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Allometric and Stoichiometric Traits Predict Nutrient Excretion Rates by Benthic Consumers

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Benthic consumers profoundly impact nutrient regeneration in coastal marine ecosystems. The concurrent nutrient imbalance and warming of our coastal seas will change the nutritional requirements and metabolic demands of these consumers, which may affect their ability to recycle nitrogen and phosphorous. Here we explore whether nutrient excretion rates of two benthic consumers, the Baltic clam (*Macoma balthica*) and the invasive spionid polychaete (*Marenzelleria* spp.) can be quantified with basic biological traits across seasons using allometric and stoichiometric relationships. We found species-specific N and P excretion rates that positively link to allometric traits, i.e., per individual rates increased with body mass and temperature; thus, high mass-specific excretion rates characterized small relative to large macrofaunal individuals. Interestingly, our body size scaling coefficients diverge from predictions by the metabolic theory of ecology (MTE) and the universal model of excretion. Furthermore, stoichiometric traits and stable isotope signatures ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) explained a minor additional proportion of variability in excretion rates among species. The excretion rates also varied strongly seasonally, with the highest nutrient recycling rates during summer months, when community $\text{NH}_4\text{-N}$ and $\text{PO}_4\text{-P}$ excretion clearly exceeded net sediment efflux. The seasonal pattern emphasized that changes in temperature and food availability drove metabolic processes and thus excretion rates of the benthic consumers, and indicated that these effects could outweigh the importance of animal biomass. Our results highlight the benefits of using allometric and stoichiometric traits when quantifying species-specific contributions to nutrient recycling in coastal marine environments, and in predicting alteration of function in response to environmental change.

Keywords: nutrient recycling, coastal ecosystems, Baltic Sea, biological traits, seasonal change, stoichiometry, macrofaunal communities

INTRODUCTION

The functional trait characteristics of organisms in combination with the environment define ecosystem process rates (Villnäs et al., 2013; Atkinson et al., 2017). By merging ecological stoichiometry with the metabolic theory of ecology (MTE), recent research has provided mechanisms to quantify the influence of consumers on elemental recycling rates on an

ecosystem level (Meunier et al., 2017). Still, few studies have addressed the applicability of these theories in resolving species contribution to ecosystem functions within and across taxa under differing environmental conditions (Allgeier et al., 2015; Atkinson et al., 2017, but see Sharitt et al., 2021).

Diverse assemblages of benthic consumers underpin nutrient recycling, a fundamental process of marine ecosystems. The acceleration of human impacts that alter the elemental balance (Carnicer et al., 2015; Peñuelas et al., 2020), temperature, and biodiversity of our coastal seas (Lotze et al., 2006; Cloern et al., 2016), may substantially alter the role of benthic consumers in biogeochemical cycling. Benthic fauna can affect ecosystem nutrient cycling indirectly through bioturbation, and directly through feeding, by retaining nutrients in their tissues, and by supplying nutrients *via* excretion and egestion (Vanni, 2002). Although many studies, including laboratory experiments (Norling et al., 2007), *ex situ* (Kauppi et al., 2017; Gammal et al., 2019) and *in situ* incubations (Villnäs et al., 2013; Norkko et al., 2013) confirm the contribution of benthic biodiversity and traits to sediment nutrient fluxes, they yield frequently complex and context-dependent results, both within and among ecosystems (Gammal et al., 2019). This inconsistency emphasizes the need to quantify the direct contribution of benthic consumers to nutrient recycling in coastal marine habitats, to predict how environmental change alters their impact across temporal and spatial scales (Snelgrove et al., 2014; Lohrer et al., 2015; Vanni & McIntyre, 2016; Allgeier et al., 2017). In this study we show that excretion rates of benthic communities, which represent important fluxes that affect ecosystem-level nutrient dynamics (Atkinson et al., 2017), can be quantified with basic biological traits when using allometric and stoichiometric relationships.

Theoretical ecological frameworks can help to predict the physiological process of excretion of inorganic nutrients, such as ammonium (NH_4^+) and phosphate (PO_4^{3-}) (Vanni et al., 2002). The metabolic theory of ecology (MTE; Gillooly et al., 2001; Brown et al., 2004), predicts that consumer metabolic rates, including excretion rates, scale predictably with body size and temperature, thus expressing an allometric relationship (Vanni et al., 2002; Allen & Gillooly, 2009). The allometric scaling of excretion (E) to body mass (M) is expressed as $E = aM^b$, where a and b are constants (Hall et al., 2007). The constant b, often called the allometric scaling coefficient, is < 1 for most aquatic animals as predicted by theory (Gillooly et al., 2001), but varies greatly among taxa (Vanni and McIntyre, 2016). This value implies that excretion rates do not increase proportionally to body size, instead smaller animals should have higher excretion rate per body mass unit (i.e., mass-specific excretion rates) than larger conspecifics (Vanni, 2002; Hall et al., 2007; Alves et al., 2010). Hence, when the biomass of two different communities are the same, a community dominated by many small individuals should exhibit higher excretion rates compared to one where large individuals dominate (Hall et al., 2007). Moreover, for poikilotherms, metabolism and hence excretion rates are temperature dependent and increase as temperatures rise (Allen & Gillooly, 2009).

In addition to MTE, ecological stoichiometry, which describes the balance of elements in an organism and related ecological processes (Sterner and Elser, 2002), likely affects species' excretion rates. The theory of ecological stoichiometry suggests that animals should be homeostatic in body nutrient composition, at least over short time scales (Persson et al., 2010; but see e.g. Liess & Hillebrand, 2005, Mäkelin and Villnäs 2022). Therefore, the rates and ratios at which animals excrete and egest nutrients should reflect any differences in nutrient composition between animals and their food sources. Whereas the C:N and C:P ratios of consumer tissues can reflect the balance of energy versus nutrient limitation, the N:P ratio of consumers should be directly relevant for predicting excretion rates of these two elements and the ratio at which they are excreted (Frost et al., 2006; Alves et al., 2010; Vanni & McIntyre, 2016). For example, the N:P excretion ratio could potentially be higher in small consumers, because of the anticipated lower N:P ratio in their tissues; small animals often grow faster, increasing their demand for P (allocated to ribosomal RNA during rapid growth; growth rate hypothesis; Elser et al., 2003).

Recent comprehensive data syntheses regarding excretion rates by aquatic animals show that metabolic parameters, such as body mass and temperature, perform better than stoichiometric parameters in predicting animal excretion rates (Allgeier et al., 2015; Vanni & McIntyre, 2016). Notably, these studies also found strong species identity effects on nutrient recycling (Vanni et al., 2002; Allgeier et al., 2015; Vanni & McIntyre, 2016), presumably reflecting species-specific nutrient requirements as well as on ontogenetic shifts in dietary nutrients (Isaac and Carbone, 2010; Allgeier et al., 2015). Stable isotope signals ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of consumer tissues may mirror changes in diets, where $\delta^{13}\text{C}$ responds to the carbon (energy) source for the organism while $\delta^{15}\text{N}$ reflects the trophic position of the consumer, which is typically enriched 3.4 ‰ relative to its diet (Post, 2002; Fry, 2006). Following “you are what you eat less excrete” (Fry, 2006), variation in tissue stable isotope signatures can potentially influence both inter- and intraspecific excretion rates, although isotopic composition typically integrates variation in diet over a longer period of time than the time scale of most excretion rate estimates.

