and possible control strategies. *Hydrobiologia* **2014**, *735*, 233–251. [CrossRef]

11. Fahnenstiel, G.L.; Lang, G.A.; Nalepa, T.F.; Johengen, T.H. Effects of Zebra Mussel (*Dreissena polymorpha*) Colonization on Water Quality Parameters in Saginaw Bay, Lake Huron. *J. Great Lakes Res.* **1995**, *21*, 435–448. [CrossRef]

12. Cha, Y.; Stow, C.A.; Bernhardt, E.S. Impacts of dreissenid mussel invasions on chlorophyll and total phosphorus in 25 lakes in the USA. *Freshw. Biol.* **2013**, *58*, 192–206. [CrossRef]

13. Gardner, W.S.; Cavaletto, J.F.; Johengen, T.H.; Johnson, J.R.; Heath, R.T.; Cotner, J.B., Jr. Effects of the zebra mussel, *Dreissena polymorpha*, on community nitrogen dynamics in Saginaw Bay, Lake Huron. *J. Gt. Lakes Res.* **1995**, *21*, 529–544. [CrossRef]

14. Caraco, N.F.; Cole, J.J.; Strayer, D.L. Top down control from the bottom: Regulation of eutrophication in a large river by benthic grazing. *Limnol. Oceanogr.* **2006**, *51*, 664–670. [CrossRef]

15. Kristensen, E.; Hansen, T.; Delefosse, M.; Banta, G.T.; Quintana, C.O. Contrasting effects of the polychaetes *Marenzelleria viridis* and *Nereis diversicolor* on benthic metabolism and solute transport in sandy coastal sediment. *Mar. Ecol. Prog. Ser.* **2011**, *425*, 125–139. [CrossRef]

16. Bonaglia, S.; Bartoli, M.; Gunnarsson, J.S.; Rahm, L.; Raymond, C.; Svensson, O.; Yekta, S.S.; Brüchert, V. Effect of reoxygenation and *Marenzelleria* spp. bioturbation on Baltic Sea sediment metabolism. *Mar. Ecol. Prog. Ser.* **2013**, *482*, 43–55. [CrossRef]

17. Quintana, C.O.; Kristensen, E.; Valdemarsen, T. Impact of the invasive polychaete *Marenzelleria viridis* on the biogeochemistry of sandy marine sediments. *Biogeochemistry* **2013**, *115*, 95–109. [CrossRef]

18. Kauppi, L.; Norkko, A.; Norkko, J. Large-scale species invasion into a low-diversity system: Spatial and temporal distribution of the invasive polychaetes *Marenzelleria* spp. in the Baltic Sea. *Biol. Invasions* **2015**, *17*, 2055–2074. [CrossRef]

19. Benelli, S.; Bartoli, M.; Zilius, M.; Vybernaite-Lubiene, I.; Ruginis, T.; Petkuviene, J.; Fano, E.A. Microphytobenthos and chironomid larvae attenuate nutrient recycling in shallow-water sediments. *Freshw. Biol.* **2018**, *63*, 187–201. [CrossRef]

20. Vanni, M.J. Nutrient cycling by animals in freshwater ecosystems. *Annu. Rev. Ecol. Syst.* **2002**, *33*, 341–370. [CrossRef]

21. Rota, E.; Benelli, S.; Erséus, C.; Soors, J.; Bartoli, M. New data and hypotheses on the invasiveness, habitat selection, and ecological role of the limicolous earthworm *Sparganophilus tamesis* Benham, 1892. *Arch. Hydrobiol.* **2018**, *192*, 129–136. [CrossRef]

22. Ribaudo, C.; Bertrin, V.; Jan, G.; Anschutz, P.; Abril, G. Benthic production, respiration and methane oxidation in *Lobelia dortmanna* lawns. *Hydrobiologia* **2017**, *784*, 21–34. [CrossRef]

23. Dalsgaard, T.; Underwood, J.C.; Nedwell, D.B.; Sundbäck, K.; Rysgaard, S.; Miles, A.; Bartoli, M.; Dong, L.; Thornton, D.C.O.; Ottosen, L.D.M.; et al. *Protocol Handbook for NICE-Nitrogen Cycling in Estuaries*; National Environmental Research Institute: Silkeborg, Denmark, 2000.

24. Golterman, H.L.; Clymo, R.S.; Ohnstad, M.A.M. *Methods for Physical and Chemical Analysis of Fresh Waters*, 2nd ed.; IBP Handbook; Blackwell Scientific: Oxford, UK, 1978; Volume 8.

25. Bower, C.E.; Holm-Hansen, T. A salicylate–hypochlorite method for determining ammonia in seawater. *Can. J. Fish. Aquat. Sci.* **1980**, *37*, 794–798. [CrossRef]

26. Nielsen, L.P. Denitrification in sediment determined from nitrogen isotope pairing. *FEMS Microbiol. Lett.* **1992**, *86*, 357–362. [CrossRef]

27. Benelli, S.; Bartoli, M.; Zilius, M.; Vybernaite-Lubiene, I.; Ruginis, T.; Vaiciute, D.; Petkuviene, J.; Fano, E.A. Stoichiometry of regenerated nutrients differs between native and invasive freshwater mussels with implications for algal growth. *Freshw. Biol.* **2019**. [CrossRef]

28. Magri, M.; Benelli, S.; Bondavalli, C.; Bartoli, M.; Christian, R.R.; Bodini, A. Benthic N pathways in illuminated and bioturbated sediments studied with network analysis: Network analysis of benthic N processes. *Limnol. Oceanogr.* **2018**, *63*, S68–S84. [CrossRef]

