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# Benthic fauna as mediators of carbon and nutrient cycling in the coastal Baltic Sea

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**Academic dissertation**

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**BENTHIC FAUNA AS MEDIATORS OF CARBON AND NUTRIENT CYCLING IN THE COASTAL BALTIC SEA**

**SAARA MÄKELIN**

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# Abstract

Benthic macrofauna modifies carbon and nutrient retention and recycling processes in coastal habitats directly through their metabolism. As animal tissues have a higher concentration of carbon and nutrients compared to the surrounding environment, benthic biomass can form a substantial carbon and nutrient pool, which is especially stable in long-living species. Benthic consumers have a profound role in carbon and nutrient regeneration, as they recycle carbon and nutrients through processes such as respiration and excretion. However, the contribution of benthic fauna to coastal biogeochemical cycling is highly context-dependent and varies under different environmental conditions. Bioturbation by benthic fauna is a well-studied process, but fewer studies have investigated the carbon and nutrient stocks formed by the benthic biomass itself. By adapting the frameworks of ecological stoichiometry, i.e. the balance of elements in organisms and ecological processes, this thesis aims to quantify the amount of carbon and nutrients stored in the benthic community biomass, explore the stability of the benthic faunal carbon and nutrient pools over time and space, and study how variation in species stoichiometry is reflected in benthic community process rates.

To explore seasonal variation in benthic community carbon and nutrient pools, two aphotic muddy sediment sites were monitored for a year. Simultaneously, excretion rates by benthic fauna were measured in the laboratory to study how variation in environmental conditions alter the nutrient recycling rates by the benthic consumers. In addition, photic

shallow sandy and aphotic deep muddy sediments were sampled across a land-to-sea-gradient from the inner to outer archipelago to explore if benthic community composition, stoichiometry and process rates changed in response to spatial variation in environmental conditions and food sources.

The results indicate that the benthic macrofaunal communities can form substantial carbon and nutrient hotspots in bare soft sediment habitats of the Baltic Sea. The organic faunal carbon and nutrient stocks were highest in August containing at maximum 14.0 g C $m^{-2}$, 2.3 g N $m^{-2}$ and 0.2 g P $m^{-2}$ in deep muddy habitats, and 19.6 g C $m^{-2}$, 3.8 g N $m^{-2}$ and 0.4 g P $m^{-2}$ in shallow sandy habitats. The benthic fauna contained approximately 40 % C, 8 % N and 0.8 % P, whereas the sediment surface layer contained ca 4.6 % C, 0.6 % N and 0.03 % P at muddy sites and 0.8 % C, 0.1 % N and 0.003 % P at sandy sites. However, the faunal carbon and nutrient pools are highly dynamic and vary in response to changes in environmental conditions over temporal and spatial scales.

The results show that faunal elemental content is species-specific and follows the population dynamics of each species. As the carbon and nutrients are bound to consumer biomass, variation in benthic community composition alters the carbon and nutrient storage and process rates of the community. Benthic faunal stoichiometry also varied with individual size and reproductive state. Furthermore, the benthic faunal stoichiometry expressed seasonal variation in response to food availability. Temperature together with food availability were also important drivers of benthic faunal excretion rates. In turn, spatial variation in benthic community biomass, carbon and nutrient stocks, respiration and secondary production rates were driven by food quality, as the stocks and process rates were higher at shallow sandy compared to deep muddy sites and increased across the land-to-sea gradient. Also, biological factors such as increasing species richness and dominance patterns were significant in predicting benthic faunal stocks and process rates especially at shallow sandy habitats.

Overall, the results of this thesis conclude that changes in environmental conditions and faunal food sources may alter the elemental content and metabolic rates of benthic key species, which in turn regulate the amount and turnover rate of carbon and nutrients stored in benthic community biomass. Changes in the benthic faunal carbon and nutrient turnover could propagate to higher trophic levels and alter biogeochemical cycling on ecosystem scale. As global warming, eutrophication and increased land-

use continue to disturb the carbon and nutrient balance of coastal habitats, it is important to maintain diverse benthic communities to sustain their contribution on carbon and nutrient cycling processes.

**Keywords**: macrofauna, carbon and nutrient cycling, stoichiometry, excretion, respiration, secondary production, seasonality, homeostasis, ecosystem function

# Tiivistelmä

Hiili, typpi ja fosfori ovat kaikille eliöille välttämättömiä aineita, sillä ne säätelevät kasvua ja lisääntymistä. Pohjaeläimet ylläpitävät merenpohjan elinympäristöjen hiili- ja ravinnekiertoa sitomalla, muokkaamalla ja vapauttamalla ravinteita. Pohjaeläimet käyttävät ravinnokseen pääasiassa kasviplanktonia ja sedimentin eloperäistä ainesta, ja syömällä ne sitovat ravinnon sisältämää hiiltä ja ravinteita omaan biomassaansa. Eläinten kudoksissa on ympäristöön verrattuna korkea hiili- ja ravinnepitoisuus, joten pohjaeläinyhteisö voi toimia ainevarastona. Pohjaeläimet myös muokkaavat ja kierrättävät hiiltä ja ravinteita aineenvaihduntansa kautta; hiili vapautuu takaisin ympäristöön hengityksen yhteydessä ja ravinteet eritteiden mukana.

Tämän väitöskirjan tarkoituksena oli selvittää kuinka paljon pohjaeläinyhteisöt voivat varastoida biomassaansa hiiltä ja ravinteita, sekä tutkia miten pohjaeläimistön muodostaman hiili- ja ravinnevaraston koko vaihtelee suhteessa erilaisiin ympäristöolosuhteisiin, kuten pohjaeläinten käyttämän ravinnon määrään tai laatuun. Tutkimus toteutettiin ottamalla pohjaeläinnäytteitä eri vuodenaikoina ja erilaisissa elinympäristöissä, sekä mittaamalla laboratoriossa eri lajien aineenvaihduntaa.

Tulokset osoittavat, että pohjaeläimet voivat toimia tärkeänä hiili- ja ravinnevarastona Itämeren pehmeiden pohjien elinympäristöissä. Pohjaeläinten biomassasta keskimäärin 40 % oli hiiltä, 8 % typpeä ja 1 % fosforia. Pohjaeläinyhteisön muodostama hiili- ja ravinnevarasto oli suurimmillaan elokuussa, jolloin eläimille oli tarjolla runsaasti ravintoa. Suurimmillaan pohjaeläinbiomassa sitoi noin 20 g hiiltä, 3 g typpeä ja 0,2 g fosforia neliömetriä kohti. Pohjaeläinbiomassan sisältämä hiilen ja ravinteiden määrä kuitenkin vaihteli sekä pohjaeläinyhteisön rakenteen että ympäristöolosuhteiden mukaan. Pohjaeläinten ainekoostumus oli lajikohtaista, joten pohjaeläinyhteisön sitomaan hiili- ja ravinnemäärään vaikuttivat oleellisesti yhteisön lajikoostumus sekä populaatioiden lisääntyminen ja kuolleisuus. Lajitasolla pohjaeläinten hiili- ja ravinnekoostumus vaihteli eläimen koon, kehitysvaiheen ja lisääntymistilan mukaan. Ympäristötekijöistä etenkin ravinnon määrä ja laatu vaikuttivat merkittävästi pohjaeläimistön ainepitoisuuteen ja aineenvaihduntanopeuteen, jotka säätelevät pohjaeläinbiomassaan varastoituneiden aineiden määrää sekä elinyhteisön kykyä kierrättää hiiltä ja ravinteita.

Ilmastonmuutoksen ja rehevöitymisen takia Itämeri vastaanottaa jatkuvasti enemmän ravinnekuormitusta. Väitöskirjan tulosten perusteella ainetasapainon muuttuminen voi vaikuttaa pohjaeläinyhteisöjen kykyyn sitoa ja kierrättää hiiltä ja ravinteita. Muutokset pohjaeläinyhteisön toiminnassa voivat puolestaan vaikuttaa aineiden kiertokulkuun koko elinympäristön tai ekosysteemin tasolla.

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# 1. Introduction

## 1.1 Benthic macrofauna as mediators of coastal carbon and nutrient cycling

Coastal areas play a key role in global biogeochemical cycling (Middelburg and Herman 2007, Carstensen et al. 2020). By being at the interface between land and the open sea, coastal habitats often receive excess amounts of land-derived organic matter and nutrients (Nixon 1995, Peñuelas et al. 2019). Hence, coastal ecosystems are important in transforming, retaining, and removing carbon and nutrients through various biogeochemical processes, often referred to as the "coastal filter" (Asmala et al. 2017). Part of the carbon and nutrients entering these systems are retained and recycled by animal biomass (Allgeier et al. 2017, Carstensen et al. 2020). Indeed, animal mediated carbon and nutrient cycling can have high influence on biogeochemical processes at an ecosystem level in aquatic systems (Vanni 2002, Schmitz et al. 2018, Allgeier et al. 2017, Atkinson et al. 2017).

Benthic animals, also known as benthos, are a community of invertebrates inhabiting the seafloor. In this thesis, all benthic invertebrates larger than 0.5 mm are classified as macrofauna. These animals are either buried in the soft sediment, attached to hard substrates, crawling in top of the sediment, free swimming close to the sediment, or living among macrophytes and macroalgae. Benthic consumers are often categorized into functional feeding groups based on their food and feeding habits: filter or suspension feeders, such as bivalves, actively sieve small particles from the water column, deposit feeders like polychaetes feed on the organic matter in the sediment, grazers such as gastropods utilize plants and algae, whereas predators prey on other animals (Merritt et al. 2017). Benthic macrofauna are also an important part of coastal food webs, as they are a food source for fish and seabirds (Karlson et al. 2019).

Furthermore, benthic species have a pivotal role in sediment biogeochemical cycling (Vanni 2002, Snelgrove et al. 2014, 2018). Benthic fauna can have both indirect and direct effects on sediment organic matter mineralization processes. As the benthic animals are searching for food or digging and flushing their burrows, they simultaneously increase the exchange of water, oxygen, and nutrients between the sediment-water

interface (Kristensen et al. 2012). Active benthic communities can oxygenate the sediment, which in turn improves redox conditions, stimulates microbial activity, and increases sediment resuspension and organic matter mineralization rates (Kristensen et al. 2012, Josefson et al. 2012).