The observed intra- and interspecific variation in excretion rates points to the importance of identifying biological traits responsible for changes in animal-driven nutrient dynamics (cf. Enquist et al., 2016; Meunier et al., 2017). In this study, we explore the excretion rates of two dominant species within the Baltic Sea, the bivalve *Macoma balthica* (Linnaeus 1758) and the invasive polychaete *Marenzelleria* spp. (Mesnil 1896). We hypothesized that the excretion rates of these consumers would be species-specific and increase with body size, but with an allometric scaling coefficient < 1 , resulting in higher mass-specific excretion rates in smaller animals. We compared the N and P excretion rates of *M. balthica* and *Marenzelleria* spp. to the universal model of excretion rates developed by Vanni & McIntyre (2016). We further explored whether excretion rates and ratios change between seasons, and if variation in allometric or stoichiometric traits or differences in stable isotope signatures

of animal tissue could explain such changes. Finally, we explored the magnitude of excreted N and P at the community level, and compared the results with overall sediment nutrient fluxes measured with *ex-situ* incubations in July.

METHODS

Study Site

Our two study sites were situated in the south-western Finnish archipelago, in the brackish-water, non-tidal Baltic Sea. The sites were sampled in July, August, October and January, when bottom water temperatures were 8°C, 18°C, 12°C and 4°C, respectively (Mäkelin & Villnäs 2022, **Table S1**). Station XXVI (20 m depth) is situated in a relatively open, dynamic archipelago with a connection to the open sea. Upwelling events that bring in nutrient-rich water from deeper areas in the Gulf of Finland occasionally affect this station (Kauppi et al., 2018a). The other site, IH4 (18 m depth) is situated between islands and borders a deeper area that can experience strong seasonal stratification and periodic oxygen deficiency (see Gammal et al., 2017 for geographic positions; XXVI is O3). However, during all sampling occasions at the stations, we observed similar bottom water salinity (5.5-6.5), and oxygenated conditions (>7.8 mg/l; **Table S1**). Seasonal differences were most apparent in primary production, with reduced Chl *a* values in surface waters in January (0.3 µg l⁻¹) compared to the other sampling occasions (3.2-7.6 µg l⁻¹; **Table S1**, Mäkelin & Villnäs 2022). Silty sediments with a surface organic matter content ranging between 6-12.5% characterize both sites (averaging 7.6 ± 1.5% at XXVI and 11.7 ± 0.6% at station IH4, respectively, **Table S1**). Generally higher carbon and nitrogen content (C:N:P 106:12:1) characterized the uppermost sediment layer (0-1 cm) at the latter site compared to XXVI (C:N:P 73:8:1). In contrast, sediment P content was similar at both sites (0.15 ± 0.02% at IH4 versus 0.14 ± 0.4% at XXVI, percent of dry mass; **Table S1**).

The tellenid bivalve *Macoma balthica* dominates the benthic community at both stations (representing, in general, >50% of total macrofaunal abundance and >90% of total dry biomass, that is >60% if excluding its shell). The invasive spionid polychaete complex, *Marenzelleria* spp. accounts for 6-23% of community abundance, while its contribution to total community biomass varies between 0-3%, or 2-8% when excluding bivalve shells (**Figure S1** and **Figure 4**). *M. balthica* has high biomass and abundance within the Baltic Sea (Bonsdorff et al., 1995). This facultative deposit- and suspension-feeder (Rossi et al., 2004) plays a key role in ecosystem processes such as energy transfer in the benthic food web and in sediment biogeochemical cycling (Norkko et al., 2013). The invasive spionid polychaete, *Marenzelleria* spp. was introduced to the southern Baltic Sea in 1985 and has rapidly spread to become a dominant species throughout the entire Baltic Sea (Kauppi et al., 2015). Three species, *M. viridis* (Verrill 1873), *M. neglecta* (Sikorski and Bick 2004), and *M. arctica* (Chamberlin 1920) have been reported in our study area, where these burrowing, sub-surface deposit feeding polychaetes can occur in high densities (Kauppi et al., 2018a).

Nutrient Flux Measurements

We measured nutrient excretion (NH₄-N, PO₄-P), body (dry) mass and tissue carbon (C), nitrogen (N) and phosphorous (P) concentrations as well as stable isotope signatures (δ¹³C and δ¹⁵N) primarily of *M. balthica* and *Marenzelleria* spp. during four different sampling occasions at the two stations. We also measured excretion rates of less dominant species (the priapulid *Halicryptus spinulosus*, the isopod *Saduria entomon*, the amphipod *Monoporeia affinis* and Chironomidae) in the community if they occurred during a sampling event. Benthic sampling utilized a box corer, and we collected three replicate samples to account for variation in benthic abundance and biomass at the sites. The sediment was sieved (0.5 mm) and animals picked for excretion, elemental content, and isotope analyses were gently rinsed before incubating them in 110 ml of filtered seawater.

The animals were kept in dark conditions at *in situ* temperatures during the excretion incubations (using climate controlled rooms). It is possible that the incubations stressed the animals, noting they had no sediment available or time to acclimate. Therefore our measured rates may represent their maximum excretion potential. For *M. balthica*, we performed a minimum of nine incubations per sampling time and station, and at least five replicate measurements for *Marenzelleria* spp. When incubating several animals together, we selected individuals of similar size. We separated *M. balthica* into the following size classes; <0.5, 0.5-0.9, 1.0-1.4, 1.5-1.9, ≥2 cm shell width, and determined the size class of the polychaete *Marenzelleria* spp. by measuring the width of the 5th segment; small (<0.9 mm), medium (ca 1 mm), large (>1.1 mm). Although we incubated large individuals of *M. balthica* (> 2 cm) separately, we grouped up to 35 juveniles (<0.5 cm) (Mäkelin & Villnäs 2022). As a control treatment, we incubated *M. balthica* shells separately to evaluate the effect of any biofilm on water nutrient concentrations. Before and after 1 to 1.5 h of incubation, the water was sampled with a syringe and filtered (GF/F) to remove feces and pseudofeces prior to freezing (-20°C), pending analyses of excreted dissolved nitrogen (N as NH₄-N) and phosphorous (P as PO₄-P). Ammonium was determined manually by a spectrometric procedure, following the ISO 7150/1-1984 method. Phosphate determination used the antimony-molybdate method (Koroleff, 1983) on a Thermo Scientific Aquakem 250 analyzer.

After collecting water samples for excretion rate measurements, the animals remained in filtered seawater for a total of 24 h, to allow them to empty their guts. The animals were frozen (-70°C) before dissection to remove bivalve shells. After freeze drying (-60°C during 24h), we measured the dry weights and prepared the specimens for elemental and stable isotope (δ¹³C and δ¹⁵N) analyses as described in Mäkelin and Villnäs (2022).