29. Marzocchi, U.; Benelli, S.; Larsen, M.; Bartoli, M.; Glud, R.N. Spatial heterogeneity and short-term oxygen dynamics in the rhizosphere of *Vallisneria spiralis*: Implications for nutrient cycling. *Freshw. Biol.* **2019**. [CrossRef]

30. Nizzoli, D.; Bartoli, M.; Cooper, M.; Welsh, D.T.; Underwood, G.J.C.; Viaroli, P. Implications for oxygen, nutrient fluxes and denitrification rates during the early stage of sediment colonisation by the polychaete *Nereis* spp. in four estuaries. *Estuar. Coast. Shelf Sci.* **2007**, *75*, 125–134. [CrossRef]

31. Bartoli, M.; Longhi, D.; Nizzoli, D.; Como, S.; Magni, P.; Viaroli, P. Short term effects of hypoxia and bioturbation on solute fluxes, denitrification and buffering capacity in a shallow dystrophic pond. *J. Exp. Mar. Biol. Ecol.* **2009**, *381*, 105–113. [CrossRef]

32. Pinardi, M.; Bartoli, M.; Longhi, D.; Marzocchi, U.; Laini, A.; Ribaudo, C.; Viaroli, P. Benthic metabolism and denitrification in a river reach: A comparison between vegetated and bare sediments. *J. Limnol.* **2009**, *68*, 133–145. [CrossRef]

33. Pelegri, S.P.; Nielsen, L.P.; Blackburn, T.H. Denitrification in estuarine sediment stimulated by the irrigation activity of the amphipod *Corophium volutator*. *Mar. Ecol. Prog. Ser.* **1994**, *105*, 285–290. [CrossRef]

34. Aller, R.C. Benthic fauna and biogeochemical processes in marine sediments: The role of burrow structures. In *Nitrogen Cycling in Coastal Marine Environments*; Blackburn, T.H., Sorensen, J., Eds.; John Wiley & Sons Ltd.: Hoboken, NJ, USA, 1988; pp. 301–338.

35. Pelegrí, S.P.; Blackburn, T.H. Effect of Bioturbation by *Nereis* sp., *Mya Arenaria* and *Cerastoderma* sp. on nitrification and denitrification in estuarine sediments. *Ophelia* **1995**, *42*, 289–299. [CrossRef]

36. Nielsen, O.; Gribsholt, B.; Kristensen, E.; Revsbech, N. Microscale distribution of oxygen and nitrate in sediment inhabited by *Nereis diversicolor*: Spatial patterns and estimated reaction rates. *Aquat. Microb. Ecol.* **2004**, *34*, 23–32. [CrossRef]

37. Kristensen, E.; Penha-Lopes, G.; Delefosse, M.; Valdemarsen, T.; Quintana, C.O.; Banta, G.T. What is bioturbation? The need for a precise definition for fauna in aquatic sciences. *Mar. Ecol. Prog. Ser.* **2012**, *446*, 285–302. [CrossRef]

38. Kristensen, E.; Delefosse, M.; Quintana, C.O.; Flindt, M.R.; Valdemarsen, T. Influence of benthic macrofauna community shifts on ecosystem functioning in shallow estuaries. *Front. Mar. Sci.* **2014**, *1*, 41. [CrossRef]

39. Jensen, K.; Jensen, M.; Kristensen, E. Nitrification and denitrification in Wadden Sea sediments (Königshafen, Island of Sylt, Germany) as measured by nitrogen isotope pairing and isotope dilution. *Aquat. Microb. Ecol.* **1996**, *11*, 181–191. [CrossRef]

40. Svensson, J.M. Influence of *Chironomus plumosus* larvae on ammonium flux and denitrification (measured by the acetylene blockage-and the isotope pairing-technique) in eutrophic lake sediment. *Hydrobiologia* **1997**, *346*, 157–168. [CrossRef]

41. Karlson, K.; Hulth, S.; Ringdahl, K.; Rosenberg, R. Experimental recolonisation of Baltic Sea reduced sediments: Survival of benthic macrofauna and effects on nutrient cycling. *Mar. Ecol. Prog. Ser.* **2005**, *294*, 35–49. [CrossRef]

42. Na, T.; Gribsholt, B.; Galaktionov, O.S.; Lee, T.; Meysman, F.J.R. Influence of advective bio-irrigation on carbon and nitrogen cycling in sandy sediments. *J. Mar. Res.* **2008**, *66*, 691–722. [CrossRef]

43. Risgaard-Petersen, N. Coupled nitrification-denitrification in autotrophic and heterotrophic estuarine sediments: On the influence of benthic microalgae. *Limnol. Oceanogr.* **2003**, *48*, 93–105. [CrossRef]

44. Richards, F.A. *Anoxic basins and fjords. Chemical Oceanography*; Ryley, J.P., Skirrow, G., Eds.; Academic Press: London, UK, 1965; pp. 611–645.

45. Sand-Jensen, K.; Borum, J.; Binzer, T. Oxygen stress and reduced growth of *Lobelia dortmanna* in sandy lake sediments subject to organic enrichment. *Freshw. Biol.* **2005**, *50*, 1034–1048. [CrossRef]

46. Schuurkes, J.A.A.R.; Kok, C.J.; Den Hartog, C. Ammonium and nitrate uptake by aquatic plants from poorly buffered and acidified waters. *Aquat. Bot.* **1986**, *24*, 131–146. [CrossRef]