Importantly, benthic fauna also mediates carbon and nutrient storage and recycling directly through their metabolic processes (Vanni 2002). Benthic animals remove carbon and nutrients from the surrounding environment by feeding and store them in their biomass (Fig. 1). Thus, the size of the consumer-bound carbon and nutrient stocks are regulated by population dynamics, such as reproduction, mortality, immigration, and emigration (Vanni 2002). Carbon is recycled back to the environment through respiration, whereas nutrients are returned through egestion and excretion (Vanni 2002, Allgeier et al. 2017, Fig. 1). As these physiological processes are species-specific and size-dependent, the benthic community composition regulates the turnover of benthic carbon and nutrients pools (Atkinson et al. 2017). The faunal tissues often have higher concentration of carbon and nutrients compared to the surrounding environment (Sterner and Elser 2002), thus, benthic biomass can form a substantial carbon and nutrient pool, which is particularly stable in long-living species (Griffiths 2006). In this thesis, faunal carbon and nutrient pools are defined as a reservoir of elements in animal biomass, whereas stocks are a certain amount of carbon and nutrients held in this pool at a certain time.

Benthic macrofauna are typically dominating the living organic carbon pool in many coastal habitats. For instance, in the Baltic Sea they can contribute >25 % to the total sediment organic carbon pool, and even > 40 % to the seafloor respiration (Rodil et al. 2020a, 2022). However, the contribution of benthic communities to carbon and nutrient recycling is context-dependent and highly variable over environmental scales (Gammal et al. 2019). The carbon and nutrient recycling by the benthic fauna are strongly correlated to community biomass (Snelgrove et al. 2018), but the benthic community composition varies in response to differences in environmental conditions, such as sediment characteristics (Thrush et al. 2003, Pratt et al. 2014), and available food sources (Marcelina et al. 2018).

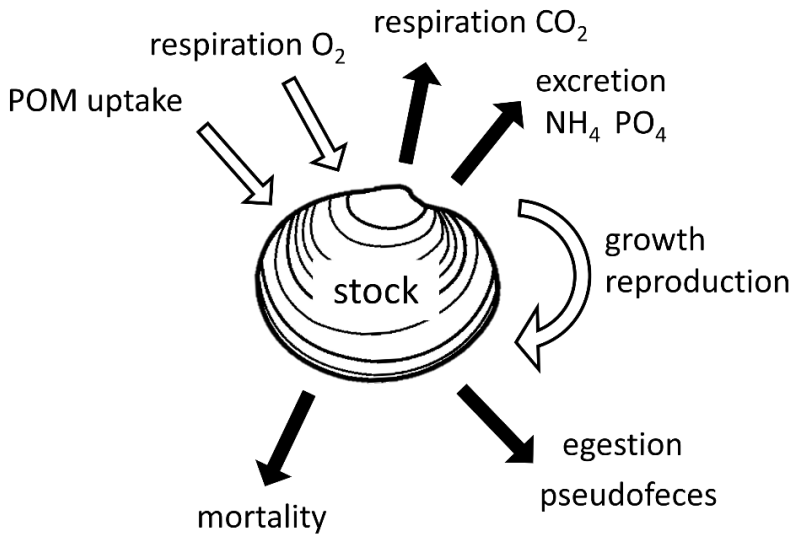


**Figure 1.** Carbon and nutrient storage and recycling processes by benthic animals. White arrows describe the carbon and nutrient binding and black arrows the release processes.

In shallow euphotic areas, the benthic fauna is supported by benthic primary production, but in deep aphotic sediments, strong benthic-pelagic coupling is the key for sustaining benthic communities (Graf 1992, Frost et al. 2002). The importance of benthic-pelagic coupling is emphasized in the Baltic Sea, as it is a relatively shallow and highly productive ecosystem (Graf 1992). The sedimentation of organic matter is the most important driver of benthic community biomass and abundance (Graf 1992, Ehrnsten et al. 2019). Because benthic macrofauna in deep sediments rely on sinking organic matter as their primary food source, they show strong responses to variation in pelagic production (Graf 1992) and can be food limited (Josefson and Rasmussen 2000, Ehrnsten et al. 2019). High resource availability and quality are likely to increase community biodiversity, and biodiversity in turn often shows a positive correlation with productivity (Cardinale et al. 2009). The capacity of the benthic communities to take up, store and recycle material is essential in directing ecosystem processes such as the storage and turnover of carbon, which will transcend to higher trophic levels and affect biogeochemical cycling on an ecosystem scale (Snelgrove et al. 2014). Therefore, shifts in resources that change benthic community composition can alter the carbon and nutrient recycling on

ecosystem level (Sobral et al. 2023). As multiple stressors continue to affect the coastal ecosystems, it is central to quantify the capacity of benthic consumers to store and recycle carbon and nutrient in coastal habitats. By adapting the theory of ecological stoichiometry, i.e. the balance of elements in organisms and ecological processes (Sterner and Elser 2002), this thesis aims to quantify the size and stability of benthic faunal carbon and nutrient pools over temporal and spatial scales.

## 1.2 Frameworks of trait ecology, ecological stoichiometry, and the metabolic theory of ecology

Traits are a commonly used tool in ecology to describe organisms and their effects on the surrounding environment (Violle et al. 2014). Allometric traits define organisms' morphological and physiological attributes such as body mass, length, and elemental composition, while functional traits such as feeding mode or position in the sediment characterize the interaction between the species and its habitat (Snelgrove et al. 2014, Violle et al. 2014). As all species have their unique set of traits, more diverse communities commonly have higher trait distribution (Sobral et al. 2023). Therefore, changes in species composition can alter both the structure and function of the benthic communities (Lohrer et al. 2004).

All species have their optimal elemental composition and ratios, also described as stoichiometric traits, and they utilize different food sources to fulfil their energy and nutrient requirements (Sterner and Elser 2002). Ecological stoichiometry explores the elemental balance between organisms and their habitats, particularly for carbon (C), nitrogen (N) and phosphorus (P), as all organisms need these elements to maintain growth and reproduction (Sterner and Elser 2002). For the benthic consumers, carbon is the main source of energy and the main building block of their tissues. Nitrogen is required for amino acid production to form proteins, whereas phosphorus is needed for DNA, RNA and ATP (Sterner and Elser 2002). According to the theory of ecological stoichiometry, organisms can regulate their inner elemental balance, a concept called homeostasis (Sterner and Elser 2002). Benthic consumers are often considered stoichiometrically homeostatic, indicating the animals can keep their body composition stable despite changes in nutrient availability (Sterner and Elser 2002, Persson et al. 2010). This is achieved by regulating the metabolic rate, i.e. the rate at which the animal uptakes and utilizes energy and material. The faunal body C:N and C:P ratios reflect the balance of

energy versus nutrient limitation, whereas the excretion of N:P is important in determining the limiting nutrient (Vanni 2002, Frost et al. 2006, Vanni & McIntyre, 2016). For instance, a species with high body N content also has high demand for N-rich food, and it excretes on low N:P ratio (Sterner and Elser 2002).

The growth rate hypothesis states that fast-growing animals, such as juveniles, generally have higher P requirements, because during rapid growth the demand of P allocated to ribosomal RNA increases (Elser et al. 2003, Frost et al. 2006, Meunier et al. 2016). These animals also have high body P content and are often P-limited (Sterner and Elser 2002). Therefore, maximum growth rates are commonly correlating with the P content in food supply (Sterner and Elser 2002). Moreover, aquatic species undergo ontogenetic changes in their morphology and diet, which are likely to influence their stoichiometric characteristics (Rossi et al. 2004, Pilati and Vanni 2007, Atkinson et al. 2017), indicating that the elemental composition of these animals change along development. For example, juvenile bivalves, such as the Baltic clam *Macoma balthica*, have higher body lipid and lower body water content compared to adults, which can also be visible in their body C:N:P ratios (Pekkarinen 1983). In addition, the juvenile bivalves can be restricted by their short siphons to only ingest small particles from the sediment surface, whereas adults can feed on larger variety of organic material and switch between deposit and suspension feeding modes (Rossi et al. 2004). The difference in feeding habits could lead to difference in the dietary carbon between adults and juveniles and be reflected in their tissue stoichiometry (Rossi et al. 2004, Rodil et al. 2020b).

The ecological stoichiometry is commonly paired with the metabolic theory of ecology (MTE), which predicts how the faunal metabolic rates regulate ecological processes from individuals to ecosystem level (Brown et al. 2004). The theory also quantifies the rates for resource uptake and how the resources are directed for growth, reproduction, and survival. As the theory links the relationships between organismal body size, body temperature and metabolic rates (Brown et al. 2004), it is possible to predict faunal metabolic rates, such as excretion rates, from information regarding body size and temperature (Vanni et al. 2002). The theory states that small individuals have higher mass-specific metabolic rates than larger conspecifics (Vanni 2002), for example, if two biological communities have the same biomass, the community dominated by smaller individuals has higher excretion rate (Hall et al. 2007). Furthermore, the theory shows that metabolic activity is strongly temperature-dependent (Allen and

Gillooly 2009). Ectothermic animals, such as the benthic fauna, cannot regulate their body temperature and are prone to temperature variation in the surrounding environment. Thus, the metabolic activity of benthic consumers increases with increasing water temperatures and decreases with cooling water. As higher temperatures result, for example, in higher excretion rates, the increase in metabolic activity most likely influences faunal body stoichiometry (Atkinson et al. 2017). Therefore, the MTE should always be taken into consideration while predicting the role of consumers in nutrient retention and recycling (Vanni 2002).

Although benthic consumers store carbon and nutrients in their body tissues at theoretically homeostatic rates, previous studies have reported temporal and spatial variation in faunal tissue C:N:P (i.e. Cross et al. 2003, Liess and Hillebrand 2005). Environmental conditions can modify benthic faunal carbon and nutrient pools directly by changing the benthic habitat, and indirectly by altering the stoichiometry of food sources for the benthic consumers (Sardans et al. 2012). Changes in environmental conditions such as in temperature and nutrient availability can affect the physiology of the benthic species and consequently alter process rates such as respiration (Rodil 2020a) and excretion (Vanni and McIntyre 2016), which are reflected to ecosystem function (Fig. 2). For instance, high sea water temperatures due to global warming could increase the metabolic rates of benthic consumers (Brown et al. 2004), resulting in faster growth and increased nutrient demand (Persson et al. 2011, Wojewodzic et al. 2011). As a response, benthic consumers tend to shift towards carbon rich diet to support the increased energy requirements (Boersma et al. 2016, Malzahn et al. 2016).