Additionally, in July, we incubated four intact sediment cores at each sampling site to measure sediment oxygen consumption and solute fluxes (NH₄-N, (NO₃⁻+NO₂⁻)-N, PO₄-P) across the sediment-water interface. Following collection of split cores (ø 90 mm, obtained with a Gemax twin corer), we sealed the upper section of the cores for use as a flux chamber, incubating the cores for 4 h in darkness at *in situ* temperature conditions under constant stirring. Water samples obtained at the start and end of the incubation were analyzed for oxygen concentration (Winkler

procedure) and for dissolved nutrients (as described above). Differences in concentration between start and end were used to calculate the sediment oxygen and nutrient fluxes ($\text{mol m}^{-2} \text{h}^{-1}$). For further description of the method, see Gammal et al. (2016). After incubation, we sieved the sediment (0.5 mm) and determined the abundance and biomass of the benthic animals in the cores.

Statistical Analyses

Variation in benthic tissue C:N:P content and stable isotope signatures ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) between the two species, the two sites and between the four sampling times was analyzed with a three-way permutational analysis of variance (PERMANOVA, PRIMER v7, Anderson et al., 2008) and illustrated with box-plots diagrams. For further information on variation in stoichiometric traits at the study sites see Mäkelin and Villnäs (2022). We calculated excretion rates as the difference between final and initial nutrient concentrations per hour, expressed either per individual ($\mu\text{mol N or P ind}^{-1} \text{h}^{-1}$) or per dry mass ($\mu\text{mol N or P g}^{-1} \text{h}^{-1}$). The excretion calculations did not consider the effect of the control incubations (*M. balthica* shells) because we most often detected only minor changes in nutrient concentrations. Thus, effects of shell biofilm on water nutrient concentrations are included in our results. A three-way permutational analysis of variances (PERMANOVA, PRIMER v7; Anderson et al., 2008) with individual dry mass (g) as covariate explored differences in excretion rates between species, sites and among sample dates, allowing for interactions. We did not assume homogeneity of slopes because the model included the interaction term, explicitly allowing for different slopes for different levels of the factor (Anderson et al., 2008). Pairwise post-hoc tests pinpointed differences between sampling occasions. A three-way PERMANOVA ensured detection of similar patterns for biomass-corrected excretion rates. Prior to the analyses, PERMDISP tests checked homogeneity of dispersions.

We compared our measured excretion rates for *M. balthica* and *Marenzelleria* spp. to estimates obtained using the universal excretion model by Vanni & McIntyre (2016). By applying an extensive global data set to explore excretion rates of aquatic consumers (marine and freshwater), Vanni and McIntyre (2016) found that body mass, water temperature and trophic guild likely yield sufficient general excretion estimates for invertebrates. Specifically, we used the equation for invertebrates feeding on algae or detritus; $\log_{10}(\text{N excretion } \mu\text{g ind}^{-1} \text{h}^{-1}) = 1.4610 + 0.6840 * \log_{10}(\text{dry mass}) + 0.0246 * \text{Temperature } (^{\circ}\text{C}) - 0.0389$, whereas $\log_{10}(\text{P excretion } \mu\text{g ind}^{-1} \text{h}^{-1}) = 0.6757 + 0.5656 * \log_{10}(\text{dry mass}) + 0.0194 * \text{Temperature } (^{\circ}\text{C}) + 0.0173$ (for further details see Vanni & McIntyre, 2016). The first factor describes the intercept and the last accounts for the trophic guild of the animal. Note that dry mass for bivalves should exclude the shell (Vanni & McIntyre, 2016).

Distance-based linear models (DistLM in PERMANOVA+ for PRIMER v7, Anderson et al., 2008) determined the importance of variation in allometric (biomass, temperature) and stoichiometric (tissue C, N, P % and ratios) traits as well as stable isotope signatures ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) on excretion rates of N and P ($\mu\text{mol ind}^{-1} \text{h}^{-1}$). An average value per size class of each species at a sampling site and

time represented the stoichiometric parameters and stable isotope signatures. \log_{10} transformations of the predictors reduced skewness. To explore whether predictors varied in importance between the two species we analyzed them together as well as separately.

Finally, we calculated a maximum community excretion potential ($\mu\text{mol NH}_4\text{-N or PO}_4\text{-P m}^{-2} \text{h}^{-1}$) using the biomass-corrected excretion rates for the six dominant species at the two sites (*M. balthica*, *Marenzelleria* spp., *H. spinulosus*, *S. entomon*, *M. affinis* and Chironomidae), which comprised over 90% of the total biomass at each sampling occasion. The average excretion rate per size class ($\mu\text{mol g}^{-1} \text{h}^{-1}$) of a species at a sampling occasion was multiplied by the corresponding biomass per replicate sample. Differences between sites and time-points were analysed with PERMANOVA as described above. In addition, in July we calculated excretion rates for the fauna within the incubated cores and compared their excretion to total sediment nutrient fluxes.

RESULTS

Stoichiometric Traits and Stable Isotope Signatures of the Benthic Animals

Overall, the bivalve *M. balthica* had a slightly higher tissue carbon content and lower nitrogen and phosphorous content (on average C: 43.3 ± 3.0 , N: 8.5 ± 1.3 , P: $0.7 \pm 0.3\%$ of dry mass) compared to *Marenzelleria* spp. (C: 42.2 ± 2.8 , N: 9.9 ± 0.7 , P: $0.8 \pm 0.2\%$, **Table S2**; $p < 0.05$, for further information, see Mäkelin & Villnäs 2022). Hence, we observed higher C:N and C:P ratios on average for the bivalve, but detected no difference in body N:P ratio among the species (**Figure S2** and **Table S2**; $p > 0.05$). The C:N ratios correlate significantly and positively with body mass, and show significant seasonal variation, with lower C:N and C:P ratios during winter months (**Figure S2** and **Table S2**, Mäkelin & Villnäs 2022). Values of $\delta^{15}\text{N}$ yielded the most pronounced difference in stable isotope signatures between the two species, with significantly lower values in *M. balthica* (on average $\delta^{15}\text{N}$: 8.0 ± 0.5) compared to the polychaete ($\delta^{15}\text{N}$: 9.8 ± 0.6 , **Figure S2** and **Table S2**). Seasonal differences in $\delta^{13}\text{C}$ were also noted, with slightly lower $\delta^{13}\text{C}$ values measured in July (**Figure S2**; Mäkelin & Villnäs 2022).

Inter- and Intraspecific Differences in Excretion Rates

The individual excretion rates of N and P co-varied (**Table 1**) and increased with body mass for both species (**Figures 1A, B**). Not surprisingly, given its larger size range, greater variation in excreted nutrients per individual characterized *M. balthica* compared to *Marenzelleria* spp. (PERMDISP $p < 0.01$ for N excretion $\text{ind}^{-1} \text{h}^{-1}$). Nonetheless, we detected significant differences in N and P excretion rates between the two species, both when considering individual (**Table 1** and **Figures 1A, B**) and mass-specific nutrient excretion rates (PERMDISP; $p > 0.05$, PERMANOVA; $p < 0.001$, **Table S3**). *Marenzelleria* spp. mass-specific excretion rates exceeded those in the bivalve (cf. **Figure 1, Table S3**), and although the average N:P excretion ratio was

TABLE 1 | Three-way PERMANOVA with individual biomass (dw, g) as co-variate exploring differences in N and P excretion rates ($\mu\text{mol ind.}^{-1} \text{h}^{-1}$) between species, seasons (month) and sampling stations.

Source of variation	N excretion ($\mu\text{mol ind.}^{-1} \text{h}^{-1}$)				P excretion ($\mu\text{mol ind.}^{-1} \text{h}^{-1}$)				Excretion N:P			
	df	MS	pseudo-F	p-value	df	MS	pseudo-F	p-value	df	MS	pseudo-F	p-value
Biomass (\log_{10})*	1	14.86	416.93	<0.001	1	12.45	153.85	<0.001	1	0.04	0.83	0.376
Species (Sp)	1	1.42	39.81	<0.001	1	4.81	59.41	<0.001	1	0.67	12.38	0.001
Month	3	1.72	48.14	<0.001	3	0.74	9.09	<0.001	3	0.59	10.94	<0.001
Station	1	0.16	4.59	0.044	1	0.50	6.16	0.016	1	1.65	30.65	<0.001
Biomass x Sp	1	0.15	4.19	0.056	1	0.46	5.66	0.018	1	0.54	9.99	0.002
Biomass x Month	3	0.58	16.18	<0.001	3	1.18	14.59	<0.001	3	0.38	7.05	0.001
Biomass x Station	1	0.21	5.81	0.027	1	0.00	0.01	0.930	1	0.02	0.32	0.571
Species x Month	3	0.23	6.54	0.002	3	0.08	0.97	0.404	3	0.05	0.93	0.427
Species x Station	1	0.07	1.82	0.209	1	0.03	0.33	0.574	1	0.02	0.33	0.569
Month x Station	3	0.08	2.21	0.123	3	0.09	1.11	0.355	3	0.14	2.51	0.065
Sp x Month x Station	3	0.07	1.85	0.175	3	0.02	0.31	0.806	3	0.04	0.79	0.488
Residuals	103	0.04			94	0.08			91	0.05		
Total	133				124				121			
<i>post hoc</i> :	<i>Macoma</i> : all months differ				<i>Macoma</i> : July-Oct, Jan;				Month: July-Oct, Jan;			
	<i>Marenzelleria</i> : July-Oct, Jan; Aug-Oct, Jan				Aug - Oct, Jan				Aug - Oct, Jan			
					<i>Marenzelleria</i> : ns							

*Non-significant interaction terms with the co-variable (biomass) are not presented. Excretion rates and dry mass are \log_{10} transformed.

highly variable, it was slightly lower for the polychaete compared to the bivalve (Figure 1C and Table 1). We found no significant relationships between the excreted N:P ratio vs. body mass (Figure 1C) or tissue N:P ratio considering both species (Pearson correlation $p > 0.5$). However, we noted an overall difference in excreted N:P between sites, with slightly higher ratios at site XXVI compared to IH4 (Table 1).

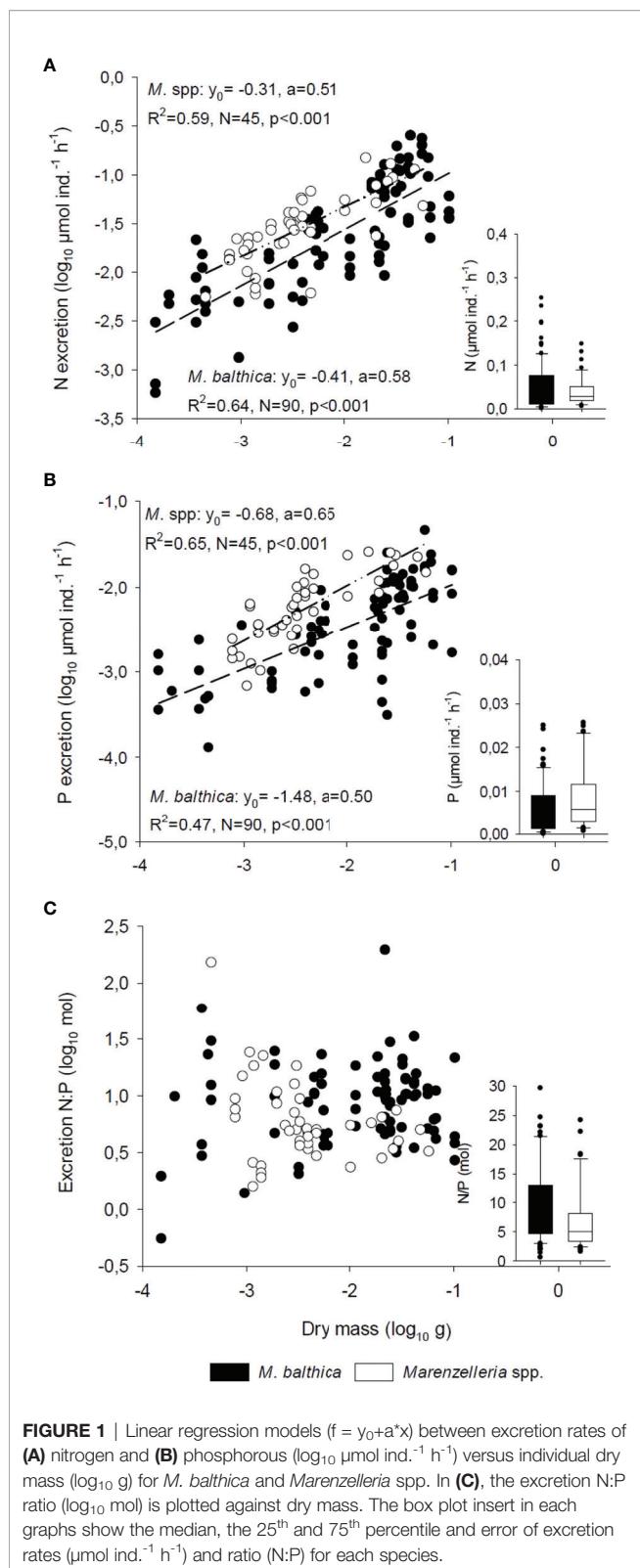
We found species-specific seasonal differences in excretion rates of both N and P (Table 1, Table S3 and Figures 2A, B). For N excretion, all time-points differed significantly for *M. balthica* (*post hoc* $p < 0.05$), whereas the greatest differences for *Marenzelleria* spp. occurred between the summer (July-August) vs. winter (October-January) months (Table 1 and Table S3). Monthly regressions between N excretion rates ($\mu\text{mol ind.}^{-1} \text{h}^{-1}$) and dry mass showed the highest slopes in July, whereas the intercept decreased from summer to winter (Table S4). We also detected positive slopes between individual N excretion rates and tissue N content (July: $R^2 = 0.41$, $p < 0.05$, August: $R^2 = 0.22$, $p < 0.01$, October: $R^2 = 0.58$, $p < 0.001$, January $R^2 = 0.34$ $p < 0.001$), whereas the intercept showed a notable decrease from summer to winter months (Figure 2C). Overall, patterns in P excretion rates resembled those in N over time (Figure 2B and Table 1). The highest P excretion rates of *M. balthica* occurred in summer, with significantly lower rates in January compared to both August and July (Table 1 and Figure 2B). Interestingly, *post hoc* comparisons detected no seasonal differences in P excretion of *Marenzelleria* spp. (Table 1). The slopes and intercepts of monthly regressions between individual P excretion rates and body mass decreased from summer to winter (Table S4). We did not observe strong relationships between P excretion rate and P tissue content (Figure 2D), detecting only a weak positive trend in July ($R^2 = 0.2$, $p < 0.05$) and a negative relationship in January ($R^2 = 0.39$, $p < 0.001$) and non-significant relationships for August and October ($p > 0.05$).