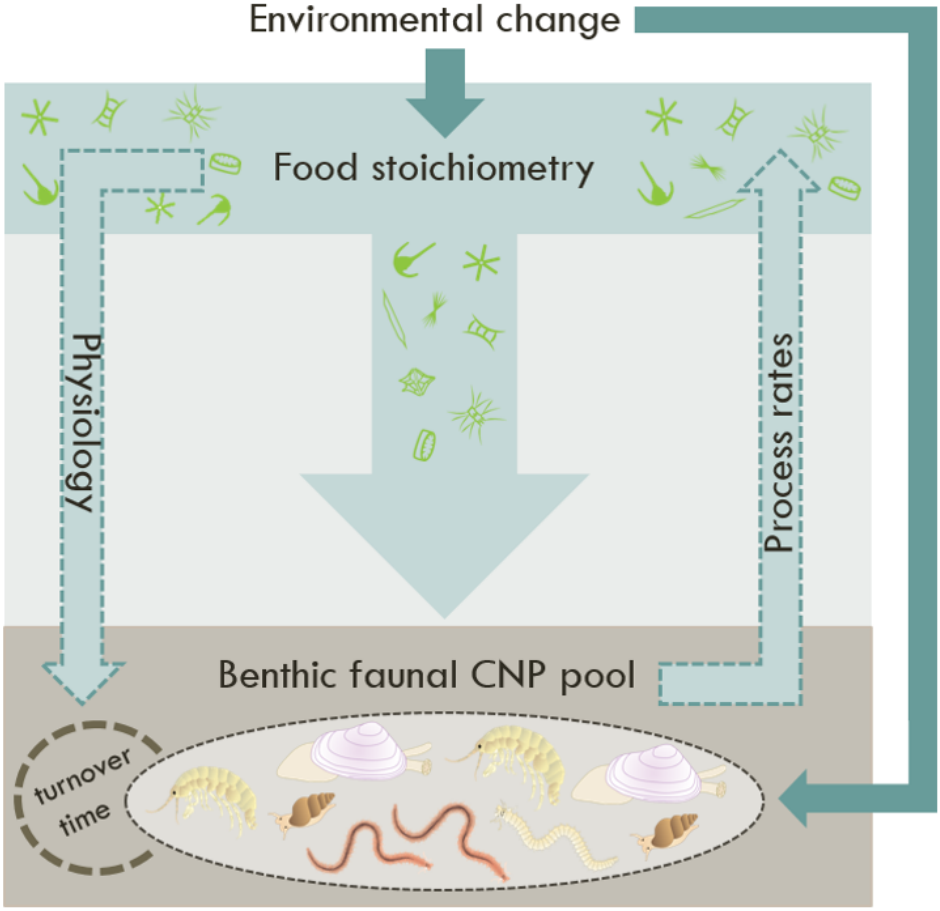


**Figure 2.** Conceptual graph illustrates how changes in environmental conditions may alter benthic faunal carbon and nutrient pools directly by formatting the habitat of the benthos, and indirectly by changing the stoichiometry of food sources for the consumers, for example by changing the phytoplankton community composition. This will alter benthic faunal stocks and turnover rates, and related processes such as respiration and excretion. Shifts in these process rates reflect to primary production and other fundamental ecosystem processes. Images in courtesy of the Integration and Application Network, University of Maryland Center for Environmental Science (ian.umces.edu/symbols/).

Moreover, phytoplankton community composition is shifting in face of environmental change (Spilling et al. 2018). For example, in the Baltic Sea, eutrophication and seawater warming have shifted the spring blooms from diatom to dinoflagellate domination (Spilling et al. 2014, 2018), and the summer blooms are increasingly dominated by filamentous cyanobacteria (Vahtera et al. 2007). Diatoms are considered as high-quality food for the benthic consumers, as they are rich in fatty-acids and have higher C:N:P compared to dinoflagellates (Spilling et al. 2014, 2018, Lipsewers et al. 2020). In turn, cyanobacteria lack vital biomolecules such as fatty acids, amino acids, and vitamins, which are essential to consumer growth and health (Nascimento et al. 2009, Winder et al. 2017). The amount and quality of the sinking organic material are directing the benthic macrofaunal biomass (Johnson et al. 2007). Thus, shifts in resource stoichiometry could create mismatches between the nutritional requirements of consumers and available resources, potentially reducing growth and reproduction of species, leading to changes in benthic community composition and dominance patterns (Fink et al. 2006, Nascimento et al. 2009, Hedberg et al. 2021).

As coastal habitats are continuously affected by multiple stressors, such as increasing land-use, eutrophication and seawater warming (Carstensen et al. 2020), the quantity and quality of organic matter inputs to the seafloor are rapidly changing (Griffiths et al. 2017, Peñuelas et al. 2019). Consequently, the benthic communities are shifting towards faster growing and short-living species, with faster fluxes and smaller C, N and P pools (Villnäs et al. 2019, Carstensen et al. 2020). For example, by examining the biological trait composition of the benthic communities all around the Baltic Sea, Villnäs et al. (2019) showed that eutrophicated inner coastal sites were typically dominated by small individuals that had lower total energy and nutrient content compared to individuals at less nutrient-enriched outer sites. These shifts in trait composition could ultimately alter the carbon and nutrient retention and recycling capacity of the benthic communities (Villnäs et al. 2019, Carstensen et al. 2020, Sobral et al. 2023). Therefore, the ability of benthic consumers to adapt their stoichiometry to environmental change is essential in maintaining their contribution to ecosystem processes. This emphasizes the need to study the link between biodiversity, stoichiometry, and process rates of benthic communities, and how these are affected by changing environmental conditions.

## 1.3 Soft-sediment benthic communities in the Baltic Sea

The Baltic Sea is a non-tidal and semi-enclosed coastal sea characterized by strong gradients in salinity and species diversity. The salinity varies between freshwater in the north to fully marine conditions in the south, additionally, the salinity increases with increasing depth, with a permanent halocline in deeper basins (Leppäranta and Myrberg 2009). For example, in the western Gulf of Finland (GoF) the surface water salinity typically varies between 5-7, while it ranges between 0-3 in the eastern GoF. In the western GoF, the bottom water salinity ranges between 7-8, but can reach up to 10 after saltwater inflows, when significant influx of saline ocean water enters the Baltic Sea through Danish Straits (Alenius et al. 1998). The water column is also seasonally stratified by temperature with cold water closer to the seafloor. Due to the varying brackish water conditions, the Baltic Sea taxa is dominated by freshwater species in the north, and marine species in the south. As only few species can tolerate the brackish conditions, the diversity is low, but key species can occur in great densities (Bonsdorff 2006). Because of the low biodiversity, the ecosystem is vulnerable to environmental change (Karlson et al. 2002). The Baltic Sea is heavily affected by human impact, most severely by eutrophication caused by excessive land-derived nutrient loading (Karlson et al. 2002, Carstensen et al. 2020). The Baltic also suffers from increasing seawater temperatures (Göbeler et al. 2022), which is changing the phytoplankton community composition (Spilling et al. 2018) and the timing of spring blooms (Kahru et al. 2016).

The soft-sediment habitats in the Baltic Sea area are commonly dominated by the Baltic clam *Macoma balthica* (Linnaeus, 1758), the invasive polychaete genus *Marenzelleria* spp. (Mesnil, 1896), the amphipods *Monoporeia affinis* (Lindström, 1855) and *Pontoporeia femorata* (Krøyer, 1842), the priapulid *Halicryptus spinulosus* (von Siebold, 1849), and the isopod *Saduria entomon* (Linnaeus, 1758). In addition, the ragworm *Hediste diversicolor* (Müller, 1776), the gastropods Hydrobiidae (Stimpson, 1865), and the clams *Cerastoderma glaucum* (Poiret, 1789) and *Mya arenaria* (Linnaeus, 1758) are common at sandy sediments. The highest abundance of animals is found from the top 3 cm sediment surface layer, but burrowing species such as bivalves, polychaetes and the priapulid can dig 10 cm down into the sediment (Kristensen and Kostka 2005).

The bivalve *M. balthica* and the polychaete genus *Marenzelleria* spp. are commonly the two most abundant species dominating the soft-sediment

benthic communities throughout the Baltic Sea (Gogina et al. 2016). The Baltic clam *M. balthica* is a key species in the Baltic Sea as it inhabits all soft sediment habitats (Segerstråle 1960, Gogina et al. 2016). The species shows plasticity to different environmental conditions, as it can thrive in varying salinity conditions and switch between suspension feeding and deposit feeding modes (Segerstråle 1960, Rossi et al. 2004). The invasive polychaete complex *Marenzelleria* spp. was introduced in the Baltic Sea in 1985 and it rapidly became highly abundant in muddy and sandy sediments (Bick and Burckhardt 1989, Gogina et al. 2016). Three species of this genus, *M. viridis* (Verrill, 1873), *M. neglecta* (Sikorski and Bick, 2004) and *M. arctia* (Chamberlin, 1920), are present in the northern Baltic Sea, but these species can only be identified by genetic analysis (Kauppi et al. 2018). The *Marenzelleria* polychaetes are commonly sub-surface deposit feeders (Dauer et al. 1981). Even though invasive species are often considered to be harmful, bioturbation by the polychaete genus *Marenzelleria* spp. has improved the sediment oxygen conditions and phosphorus retention in many areas of the Baltic Sea (Norkko et al. 2012). Moreover, *Marenzelleria* spp. has invaded an empty niche and does not seem to compete for resources with the native species (Karlson et al. 2015). However, in the Baltic Sea eutrophication and global warming are major threats to these benthic key species (Villnäs et al. 2012, Jansson et al. 2015) as hypoxic and anoxic areas have expanded over the past decades reducing benthic communities (Conley et al. 2009).

In bare sediment habitats, potential food sources for the suspension and deposit feeding benthic fauna are phytoplankton, benthic microalgae, bacteria, and detritus from autochthonous or allochthonous origin (Marcelina et al. 2018). In addition, organic matter derived from drifting macroalgae can provide a source of energy and nutrients for the macrofauna especially in shallow habitats (Kahma et al. 2020). Deposit-feeders may also ingest meiofauna, such as cladocera (Viitasalo 2007) and copepod eggs (Parker et al. 1996). However, benthic macrofaunal communities living in deep aphotic habitats rely on sinking organic matter as their food source (Griffiths et al. 2017). Because the Baltic Sea is located in the high latitudes, the strong seasonal variation in temperature and light conditions regulate primary production (Kahru et al. 2016, Spilling et al. 2018) and consequently sedimentation rates (Rodil et al. 2020b). Typically, the primary production in the Gulf of Finland peaks in March – April, after the light availability increases with longer days and melting ice cover (Svedrup 1953). The high phytoplankton production also increases sedimentation of organic matter (Rodil et al. 2020b). In the summer, the

phytoplankton communities are first dominated by flagellates, followed by cyanobacteria, after the nitrogen is depleted from the water (Spilling et al. 2018). During winter, the ice cover and shorter day length reduce primary production and the organic matter sedimentation (Rodil et al. 2020b). As the benthic fauna experiences strong variation in food availability, they can in cases be food limited (Josefson and Rasmussen 2000, Ehrnsten et al. 2019). Particularly nitrogen is often the limiting nutrient for both the primary producers (Savchuk 2005, Tamminen and Andersen 2007) and the benthic fauna (Tenore 1988).