The excreted N:P ratio was, overall, slightly higher in July and August compared to October and January for the two species (Table 1).

We compared our measured excretion values to the universal excretion model by Vanni and McIntyre (2016). When testing their model on our two dominant species (Figure 3), we found similar slopes for *M. balthica* measured versus modelled excretion rates ($\mu\text{g N or P ind.}^{-1} \text{h}^{-1}$), but markedly lower intercepts for the measured than for the modelled rates (Figure 3). In contrast, the slope of N and P excretion of *Marenzelleria* spp. differed from the modelled value (lower for N, higher for P) and the measured N intercept did not converge with the modelled values (Figure 3).

Predictors of Individual Excretion Rates

Allometric variables, particularly size (individual tissue biomass) and temperature were the most important factors explaining variation in species N and P excretion rates. Whereas individual mass alone often accounted for over half of the explained variation, temperature differences had less impact, explaining only 4-14% of the variation (Table 2). In addition, temperature was not a significant predictor of P excretion by *Marenzelleria* spp., nor of the N:P excretion ratio of the two species. Interestingly, when considering both species, the model included $\delta^{15}\text{N}$ as an explanatory variable for both N and P excretion (accounting for 4.6 and 12.4% of the total explained variation, respectively). Different stoichiometric variables increased the explained variation foremost in N excretion; specifically, the tissue C:N ratio explained 4.5% of the overall variation in *M. balthica* N excretion, whereas the model explaining variability in N excretion by *Marenzelleria* spp. included the tissue N:P ratio and the tissue N content (%) (Table 2, Figure 2C). Regarding P excretion by both species, the overall model included $\delta^{13}\text{C}$ signature and tissue C content



(%), and C content was also a significant predictor variable when considering P excretion of *M. balthica* (Table 2). We could not find a satisfactory explanatory model for the excreted N:P ratio,

but species identity and biomass together explained 12.4% of the variation (Table 2).

Seasonal Variation in Community Excretion Rates

Overall, benthic community abundance and biomass were high at both stations in this productive coastal area (Figure S1, Figure 4), but community excretion rates showed clear seasonal differences (PERMANOVA; $p < 0.001$, Table S5). Substantially higher rates occurred in summer (July, August) compared to October and January (Figure 4). Despite high variability in excretion, especially during the summer months, we nonetheless observed a difference in community P excretion rate between stations (Table S5), with a generally higher excretion N:P ratio at station XXVI (on average 12.8 ± 3.3) compared to IH4 (6.0 ± 1.9 ; October values excluded from both stations). Although *M. balthica* and *Marenzelleria* spp. accounted for at least 70% of the total invertebrate community excretion rate at all times, the crustaceans *M. affinis* and *S. entomon* as well as Chironomidae also contributed significantly when present (Figure 4). However, we noted a deteriorated community with lower excretion rates at IH4 in October (Figure 4).

In July, the benthic community N excretion rates calculated for animals within the flux core incubations were substantially higher (station XXVI: 78.1 ± 44.2 , IH4: $44.9 \pm 19.2 \mu\text{mol h}^{-1} \text{m}^{-2}$) than the measured sediment efflux of $\text{NH}_4\text{-N}$ at both stations (efflux at station XXVI: 18.5 ± 5.4 , IH4: $11.4 \pm 22.2 \mu\text{mol h}^{-1} \text{m}^{-2}$, Figure S3). We measured ($\text{NO}_3^- + \text{NO}_2^-$)-N effluxes of similar magnitude at the two sites (XXVI: 11.52 ± 2.24 , IH4: $7.58 \pm 2.15 \mu\text{mol h}^{-1} \text{m}^{-2}$, Figure S3). Regarding phosphate, the benthic community P excretion rates in the cores (XXVI: 5.1 ± 2.8 , IH4: $10.7 \pm 4.7 \mu\text{mol h}^{-1} \text{m}^{-2}$) generally resembled the measured P efflux from sediments (XXVI: 8.8 ± 5.3 , IH4: $4.6 \pm 1.7 \mu\text{mol h}^{-1} \text{m}^{-2}$, Figure S3).

DISCUSSION

Our study confirms that benthic consumers profoundly impact nutrient regeneration in productive coastal marine ecosystems. We show high and variable maximum potential excretion rates from aphotic benthic communities, depending on community composition and season. As expected, excretion rates decreased from summer to winter months, but the dominant species, *M. balthica* and *Marenzelleria* spp., primarily drove community nutrient recycling rates at all times. In line with current literature (e.g. Vanni et al., 2002; Alves et al., 2010; Allgeier et al., 2015; Vanni & McIntyre, 2016), individual excretion rates of both N and P were species-specific and positively related to increases in traits relevant to metabolic ecology, i.e. to individual body mass and temperature, whereas small individuals had higher mass-specific excretion rates than larger ones. Interestingly, stoichiometric traits and stable isotope signatures explained a minor, but significant, amount of the variability in excretion, particularly between species. Our results emphasize the benefits of using allometric and stoichiometric traits of benthic consumers when quantifying species-specific contributions to nutrient