## 1.4 Tracing of benthic faunal food sources

Imbalances in the availability and quality of food sources can affect consumer-driven carbon and nutrient retention and recycling processes as well as energy and nutrient flow in coastal food webs (Fink et al. 2006, Snelgrove et al. 2014, Peñuelas et al. 2020). Therefore, it is important to understand how variation in basal resources can alter the function of coastal ecosystems. Because of the low biodiversity, food webs in the Baltic Sea are relatively simple (Karlson et al. 2002), making the assessing of consumer-resource relationships more feasible. To explore variation in the available food sources for the benthic consumers, several organic compounds can be used as indicators of quantity and quality of the organic matter (Le Guitton et al. 2015). Chlorophyll *a* (Chl a) i.e. the main photosynthetic pigment in primary producers and cyanobacteria, can be used to indicate the quantity of the autochthonous organic matter, whereas phaeopigments, the degradation products of algal chlorophyll, describe the "freshness" of this material (Boon and Duineveld 1996).

In turn, the C:N:P ratios of seston, i.e. the suspended particles in the water column, and sediment reflect the energy and nutrient quality of the food for the benthic consumers. Low C:N and C:P ratios indicate high nutrient content and thus high food quality, whereas high C:N and C:P ratios indicate lower quality food. For example, seston C:N and C:P ratios often increase during cyanobacteria blooms implying nitrogen limitation (Walve and Larsson 2010). Also terrestrial inputs can increase seston and sediment C:N and C:P (Walve and Larsson 2010). Compared to phytoplankton, land-derived organic matter is lower quality food for the benthic consumers, as it is low in protein sources and the nutrients are bound in large macromolecules, such as cellulose or lignin, which require time and energy to process (Pardo et al. 2023). Therefore, terrestrial runoff is likely to

increase the quantity but lower the quality of organic matter in the water column. Although benthic consumers can utilize particulate allochthonous organic matter, they tend to select autochthonous resources (Antonio et al. 2010, Bartels et al. 2018).

In addition, stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) are commonly used to study food webs and trophic interactions as the stable isotope signatures of consumer tissues reflect those of their diet (Fry 2006). Both carbon and nitrogen have two stable isotopes, $^{12}C$ and $^{13}C$, and $^{14}N$ and $^{15}N$, which differ in atomic mass. This difference in mass can be measured with a mass spectrometer, and the ratio between the heavy and the light isotope ($^{13}C$:$^{12}C$ or $^{15}N$:$^{14}N$) is compared to international standards (Vienna Pee Dee belemnite for C and air for N) and expressed as deviation from the reference value $\delta$ (‰). The $\delta^{13}C$ signatures are highly variable among primary producers, therefore $\delta^{13}C$ is used to identify the main dietary carbon source of a consumer. In turn, $\delta^{15}N$ enriches stepwise through trophic levels and is used to define the trophic position of a consumer (Fry 2006, Bouillon et al. 2011). Stable isotopes signatures can also aid to identify spatial differences in organic matter. Land-derived carbon can be discerned from autochthonous organic matter based on more depleted $\delta^{13}C$ signatures (Fry 2006), while microbially processed organic matter, such as treated sewage, can be detected by its elevated $\delta^{15}N$ values in comparison to autochthonous sources (Savage 2005).

## 1.5 Knowledge gaps and aims of the thesis

Bioturbation by benthic fauna is a well-studied process, but fewer studies have investigated the carbon and nutrient stocks formed by the benthic biomass itself. The benthic faunal carbon and nutrient pools are highly dynamic, and we lack an understanding of their variation under different environmental conditions. The overall aim of this thesis was to quantify the magnitude and stability of the benthic faunal carbon and nutrient pools and explore the main drivers for this variation. Another aim was to quantify the benthic community process rates such as excretion, respiration, and secondary production, which are essential for nutrient recycling and carbon turnover. Specifically, the main objectives were;

- *What is the elemental composition of the Baltic benthic fauna?*
  The benthic faunal stoichiometry was expected to show intra- and interspecific variation. The hypotheses was that the benthic faunal

stoichiometry would be species-specific and size dependent as the juveniles would have higher body P content as suggested by the growth rate hypotheses (study I).

- *Does benthic faunal stoichiometry vary under changing environmental conditions?*
  The faunal tissue C, N, P content and ratios were expected to vary seasonally and spatially in response to variation in food availability (I), food quality (III) and temperature (II), which would drive the faunal metabolism and excretion rates.

- *What are the main drivers of benthic faunal process rates on species and community levels?*
  As suggested by the metabolic theory of ecology, the benthic faunal excretion rates were expected to depend on the individual size and seawater temperature (II) on species level. On community level, the process rates (excretion, respiration, and secondary production rates) were expected to vary in response to changes in benthic community composition (II, III), seasonally in response to changes in water temperature and food availability and quality (II), and spatially in response to changes in food availability and quality (III).

The thesis is based on three original papers. Study I quantifies the magnitude and temporal variability of benthic faunal carbon and nutrient pools. Study II investigates intra- and interspecific variation in excretion rates of benthic consumers. Study III explores how the benthic community composition, stoichiometry, and process rates (respiration and secondary production) vary across a gradient from the inner to the outer archipelago in response to variation in environmental conditions.

# 2. Materials and methods

## 2.1 Study area

All studies in this thesis were conducted in Ekenäs-Tvärminne archipelago in western Gulf of Finland, the Baltic Sea (Fig. 3). To identify temporal changes in the stoichiometric traits of benthic animals in study I, two 18 m deep muddy sediment sites were sampled six times (May, July, August, October, January, March) in years 2019 - 2020. Both sites are located in the outer archipelago zone (Fig. 3). Site IH4 is a sediment accumulation site enclosed by islands (Gammal et al. 2017), in contrast, site XXVI is exposed to open sea and has highly variable environmental conditions affected by regular upwelling events (Haapala 1994). The excretion rates by benthic fauna in study II were measured during four of the six different sampling occasions at these two stations.

To explore how benthic community stoichiometry and functions varied over spatial scales in study III, two contrasting habitats, photic shallow sandy and aphotic muddy sediments, were sampled across a land-to-sea gradient in late August 2020 (Fig. 3). The innermost sites were located outside the city of Ekenäs, whereas the outermost sites were in the exposed outer archipelago with connection to the open sea. The enclosed inner archipelago receives high amounts of allochthonous organic matter from riverine and terrestrial runoff, additionally two water treatment plants at the river estuary increase the nutrient loading (Asmala et al. 2013, Jilbert et al. 2018, Asp et al. 2020). In contrast, the outer sites have a direct connection to the open sea and are characterized by highly variable environmental conditions affected by regular upwelling events (Haapala 1994). The soft-sediment habitats in the study area are dominated by the typical Baltic Sea taxa described in section 1.3.

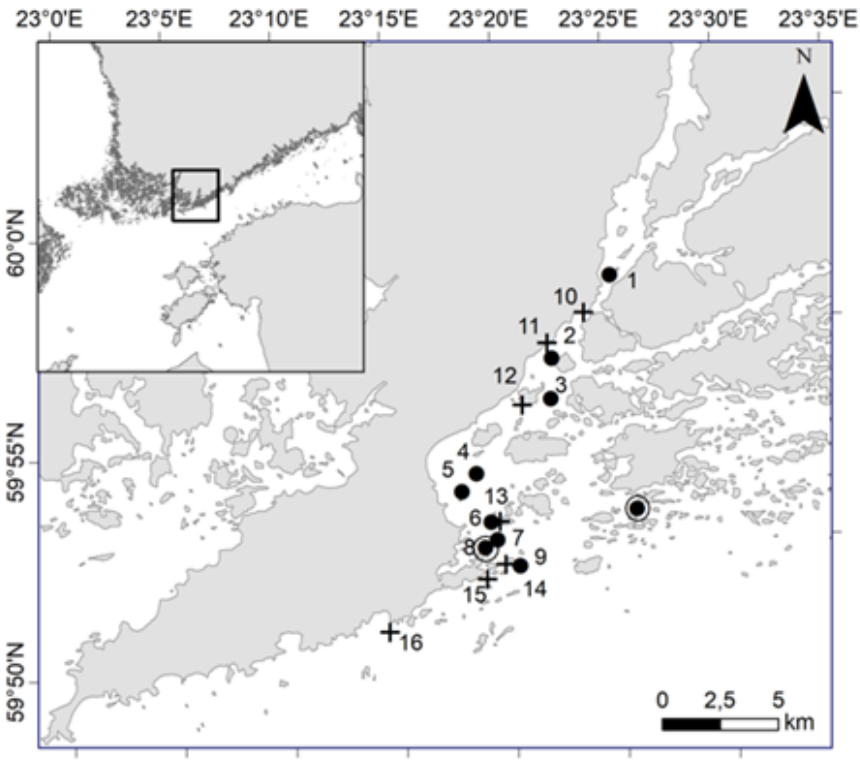


**Figure 3.** Map of the study sites. Deep muddy sites are marked with a black circle, while shallow sandy sites are marked with a cross. Circled sites were monitored for seasonal variation in studies I and II, numbered sites were used to explore spatial variation in study III.

## 2.2 Environmental conditions

The sampling procedure was similar in studies I and III. Water depth, temperature, salinity, and bottom water oxygen concentration were measured with CTD and YSI proODO sondes. Water samples were collected from the surface and at the bottom with a Limnos water sampler and analyzed for total (Koistinen et al. 2017, 2019) and dissolved nutrients (Koroleff 1979, Table 1). To estimate the amount of fresh pelagic food source available for the benthic fauna, chlorophyll a (Chl a) and pheophytin (pheo) concentrations were measured from the surface water after ethanol extraction from the GF/F filters (Lorenzen 1967). Another set of filters were dried for 24 h at 60 °C, capsulated in tinfoil and analyzed for seston C and N content, and stable isotopes $\delta^{13}C$ and $\delta^{15}N$ using an elemental

analyzer interfaced to an isotope-ratio mass spectrometer at UC Davis Stable Isotope Facility. Stable isotopes are expressed using δ notation, δ (‰) = [($R_{sample}/R_{standard}$) -1] x 103, where R is the ratio between the heavy and the light isotope $^{13}C:^{12}C$ or $^{15}N:^{14}N$, and δ (‰) the difference from the international standards Vienna Pee Dee belemnite (for C) and air (for N). Seston P content was analyzed as particulate organic phosphorus at the Tvärminne Zoological Station (TZS, Solórzano and Sharp 1980).

Sediment samples were collected with a Gemax twin corer (64 $cm^2$) at muddy sites and with a Haps corer (145 $cm^2$) at sandy sites. Sediment was sliced to obtain the surface layer (0 - 1 cm), which was measured for organic matter (OM) content, Chl a, pheo, C, N and P content, and stable isotopes $\delta^{13}C$ and $\delta^{15}N$. The sediment organic matter content was measured as loss of ignition (LOI ) by first drying the samples for 24 h in 60 °C, followed by combustion for 3h in 500 °C. Prior to pigment and elemental analysis, the sediment was freeze-dried and homogenized. Sediment Chl a was determined by extracting with 90 % acetone for 24h and measured spectrophotometrically. Acidification was added to identify phaeopigments from Chl a (Lorenzen 1967). Sediment was analyzed for C and N content, and stable isotopes $\delta^{13}C$ and $\delta^{15}N$ using an elemental analyzer connected to a continuous flow stable isotope-ratio mass spectrometer at University of Jyväskylä. In study I, sub-samples for sediment P were analyzed for total phosphorus ($P_{tot}$, Solórzano and Sharp 1980), while in study III the sediment P content was determined as $P_{tot}$ and as organic phosphorus ($P_{org}$) at TZS (Ruban et al. 1999).

**Table 1.** Overview of the parameters measured in each study.

| **Environmental conditions** | **I** | **II** | **III** |
|---|---|---|---|
| depth | x | x | x |
| temperature | x | x | x |
| salinity | x | x | x |
| $O_2$ | x | x | x |
| water nutrients | | | |
| total nitrogen (totN), total phosphorus (totP) | | x | x |
| dissolved ($NH_4^+$-N, $NO_{3-+}NO_{2-}$-N, $PO_4^{3—}$P, Si) | | x | x |
| **Sediment** | | | |
| organic matter (LOI) | x | x | x |
| Chl *a* | x | x | x |
| Phaeophytin | x | x | x |
| C:N:P | x | x | x |
| $\delta^{13}C$ and $\delta^{15}N$ | x | x | x |
| $O_2$ flux | | x | |
| $NH_4$-N flux | | x | |
| $NO_3$+$NO_2$-N flux | | x | |
| PO4-P flux | | x | |
| **Seston** | | | |
| Chl *a* | x | x | x |
| Phaeophytin | x | x | x |
| C:N:P | x | x | x |
| stable isotopes $\delta^{13}C$ and $\delta^{15}N$ | x | x | x |
| **Benthos** | | | |
| abundance | x | x | x |
| number of species | | | x |
| biomass | x | x | x |
| C:N:P | x | x | x |
| stable isotopes $\delta^{13}C$ and $\delta^{15}N$ | x | x | x |
| excretion ($NH_4$-N, $PO_4$-P) | | x | |
| respiration (calculated) | | | x |
| secondary production (calculated) | | | x |

## 2.3 Benthic macrofauna

Benthic fauna was sampled with a box corer (400 $cm^2$) at muddy sites and with a Haps corer (63.9 $cm^2$) at sandy sites. All faunal samples were sieved (0.5 mm), and the individuals selected for the elemental content analysis were handpicked from the sieve into filtered seawater. The rest of the sample was preserved in 70 % ethanol. The benthic samples (three replicates) were analyzed for species richness to the lowest taxonomic level possible, total abundance (ind $m^{-2}$) and biomass (dw g $m^{-2}$).

To get information on the elemental content in animal tissues, the animals were incubated for 24 h in filtered seawater, at in situ temperature, in a dark climate room to allow them to empty their guts. Following the incubation, the species were measured and divided into size classes. Fauna selected for elemental analyses were frozen, bivalves were dissected to separate the shells and tissue, freeze-dried, weighed, grinded, homogenized, and subsamples were encapsulated in tin cups. Three to five replicate samples were analyzed for each species and size class. The C and N content and stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) of the benthic fauna were determined as described for the sediment. Faunal P content was analyzed using the molybdate-blue reaction (Koistinen et al. 2019) and measured with a photometric analyzer at TZS. For the species whose biomass was too low to measure, the elemental content was estimated from literature (Carman and Cederwall 2001, Liess and Hillebrand 2005, Fink et al. 2006, Brey et al. 2010, Tatzelt 2015).

To calculate the faunal carbon and nutrient stocks in studies I and III, the C, N and P content was calculated for each taxon by multiplying the tissue dry weight by the corresponding C, N and P %. The community stocks were calculated as a sum of the elemental content of each species. The community C:N:P ratios are based on the total community C, N, and P stocks. All elemental ratios presented in this thesis are molar ratios. All ratio data were log10-transformed to fulfil the normality and variance homogeneity assumptions for statistical analyses. To explore if the elemental content ratios of the consumers followed those in their food, the stoichiometric mismatch was calculated after Bracken (2017) as mismatch = $\log_{10}(C{:}N_{resource}/C{:}N_{consumer})$ in study I.

## 2.4 Excretion incubations

The animals selected for the excretion experiment, elemental content and isotope analyses were hand-picked, gently rinsed, and placed in filtered seawater. The water had *in situ* temperature (climate-controlled room) and the animals were kept in dark conditions during excretion incubations. If several animals were incubated together, all individuals were of similar size. To measure the effect of biofilm on water nutrient concentrations, empty *M. balthica* shells were incubated as a control treatment. Before and after 1 h of incubation, the water was sampled with a syringe and filtered to remove feces and pseudofeces. After collecting water samples for the excretion rate measurements, the animals remained in filtered seawater for a total of 24 h prior the elemental analysis.

The filtered water samples were frozen (-20°C) prior to the analyses of excreted dissolved nitrogen (N as $NH_4^+$-N) and phosphorous (P as $PO_4^{3-}$-P). Ammonium was determined manually by a spectrometric procedure following the ISO 7150/1-1984 method, while phosphate was determined using the antimony-molybdate method (Koroleff 1983). The excretion rates were calculated as a difference in nutrient concentrations before and after the incubations. Because only minor changes in nutrient concentrations were observed in control trials, the effects of the biofilm in bivalve shells are included in excretion rates. However, the measured excretion rates seemed relatively high. Most likely the experimental conditions stressed the animals, thus the measured excretion rates represent their maximum excretion potential. Also, as several animals were incubated together, *M. balthica* individuals could potentially have ingested each other's pseudofeces, which might have increased the measured the excretion rates.

## 2.5 Benthic faunal respiration and secondary production rates

To calculate benthic community respiration and secondary production rates, the benthic faunal biomass was transformed into ash free dry weight (AFDW) by using conversion factors by Rumohr et al. (1987) and Brey et al. (2010). The benthic community respiration and secondary production rates were estimated from ash free dry weight by assuming 50 % of the AFDW to be carbon (Wong et al. 2011, Rodil et al. 2020a). Respiration was calculated by using the Mahault et al. (1995) formula for shallow water benthos as $R = 0.0174W^{0.844}$, where R is the respiration (C mg $m^{-2}$ $d^{-1}$) and

W is the average individual biomass as C mg. The daily individual respiration rates were multiplied by the corresponding total abundance of each species. Community respiration rates were calculated by summing up the respiration of each species. As the formula is valid for temperatures between 15-20 °C, the respiration rates were corrected for the lower temperatures assuming a $Q_{10} = 2$ (Timmermann et al. 2012).

Secondary production rates were calculated after Edgar (1990) as $P = 0.0049B^{0.80}T^{0.89}$, where P is the individual production (C µg $day^{-1}$), B is the biomass (µg AFDW), and T is the water temperature (5 – 30 °C). Similarly to respiration, the average individual secondary production rates were multiplied by the total abundance of each species, and the total community secondary production rate was calculated as a sum of the rates of each species. To assess the carbon turnover of the benthic communities, the production to biomass ratio (P:B) was calculated by dividing the community secondary production rate with the community carbon stock. High P:B ratios indicate fast growth rates, small body-size and short life span, whereas low P:B ratios are associated with slow growth, large and long-living individuals (Wetzel 2001).

## 2.6 Statistical analyses

Both univariate and multivariate statistical analysis were used in this thesis. Univariate analyses were conducted in IBM SPSS Statistics software (version 27.0), whereas PRIMER 7 with PERMANOVA+ software was used for multivariate analyses (Anderson et al. 2008, Clarke and Gorley 2015).

To describe the environmental conditions in the study area, a Principal Component Analysis (PCA) was done in all three studies. Variation in benthic community composition was explored using non-metric multidimensional scaling (nMDS) in study III based on Brey-Curtis dissimilarity index calculated on square root transformed data.

PERMDISP analysis was always run to tests homogeneity of multivariate dispersions. Permutational ANOVAs (PERMANOVAs) were used in all three studies. In study I, the PERMANOVA was used to explore if the faunal stoichiometry and stable isotope signatures varied between the species and if the benthic faunal C, N, P content, C:N:P ratios, $\delta^{13}C$ and $\delta^{15}N$ varied between seasons and sites. In study II the PERMANOVA was

run to study if the benthic faunal excretion rates varied between species, sites, and sampling times. In study III PERMANOVA was used to investigate if the benthic community diversity, biomass, C, N, P stocks, C:N:P, respiration rates, secondary production rates and P:B varied between habitat types. Pairwise post-hoc tests were used to identify the differences between timepoints to assess seasonal variability in studies I and II.

Linear regressions were used to analyze whether the faunal C:N:P ratios and excretion rates were size dependent in studies I and II. They were also used to explore if the benthic community diversity, biomass, stoichiometry, stable isotopes, respiration, secondary production rates, and the stable isotope signatures of individual species differed in response to distance, which represented the land-to-sea gradient in study III. Linear regressions were also used to test whether the $\delta^{13}C$ and $\delta^{15}N$ signatures of individual species differed across the gradient, and if the patterns observed in the faunal stable isotope values followed the isotope signatures of seston and sediment in study III.

To test whether the elemental content of a species was dependent on the C:N:P of their food, Spearman correlations were used to analyze the relationship between the resource and consumer stoichiometry in study I. Pearson correlations were used to determine whether the benthic community stoichiometry followed the C:N:P of the *M. balthica* population in study III. Correlations were also used to explore collinearity between the environmental variables to preselect predictor variables for distance based linear models in studies I and III.