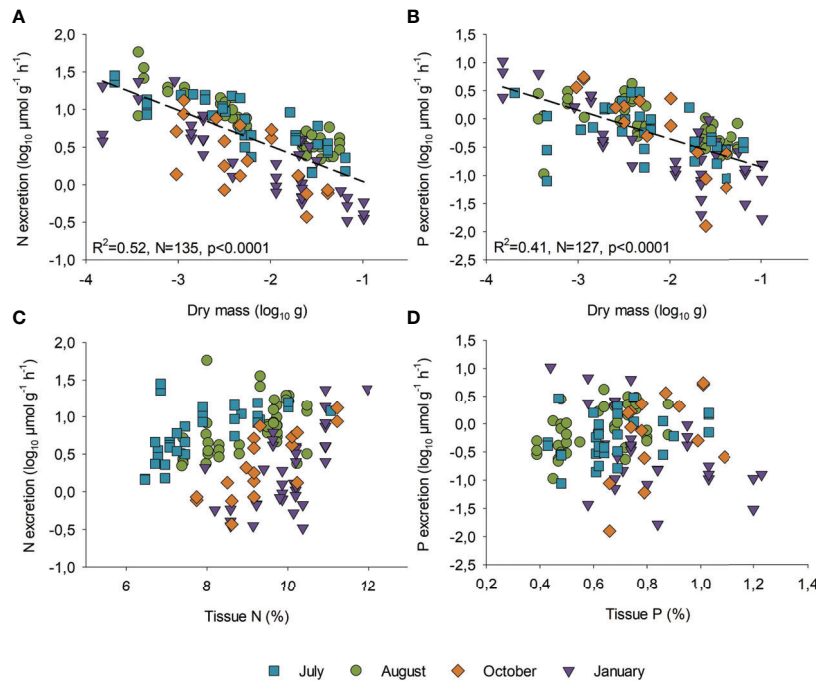


FIGURE 2 | Scatter plots presenting the seasonal differences in biomass-specific (A) nitrogen and (B) phosphorus excretion rates (log₁₀ μmol g⁻¹ h⁻¹) versus dry mass (log₁₀ g) for the two species. The linear regression ($f = y_0 + a \cdot x$) is fitted to all data. The relation between nutrient excretion rates to tissue N (%) and P (%) content are presented in (C, D), respectively.

recycling in coastal marine environments, and when predicting how function may respond to environmental change.

The metabolic theory of ecology suggests a scaling coefficient of 0.75 with body mass for predicting physiological rates of organisms (Brown et al., 2004), including excretion rates of N and P (Allen and Gillooly, 2009). On average, scaling coefficients <0.75 characterized

our study species, and these coefficients differed between the bivalve (slope: 0.62 for N and 0.55 for P) and the polychaete (0.50 for N, 0.65 for P; **Figure 3**). Importantly, our data support strong temperature dependence of metabolism and thus excretion rates (Gillooly et al., 2001). The low bottom water temperatures, especially in January (4–5°C) slow down metabolic reactions (Gillooly et al., 2001)

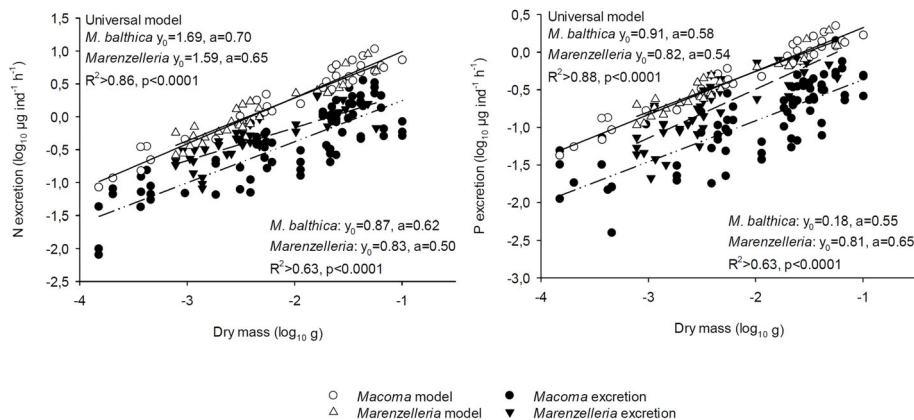


FIGURE 3 | Measured versus modelled excretion rates for *M. balthica* and *Marenzelleria* spp. Note that excretion is given as μg ind⁻¹ h⁻¹ (log₁₀). The universal excretion model by Vanni & McIntyre (2016) was used for calculating excretion rates of both N and P, accounting for variation in individual dry mass as well as temperature. Linear regression models ($f = y_0 + a \cdot x$) were used to relate the modelled excretion rates of the species to dry mass (continuous line). Regression for measured excretion values versus dry mass are marked with line-dot for *M. balthica*, while short dash were used for *Marenzelleria* spp.

TABLE 2 | Result of distance based linear models (DISTLM) shows the most important predictors for explaining variation in excretion rates (N and P $\mu\text{mol ind.}^{-1} \text{h}^{-1}$) of *Macoma balthica* and *Marenzelleria* spp. together ("both species", N=135) and separately (*M. balthica*: N=91, *Marenzelleria* spp.: N=44).

Both species	N excretion $\text{ind.}^{-1} \text{h}^{-1}$			
	Marginal	BIC	Sequential	Cumulative proportion
Body mass	0.514***	-281.0	0.514***	0.514
Temp. °C	0.082**	-309.6	0.116***	0.629
$\delta^{15}\text{N}$	0.009	-321.3	0.046***	0.676
Tissue N (%)	0.121***	-338.4	0.053***	0.729
<i>Macoma balthica</i>				
Body mass	0.553***	-178.3	0.553***	0.553
Temp. °C	0.117***	-204.3	0.137***	0.691
Tissue C/N	0.267***	-212.9	0.045***	0.735
$\delta^{15}\text{N}$	0.062*	-217.2	0.026**	0.762
<i>Marenzelleria</i> spp.				
Body mass	0.631***	-121.54	0.631***	0.631
Temp. °C	0.021	-122.68	0.042*	0.673
Tissue N/P	0.018	-123.38	0.034	0.707
Tissue N (%)	0.011	-123.92	0.029	0.737
P excretion $\text{ind.}^{-1} \text{h}^{-1}$				
Both species	Marginal	BIC	Sequential	Cumulative proportion
Body mass	0.389***	-191.73	0.389***	0.389
$\delta^{15}\text{N}$	0.066**	-214.71	0.124***	0.512
Temp. °C	0.046*	-230.82	0.076***	0.589
$\delta^{13}\text{C}$	0.011	-240.1	0.045***	0.633
Tissue C %	0.009	-244.51	0.027**	0.660
<i>Macoma balthica</i>				
Body mass	0.516***	-133.91	0.516***	0.516
Temp. °C	0.062*	-143.87	0.077***	0.593
Tissue C %	0.003	-148.79	0.043**	0.636
<i>Marenzelleria</i> spp.				
Body mass	0.653***	-105.14	0.653***	0.653
Excretion N/P ratio (mol)				
Both species	Marginal	BIC	Sequential	Cumulative proportion
Species	0.086**	-235.78	0.086**	0.086
Body mass	0.009	-236.2	0.038*	0.124

*** $p < 0.001$. ** $p < 0.01$. * $p < 0.05$.

compared to summer and autumn months (July: 8°C, October: 12°C, August 18°C), and it also affected the scaling coefficients. This interesting finding implies that temperature effects might depend on animal size, i.e. showing an interactive effect that MTE does not explicitly predict. However, based on an extensive, global data set of ectothermic aquatic invertebrates and vertebrates, Vanni & McIntyre (2016) also reported lower species-specific metabolic scaling coefficients (0.68 for N, 0.57 for P) than predicted by MTE, using data pooled from all aquatic animal species including marine and freshwater invertebrates and vertebrates. Importantly, the slopes calculated by Vanni & McIntyre accounted for variables such as temperature, biomass, trophic guild as well as invertebrate and vertebrate classification. When adapting the universal model to our data, we found that *M. balthica* yielded a similar slope for measured versus modeled excretion rates, but with markedly lower intercepts for regressions fitted to measured data (Figure 3). Although the universal model of excretion accounts for temperature, the lower energy content (C %) in bivalve tissue during winter in response to lower food availability (Mäkelin & Villnäs 2022) likely leads to decreased metabolism in the bivalve, thereby reducing its excretion rates below the predicted. In contrast,

the slopes of N and P excretion by *Marenzelleria* spp. differed from the modelled slopes.

Previous studies report substantial variation in species-specific scaling coefficients (slopes) when estimating excretion rates across taxa of benthic consumers (Vanni et al., 2002; Allgeier et al., 2015). Therefore, the application of a universal model should be used only as a general approximation (Vanni & McIntyre, 2016). However, the reasons for such differences between species remain unclear. Our results suggests that differences in tissue nitrogen content (N %) as well as $\delta^{15}\text{N}$ could explain part of the observed variation in N excretion rates between *M. balthica* and *Marenzelleria* spp. The polychaete likely has higher nitrogen requirements because its tissue N content averages about 10%, in contrast to a lower percentage in the bivalve (8.5%; Karlson et al., 2015; Mäkelin & Villnäs 2022). Tissue $\delta^{15}\text{N}$ values, which showed a corresponding enrichment for the polychaete compared to the bivalve support this pattern. Whereas *M. balthica* suspension or surface feed on newly settled organic matter (Kahma et al., 2020), *Marenzelleria* spp. utilizes older sedimentary carbon sources (Karlson et al., 2011; Kahma et al., 2020) that contain a higher proportion of microbially

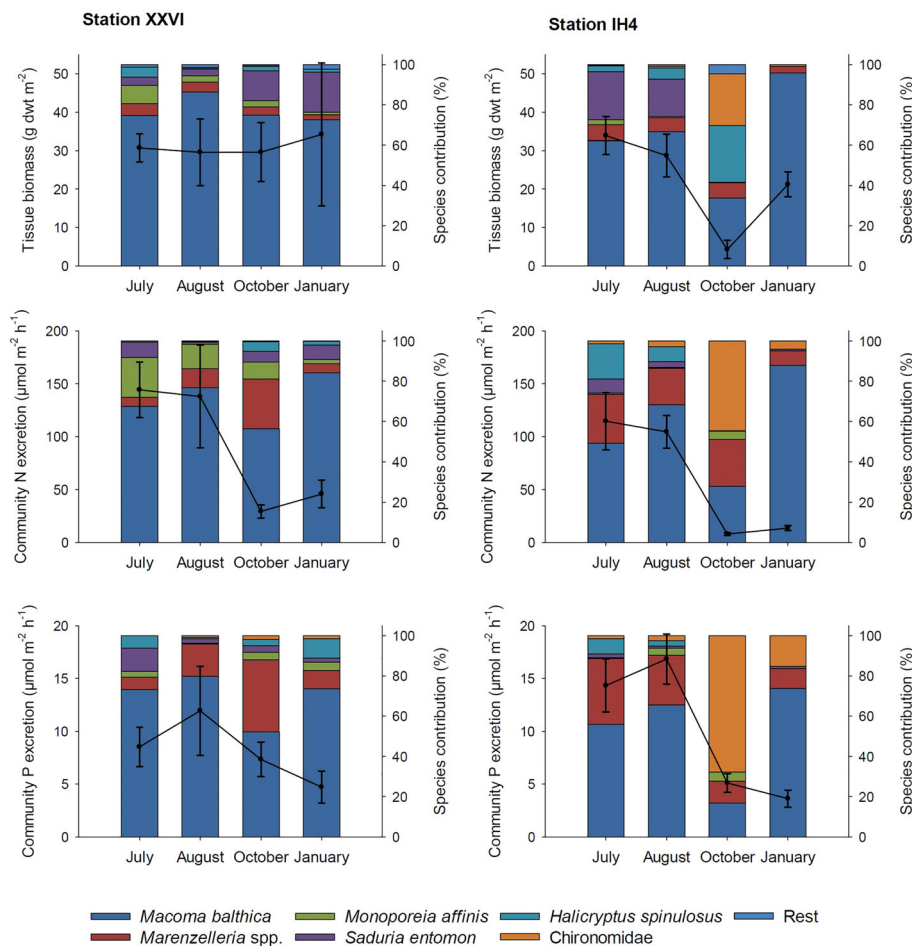


FIGURE 4 | The average tissue biomass of the benthic community (g dwt m⁻² ± std) and the maximum excretion potential of N and P (μmol h⁻¹ m⁻² ± std) per station and sampling occasion is depicted on the primary y-axis. The contribution of each species to the total biomass and excretion rate (%) is illustrated with columns at the secondary y-axis. Note that biomass does not include bivalve shells.

processed organic matter that potentially leads to $\delta^{15}\text{N}$ enrichment (Karlson et al., 2015). Interestingly, Karlson et al. (2015) suggested that $\delta^{15}\text{N}$ enrichment in *M. arctica* could also reflect higher isotopic fractionation in this polychaete than in species such as *M. balthica*, especially if a mismatch occurs between the N content of the food source of this polychaete and its high nitrogen requirements. Indeed, at our sites, we observed a lower sediment N:P than the N:P ratio of polychaete tissue (cf. **Figure S2** and **Table S1**). In line with our results, Allgeier et al. (2015) reported that $\delta^{15}\text{N}$ could explain a large part of the variance in N and P excretion of aquatic consumers, and emphasized the importance of trophic position. However, collinearity between $\delta^{15}\text{N}$ and biomass blurred the results and their study included multiple consumer feeding guilds, from algivores to carnivores (Allgeier et al., 2015).

Theoretically, if the biomass-scaling coefficients are similar for both N and P excretion, the slope of body mass vs excretion N:P ratio should be zero (Allgeier et al., 2015; Vanni & McIntyre, 2016). Despite variation in the scaling coefficients both for the bivalve and

the polychaete in our study, we found no clear relationship between the excreted N:P and consumer dry mass. In theory the excretion N:P could decrease with body size, because large animals within a species likely have higher tissue N:P (and demand) than small individuals (Elser et al., 2003; Hall et al., 2007). By utilizing a larger dataset, Mäkelin & Villnäs (2022) demonstrated a positive relationship between tissue N:P and dry mass for both *M. balthica* ($r^2 = 0.11$, $p < 0.01$) and *Marenzelleria* spp. ($r^2 = 0.51$, $p < 0.01$). However, the more limited data set of this study did not yield such a pattern, presumably as a result of the smaller size range of the animals. We could not detect any difference in tissue N:P between the bivalve and the polychaete (bivalve shell excluded; **Table S2**). In contrast, the excretion N:P differed significantly between the species and over time, emphasizing that in addition to allometric variables, other species-specific traits as well as environmental variability likely play an important role when predicting animal nutrient recycling rates.