Distance based linear modelling (DistLM) is a multiple regression approach to analyze univariate or multivariate data in response to predictor variables (Andersson et al. 2008). In study I, DistLMs were used to explore how much of the variation in benthic faunal stoichiometry could be explained by environmental parameters. In study II DistLMs were run to investigate which factors (allometric traits, stoichiometric traits, or stable isotopes) were driving the variation in benthic faunal excretion rates on species and community levels. In study III, DistLMs were used to study the contribution of biological (species richness, *M. balthica* biomass dominance) and environmental factors to benthic community C stocks, respiration, and secondary production rates. The DistLMs were run with different subsets of predictor variables using forward and stepwise selection. The best models were selected based on Akaike information

criteria (AIC) and the best goodness of fit ($R^2$). The two habitat types were analyzed separately as the they had different environmental conditions and different benthic community composition.

# 3. Results and discussion

This thesis aimed to quantify benthic faunal carbon and nutrient stocks in the soft-sediment habitats of the costal Baltic Sea and to explore how variation in environmental conditions alter the benthic community stoichiometry and process rates such as excretion, respiration, and secondary production. The results show that benthic macrofauna can function as hotspots of carbon and nutrients in coastal soft-sediment habitats, emphasizing their importance for overall nutrient uptake and turnover. Although the benthic communities can form a substantial carbon and nutrient stock, the faunal pools are highly dynamic and show variation over temporal and spatial scales. The benthic community composition together with food availability and food quality were the most important factors regulating the amount of carbon and nutrients stored in the benthic biomass. Furthermore, individual size and temperature were, in addition to species composition, the main drivers of benthic community excretion rates and important in directing the turnover rate of the consumer-bound carbon and nutrients.

## 3.1 Benthic community carbon and nutrient stocks

Benthic macrofauna can have a profound role in coastal carbon and nutrient recycling processes, as they retain and recycle these elements through their metabolism (Vanni 2002, Allgeier et al. 2017). The results of the studies I and III confirmed that benthic macrofauna formed a substantial organic elemental pool in coastal soft-sediment habitats. In the study area in the western Gulf of Finland, the organic faunal carbon and nutrient stocks were highest in August containing at maximum 14.0 g C $m^{-2}$, 2.3 g N $m^{-2}$ and 0.2 g P $m^{-2}$ in deep muddy habitats, and 19.6 g C $m^{-2}$, 3.8 g N $m^{-2}$ and 0.4 g P $m^{-2}$ in shallow sandy habitats (Fig. 4). The benthic fauna contained c. 40 % C, 8 % N and 0.8 % P, whereas the sediment surface layer (0-1 cm, without fauna) approximately 4.6 % C, 0.6 % N and 0.03 % P at muddy sites and 0.8 % C, 0.1 % N and 0.003 % P at sandy sites. The sediment surface is often used to compare the contribution of macrofauna to total sediment carbon and nutrient stocks in the Baltic Sea (i.e. Carman and Cederwall 2001, Rodil et al. 2022), as it is the most carbon and nutrient rich layer (Carman and Cederwall 2001) and there are no estimates of the organic C, N and P in the top 10 cm (Scheffold and Hence 2020), which is the layer inhabited by the benthic fauna (Kristensen and Kostka 2005).

Hence, when comparing to sediment with no fauna, the highest faunal stocks contained c. 12 % C, 15 % N, and 30 % P of the total organic carbon and nutrient storage in the muddy sediments, and c. 20 % C, 23 % N and 130 % P of the total stocks in sandy sediments. Even if the sediments of the Gulf of Finland are rich in nutrients, majority of the P is in inorganic form (Jilbert et al. 2011). Therefore, when focusing on the organic fraction, faunal communities contained more organic P than the sediment surface at sandy sites, which were the sites having the highest benthic diversity and biomass. Although the benthic fauna forms carbon and nutrient hotspots in the unvegetated coastal sediments, the pool is dynamic as the benthic species show intra- and interspecific variation in their stoichiometry in response to environmental and biological factors over temporal and spatial scales.

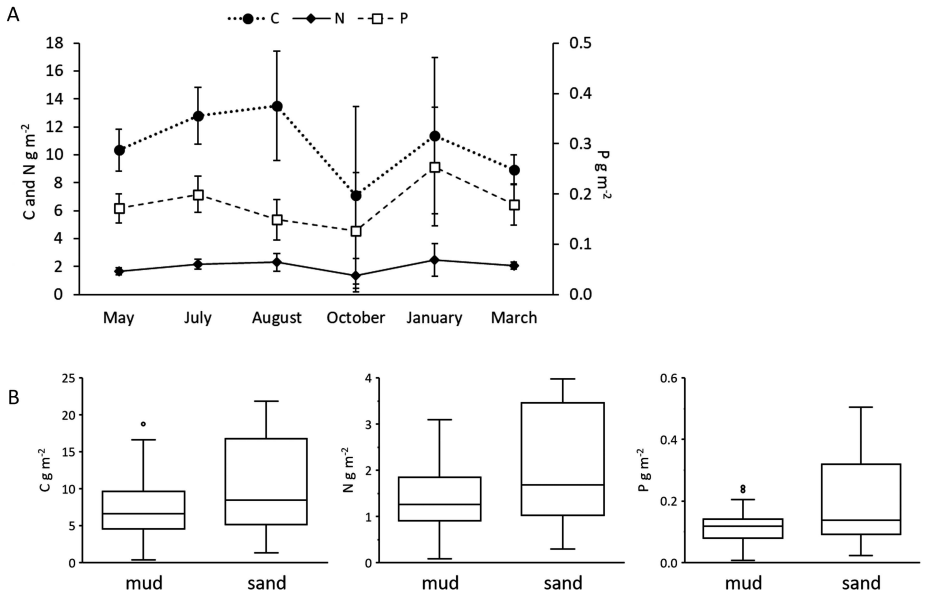


**Figure 4.** Benthic macrofaunal carbon and nutrient stocks varied A) over seasons at aphotic muddy sediments and B) between sandy and muddy habitats. The boxplots present variation between the two sediment types in August, when the highest C, N and P stocks were measured.

### 3.1.1 Intraspecific variation in faunal stoichiometry

Body size is an important factor in predicting the faunal stoichiometry. As large individuals have higher biomass, they also contain more carbon and nutrients. However, the small individuals often have higher mass-specific P content and the C:N:P ratios can change with body size (Sterner and Elser 2002). The growth rate hypothesis states that fast-growing organisms have relatively higher RNA and phosphorus content because they need more phosphorus to support fast rates of protein synthesis (Elser et al. 2003). In addition, benthic consumers undergo ontogenetic changes in their morphology and diet, which alters their stoichiometry (Elser et al. 2003, Pilati and Vanni 2007). The results of study I revealed that benthic animals indeed express ontogenetic variation in tissue C:N:P as the elemental content ratios of the two most dominant species, the clam *M. balthica* and the polychaete *Marenzelleria* spp., increased with increasing body size (Fig. 5A and 5B). The body C content (%) remained relatively constant between the size classes, but the N and P content (%) decreased with increasing size. The lower C:P and N:P ratios measured in juveniles compared to adults most likely refer to an increased demand for P during rapid growth (Elser et al. 2003, Frost et al. 2006). Similar patterns have been previously recorded for other aquatic taxa, such as microbes, zooplankton, insects, decapods (Elser et al. 2003) and fish (Pilati and Vanni 2007). The variation in body stoichiometry during growth could also be linked to ontogenetic variation in faunal diet as *M. balthica* juveniles had higher $\delta^{13}C$ and lower $\delta^{15}N$ values compared to adults, suggesting that adults and juveniles feed on different food sources. Probably, juvenile *M. balthica* are limited by their gut size or siphon length to only ingest small particles from the sediment surface, whereas the adults are facultative deposit and suspension feeders able to take up a large variety of organic material (Ólafsson 1986, Rossi et al. 2004). Although the juvenile *M. balthica* and *Marenzelleria* spp. were highly abundant during July and August, their biomass was very low, therefore their contribution to the total community stoichiometry remains minor.

The reproductive cycle can also change the body elemental composition in many aquatic species (Gabbott 1975). For instance, bivalves invest a majority of their total energy intake to gametes, and the high lipid content of the eggs increases the body carbon content (Honkoop et al. 1999). The spawning of the most dominant species *M. balthica* occurs in May in the study area when the highest C content and C:N ratios were measured for the species and for the whole community. The gonads of *M. balthica*

contain more lipids compared to somatic tissues increasing the C:N and C:P ratios (Honkoop et al. 1999). A previous study by Pekkarinen (1983) recorded similar seasonal trends in *M. balthica* physiology and biochemical content, as the *M. balthica* condition indexes were lowest in May right after the spawning but increased rapidly and were highest in July. In crustaceans, the reproductive tissue has higher N and P content than somatic tissues, which decreases the body C:N and C:P ratios (Lehtonen 1996a, Færøvig and Hessen 2003).

### 3.1.2 Interspecific variation in faunal stoichiometry

The amount of carbon and nutrients stored by the benthic fauna biomass are affected by benthic community composition and population dynamics (Fig. 5). Firstly, the faunal elemental composition is species-specific, as all species have their optimal body composition, and they utilize different food sources to fulfill their energy and nutrient demands (Sterner and Elser 2002). The results of study I showed that overall the benthic invertebrates differed in their elemental composition. On average, the clam *M. balthica* had the highest tissue carbon content of c. 40 %, while the polychaete genus *Marenzelleria* spp. showed the highest body nitrogen content close to 10 % (differences in *M. balthica* and *Marenzelleria* spp. N content illustrated in Fig. 5E). Faunal tissue phosphorus content showed very little variation between the species varying between 0.6 – 1.0 %. Regarding the elemental ratios, on average the *M. balthica* had the highest body C:N, whereas *Marenzelleria* spp. and *H. spinulosus* the lowest. The C:P ratio was also highest in the clam *M. balthica* but lowest in the amphipod *M. affinis*. *M. balthica* and *Marenzelleria* spp. had the highest body N:P, whereas crustaceans such as the amphipod *M. affinis* and the isopod *S. entomon* the lowest. Anatomy and morphology are important factors determining the elemental content of a species (Elser et al. 1996), for instance, the chitinous exoskeletons of crustaceans contain mainly carbon but very little nutrients (Elser et al. 1996) increasing the carbon content and increasing the C:N of the animal. Also, the stable isotope signatures $\delta^{13}C$ and $\delta^{15}N$ differed among the species indicating difference in their diet (Fig. 5F). The relatively high contribution of the stable isotope $\delta^{15}N$ measured in the polychaete genus *Marenzelleria* spp. revealed that the species has a high nitrogen demand because of its high body nitrogen content of c. 10 % (Karlson et al. 2015).

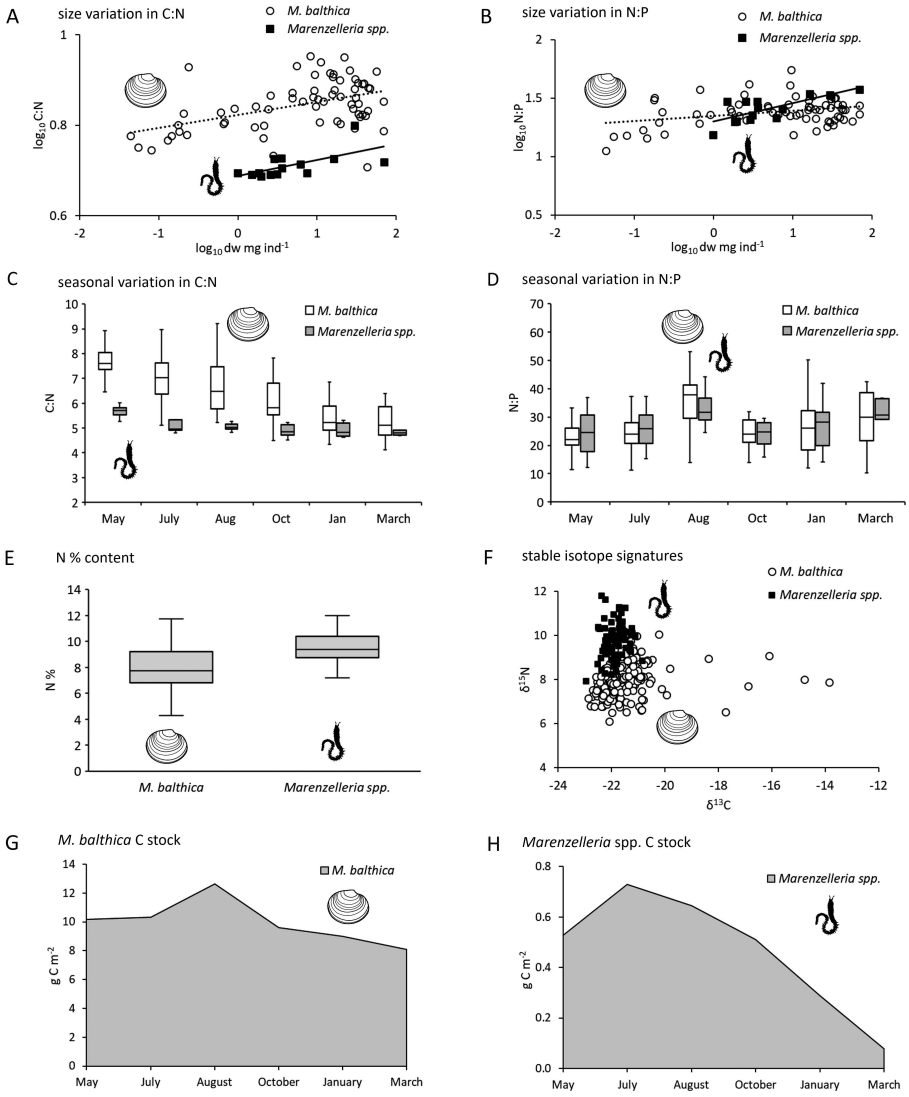


**Figure 5.** Inter- and intraspecific variation in benthic faunal stoichiometry. The faunal stoichiometry showed ontogenetic variation as their body C:N (A) and N:P (B) ratios differed in relation to individual size. The faunal stoichiometry also varied seasonally on population level (C and D). The elemental composition varied between the benthic species (E), for example, the polychaete *Marenzelleria* spp. had higher N content (%) compared to the clam *M. balthica*. Also, the stable isotope signatures varied among the species (F) indicating they utilize different food sources. The long-living *M. balthica* formed a relatively stable carbon stock throughout the year (G), whereas the biomass bound carbon stocks of the short-living *Marenzelleria* spp. practically disappeared during winter (H).

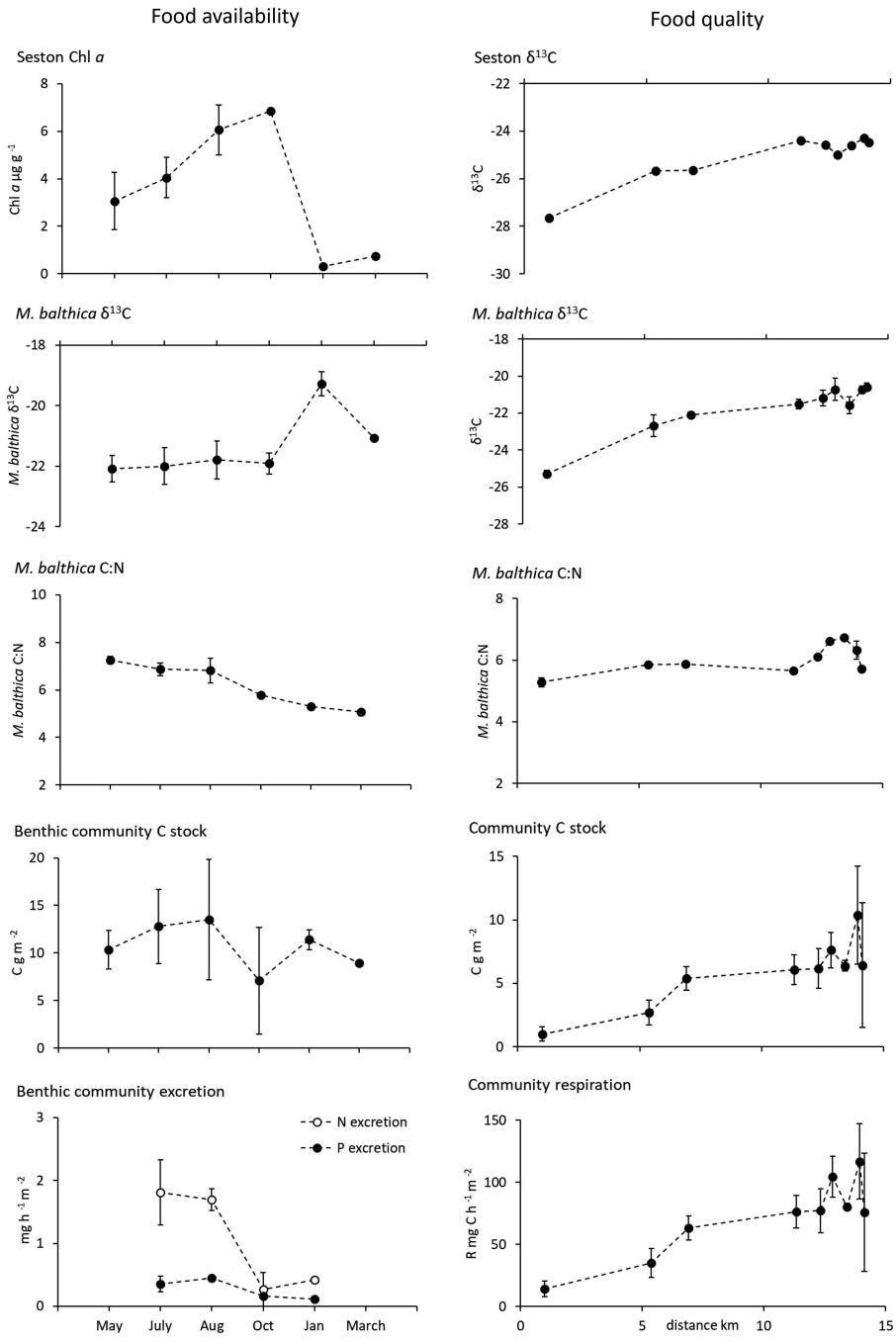


**Figure 6.** Variation in food availability (left) and quality (right) altered benthic faunal stable isotope signatures, stoichiometry, stocks, and process rates.

The different species also have very contrasting life cycles, for example, the long-living clam *M. balthica* formed a relatively stable biomass throughout the year, whereas the polychaete genus *Marenzelleria* spp. has approximately a yearlong lifespan and the population practically disappeared during the winter (Kauppi et al. 2018, Fig. 5G and 5H). In all three studies, the benthic community biomass, C, N, and P stocks followed the population dynamics of the clam *M. balthica*, which dominated the biomass and abundance on all occasions. Notably, as the benthic communities were dominated by large and long-living bivalves, the faunal biomass together with the C, N and P stocks were relatively stable (Griffiths 2006, Villnäs et al. 2019).

### 3.1.3 Seasonal variation in faunal stoichiometry in response to food availability

The benthic animals living in aphotic sediments depend on sinking organic material as their primary food source (Cross et al. 2003, Rodil et al. 2020b). As the benthic communities are subjected to variation in food availability due to strong seasonal patterns in primary production in the northern Baltic Sea (Niemi 1973, Kahru et al. 2016, Rodil et al. 2020b), also their life cycles express seasonality in growth, reproduction, and abundance (Coma et al. 2000). Variation in food sources could also alter the faunal stoichiometric traits (Sterner and Elser 2002, Frost et al. 2005), which would influence the amount of carbon and nutrient stored in faunal biomass (Atkinson et al. 2017).

Study I explored how benthic faunal stoichiometry varies over time in relation to food sources and environmental conditions. The seasonal differences in food availability were evident in measured Chl *a* concentrations of the surface water and sediment, which were high during summer compared to winter months. The faunal C:N:P ratios showed strong seasonal variation at both species and community levels (Fig. 5C and 5D, Fig. 6), which suggests that the stoichiometry of benthic consumers shift in response to food sources and environmental conditions. The faunal C:N decreased from May to March, and C:P was higher in summer compared to winter months (Fig. 6). This variation in elemental ratios was caused by decreasing body carbon content and increasing body N content from summer towards winter. Also, the faunal $\delta^{13}C$ values differed significantly between the sampling occasions with more negative values measured in the summer compared to winter months, suggesting a seasonal

change in the dietary carbon source of these consumers (Wessels and Hahn 2010).