Although our study did not test for the effects of nutrient imbalances between consumer tissue and diet, the pronounced

seasonal differences in N and P excretion rates indicate that food quantity and quality affect consumer excretion. Indeed, our model suggested that stoichiometric traits describing the energy content of the consumers (C %) and available food ($\delta^{13}\text{C}$) might slightly improve on the main predictor variables (body mass and temperature) of P excretion. Mäkelin & Villnäs (2022) showed a decrease in soft tissue C (%) in both of our study species and a simultaneous increase in N content (%) from May to March. Part of these changes related to temporal changes in food availability, i.e. less fresh food during winter time (Table S1, Rodil et al., 2020) and lower metabolic rates associated with colder temperatures (Mäkelin & Villnäs 2022). A more pronounced decrease in the C:N ratio occurred in the bivalve, whose glycogen, protein, and lipid content declines after spawning in early summer towards winter months (Beukema and De Bruin, 1977).

Overall, the biomass of our benthic communities remained high throughout the year, and at levels comparable to the high biomass levels reported for *M. balthica* during the 2000s in this productive coastal area (Ehrnsten et al., 2019). Still, the importance of physiological changes in response to colder temperatures and food availability becomes clear when considering the community excretion patterns of N and P, which decreased markedly from summer to winter months. Although the community biomass remained stable, excretion rates at station XXVI decreased towards winter (Figure 4 and Figure S1). Variation in community composition can explain some of this pattern; for example, biomass increased at station XXVI in January because of *S. entomon*, but this isopod has low excretion rates relative to its dry weight (Figure 4). Importantly, the pattern prevailed when we excluded this species from the data set.

The estimated community excretion rate of N, which as mentioned may represent a maximal rate, was about four times higher than fluxes of $\text{NH}_4\text{-N}$ across the sediment-water interface as measured by core incubations in July. The stress experienced by incubated animals associated with handling prior to the incubation likely elevated our measured excretion rates (Whiles et al., 2009), whereas animals in the intact cores might have experienced less stress from handling. Whiles et al. (2009) evaluated the effects of stress versus starvation on the excretion rates of fish and tadpoles and demonstrated that handling stress can increase ammonium excretion rates during short-term incubations. However, several studies report a positive relationship between macrofaunal biomass and sediment NH_4^+ effluxes (e.g. Gibbs et al., 2005; Norkko et al., 2013), and benthic excretion and activity have been shown to stimulate macrobenthic (Norkko et al., 2001), meiobenthic, and microbial activity and enhance sediment biogeochemical cycling (Henriksen et al., 1983; Stief, 2013; Zilius et al., 2022). In our case, the high excretion rates of $\text{NH}_4\text{-N}$ in comparison to sediment efflux emphasize that the benthic fauna likely enhances nitrification, as indicated by the effluxes of nitrate and nitrite from the sediments (Figure S3). Fecal pellets of *Macoma balthica* are hotspots for nitrification (Henriksen et al., 1983), and the excreted ammonium also likely promotes the process of nitrification-coupled denitrification (Henriksen et al., 1983; Stief, 2013; Benelli et al., 2017; Hoellein et al., 2017). This process is likely more efficient at organic-rich sites (cf. IH4 to XXVI), given the strong association of

nitrifiers and denitrifiers with the organic rich fraction of the sediment (Jäntti et al., 2011). In contrast to N, the benthic community P excretion rates were generally similar to the measured sediment P effluxes, and slightly lower than reported elsewhere in the Gulf of Finland (Berezina et al., 2019). Clearly, our sediment incubations only provide a snap-shot in time, and sediment fluxes result from both environmental and biotic variables and their interactions (cf. Belley and Snelgrove, 2016). Nevertheless, our findings are supported by Kauppi et al. (2017), who showed that the abundance of benthic consumers, such as *Marenzelleria* spp., can be a particularly important predictor of sediment nutrient fluxes across seasons.

In our coastal study area, the benthic macrofauna processes a major part of organic carbon sedimentation (Ehrnsten et al., 2019). Our study emphasizes the importance of nutrient excretion by benthic communities for nutrient recycling rates in shallow coastal ecosystems, which likely supports primary production. Multiple studies have demonstrated that benthic consumers can change nutrient concentrations and limiting nutrients in aquatic ecosystems (Atkinson et al., 2013). Our study cannot explicitly link excretion rates and ratios to primary production, which we did not measure, but the N:P ratio of benthic excretion was lower than the Redfield ratio (16:1), especially for *Marenzelleria* spp. Indeed, Berezina et al. (2019) found that *Marenzelleria arctica* contributed to 79–98% of the total benthic community P excretion rates in the eastern part of the Gulf of Finland. Berezina et al. (2019) suggest that the excretion by this invasive, but now dominant polychaete (Kauppi et al., 2015) may drive primary producers toward N limitation and away from P limitation. In theory, N limitation, together with increasing ocean temperatures can create conditions that favour harmful cyanobacterial blooms. Still, other ecological processes performed by the benthic consumers could counteract such a development. Indeed, the polychaete *Marenzelleria* spp. is a well-known bioturbator, which can oxidize the sediment and promote overall P binding (Norkko et al., 2012; Berezina et al., 2019). Still, previous work demonstrates the highly context dependent impact of this polychaete on sediment solute fluxes (Kauppi et al., 2018b). *Marenzelleria* spp. likely increases sediment nutrient cycling, but the broader ramifications of this polychaete genus for nutrient concentration and limitation on an ecosystem level remain unresolved across larger scales in the Baltic Sea.

In conclusion, we showed significant relationships between consumer nutrient recycling rates and allometric traits at a species level, whereas stoichiometric variables and tissue stable isotope signatures ($\delta^{15}\text{N}$) helped to describe differences in excretion rates and ratios between our two species. As expected, excretion rates of benthic consumers showed strong seasonality pattern, with highest rates during summer and lowest during winter, emphasizing that changes in temperature and food availability affect metabolic processes of benthic consumers. Thus, pinpointing which physiological traits and environmental variables can enhance our understanding of benthic nutrient recycling at the ecosystem level will require further research. Nevertheless, the biological trait concept is a promising tool for exploring the contribution of benthic consumers to nutrient processes over time and space, and for identifying hotspots of

change (Miatta et al., 2021). This potential is especially important in a world where climate warming and environmental change can increase the metabolism of benthic consumers, homogenize the biodiversity of species and traits, and thus disrupt the elemental balance of natural ecosystems.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

AV and SM designed the study and conducted the field- and laboratory work. AV, SM and MV structured the ms. AV performed statistical analyses, wrote the first draft of the ms. All authors provided significant intellectual input and contributed to revisions. Funding was obtained by AV. All authors contributed to the article and approved the submitted version.

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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.2022.870308/full#supplementary-material>

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