Similar seasonal patterns were observed at both species and community levels at the two study sites. During the starvation period in the winter, benthic animals burn body lipids for energy and lose carbon, decreasing the C:N and C:P ratios and increasing N content. In contrast, during summertime the benthic consumers accumulate lipids in their tissues, which can be seen as an increase in C content and C:N ratio (Lehtonen 1996a). Decreasing temperature is the main driver of these patterns, as it directly lowers the metabolism of the animals (Brown et al. 2004), and indirectly modifies the productivity and composition of phytoplankton communities in temperate seas (Clarke 1993, Lipsewers et al. 2020). Also, stoichiometric mismatches between consumers and their resources were larger in the winter compared to summer months, indicating reduced energy and nutrient availability during the winter months. These observations were supported by the statistical analyses, which showed that the most important environmental factors explaining the seasonal variation in *M. balthica* stoichiometry were proxies for food availability, such as Chl a, seston C:N:P and sediment Chl a:pheo, together with abiotic factors temperature, salinity, and oxygen concentration.

### 3.1.4 Spatial variation in community carbon and nutrient stocks in response to food quality

Coastal areas often receive increasing amounts of organic matter and nutrient inputs that may cause imbalances in consumer-resource stoichiometry (Snelgrove et al. 2014, Peñuelas et al. 2019). The structural and functional composition of benthic macrofaunal communities vary across spatial gradients (Villnäs et al. 2019), but the implications of how such shifts alter the benthic community stoichiometry remain largely unresolved (Cross et al. 2003, Atkinson et al. 2017). By sampling both shallow sandy and deep aphotic muddy sediments across a land-to-sea gradient in the coastal Baltic Sea, study III explored if benthic community composition and stoichiometry varied in response to changes in faunal food sources.

The results showed that the quality of organic matter for the benthic consumers differed between shallow sandy and deep muddy sites and across the land-to-sea gradient (Fig. 6). In aphotic muddy sites, detritus is the basal food source for the benthic consumers (Graf 1992), while the

shallow sandy sites have close benthic-pelagic coupling and light reaches the seafloor enabling benthic primary production, which enhances food quality for the benthic animals (Marcelina et al. 2018). The quality of the organic matter also changed from land towards the open sea, as the amount of land-derived organic matter decreased, and autochthonous matter increased across the gradient. The seston $\delta^{13}C$ indicated terrestrial runoff and $\delta^{15}N$ sewage input at the innermost study sites (Fig. 6), whereas increasing seston Chl a and Chl a: pheo towards the open sea suggested increasing resource quantity and quality for the benthic consumers at the outer sites.

The spatial differences in food quality were reflected in benthic community carbon and nutrient pools. The benthic community biomass together with carbon and nutrient stocks were higher at shallow sandy compared to deep muddy sites and increased across the land-to-sea gradient (Fig. 4 and 6). At the sandy sites, also the number of species increased towards the outer archipelago. The increase in community biomass towards the outer sites was mostly affected by an increase in the key species *M. balthica*. However, the biomass dominance of the clam *M. balthica* decreased over the gradient and the contribution of other species to community biomass increased.

Interestingly, despite the variation in food sources, the benthic community stoichiometry remained stable. Only the C:N of the deeper, muddy sediment communities varied across the land-to-sea gradient. However, faunal stable isotope signatures showed clear response to variation in food quality (Fig 6). The $\delta^{13}C$ of *M. balthica* and *Marenzelleria* spp. increased across the land-to-sea gradient in both habitat types, following the variation in seston and sediment $\delta^{13}C$. The $\delta^{15}N$ of *M. balthica* expressed different trends in the two habitat types, suggesting that this facultative suspension and deposit feeding clam (Ólafsson 1986) prefers filter-feeding in the nutrient poor sandy sediments, while the high availability of detritus sustains deposit feeding in muddy sites (cf. Karlson et al. 2015). Also, the $\delta^{15}N$ of *Marenzelleria* spp. decreased across the land-to-sea gradient in both habitat types indicating that the polychaete genus feeds on seston at sandy sites, but on sediment at muddy sites. Importantly, as these two key species show plasticity in their biological traits, such as in feeding modes or feeding depths, their impact on ecosystem functions could differ between habitat types (Törnroos et al. 2015).

## 3.2 Benthic community process rates

To estimate the contribution of the benthic communities to carbon and nutrient turnover, study II measured the excretion rates and study III calculated the respiration and secondary production rates by the benthic fauna. Benthic animals take up nutrients by feeding and transform them into inorganic ammonium and phosphate, which are recycled back to primary production through excretion (Josefson and Rasmussen 2000, Persson et al. 2010). In study II the measured benthic community N-excretion rates varied between 10–190 µmol $m^{-2}$ and P-excretion between 3-20 µmol $m^{-2}$. These rates were larger than the measured sediment effluxes, but it should be noted that the animals experienced stress during incubations, which increases the rates significantly. Thus, the measured nutrient excretion rates should be evaluated as maximum excretion potential by the benthic communities.

Benthic consumers can have a profound impact on nutrient regeneration in coastal marine ecosystems (Sardans et al. 2012, Atkinson et al. 2017). Although the effect of benthic faunal excretion rates on sediment nutrient fluxes are often negligible compared to microbial processes in the sediments, dense aggregations of benthic macrofauna can alter nutrient concentrations and ultimately change the limiting nutrient in an aquatic system (Atkinson et al. 2013, Atkinson and Vaughn 2015). In a sandy tidal flat, the sediment $NH_4^+$ and $PO_4^{3-}$-fluxes including bivalves were ten times higher compared to the nutrient fluxes from the same sediment without fauna (Magni et al. 2000). In previous studies from the Baltic Sea, excretion by the clam worm *Nereis virens* contribute to c. 30 % and the excretion by the amphipod *M. affinis* up to 10 % of the total of sediment-water NH4+-fluxes (Kristensen 1984, Tuominen et al. 1999). However, the benthic faunal excretion rates can show high variation depending on the faunal biomass, temperature, and food availability (Lehtonen 1996b, Magni et al. 2000, Norkko et al. 2013).

As respiration and secondary production rates are strongly related to biomass production, they are often used as measures of ecosystem function important for carbon cycling (Wong et al. 2011). In the Baltic Sea, the respiration by benthic animals is highly important in organic matter mineralization (Ehrnsten et al. 2019, Rodil et al. 2020a). At the study area in the GoF, the benthic fauna contributes between 10 to 30 % to the total seafloor respiration, although in dense blue mussel reefs their contribution might exceed 40 % (Rodil et al. 2020a). In study III, the estimated benthic

community respiration rates ranged between 7-144 mg C $m^{-2}$ $d^{-1}$ at muddy sites and between 15-232 mg C $m^{-2}$ $d^{-1}$ at sandy sites. Attard et al. (2019) measured the total respiration rates of the seafloor during the same season in the study area to c. 70 mg C $m^{-2}$ $d^{-1}$ at aphotic muddy and 39 mg C $m^{-2}$ $d^{-1}$ at bare sandy sediments. In comparison to their values, community respiration rates in study III accounted for 10-200 % of the total seafloor respiration in aphotic muddy sites, and between 30-50 % in shallow sandy sites. The calculated benthic community secondary production rates ranged between 6 - 103 mg C $m^{-2}$ $d^{-1}$ at the muddy sites and between 13 - 210 mg C $m^{-2}$ $d^{-1}$ at the sandy sites. At the study area, the benthic fauna seems to be more important for carbon cycling at the organic matter poor sandy sites compared to muddy sites. Although secondary production rates are strongly linked to faunal biomass, recent studies have shown that diversity enhances benthic community production (Rodil et al. 2020a, 2022). Commonly, the highest benthic faunal secondary production rates are measured in vegetated habitats, such as in seagrass beds, which rates range between 47 – 467 mg C $m^{-2}$ $d^{-1}$ in the study area (Rodil et al. 2020a, 2022). Overall, the results emphasize that the contribution of benthic fauna to ecosystem function is highly variable and strongly related to the community biomass (Snelgrove et al. 2018).

### 3.2.1 Seasonal variation in benthic faunal excretion rates

As the benthic animals modify sediment carbon and nutrient recycling processes directly through their metabolism (Vanni 2002), the rate at which benthic communities recycle nutrients is highly dependent on community biomass, species composition, allometric traits and environmental conditions, most importantly temperature (Vanni 2002, Brown et al. 2004, Allgeier et al. 2015, Vanni and McIntyre 2016). Study II measured the nutrient excretion rates by benthic species to determine if the excretion rates can be predicted by stoichiometric and allometric traits. As expected, the results showed that the benthic faunal N and P excretion rates are species-specific, and strongly related to species metabolism, as body size and temperature were the most important factors predicting faunal excretion rates. Small individuals had higher mass-specific excretion rates compared to larger individuals, and the N and P excretion showed a positive relationship with increasing water temperature (Fig. 7).

As the excretion rates were highly temperature-dependent, the excretion by benthic fauna showed strong seasonal variation at both species and

community levels (Fig. 6 and 7). The highest faunal excretion rates were measured in August, when the community $NH_4^+$-N excretion clearly exceeded the net sediment efflux. The excretion by benthic macrofauna can boost meiobenthic and microbial activity and speed up sediment biogeochemical cycling (Stief 2013, Zilius et al. 2022). Most likely, in the organic matter rich sediments the high faunal $NH_4^+$-N excretion stimulated nitrification and denitrification processes (Stief 2013). In contrast, the lowest excretion rates were measured in January with water temperature between 4-5 °C. Interestingly, the statistical test suggested that also stoichiometric traits such as tissue C and N %, C:N and N:P, as well as stable isotope signatures $\delta^{13}C$ and $\delta^{15}N$ explained a small, but significant, proportion of the variability in species excretion rates. This strong seasonal pattern underlines that changes in temperature, food availability and resource quality are important drivers of faunal metabolic processes, and thus excretion rates of the benthic consumers. Thus, global warming together with increasing nutrient imbalance in coastal areas will likely change the nutritional and metabolic requirements of these consumers and affect their ability to recycle nitrogen and phosphorus (Brown et al. 2004).

The most dominant species, the clam *M. balthica* and polychaete genus *Marenzelleria* spp., drove the community nutrient recycling rates at all sampling times. As both species fed on food that had lower N:P compared to the tissue N:P ratio, and excreted even lower N:P, they could have been nitrogen limited (Sterner 1990). This mismatch between the consumer-resource stoichiometry was emphasized during winter months, when the food availability was low and metabolic activity slow due to low water temperatures. Deposit feeders are often nitrogen limited, which can reduce the growth and reproduction of these species (Tenore 1988). Because the polychaete *Marenzelleria* spp. has higher nitrogen requirement due to high body N content, it is most likely more sensitive to nitrogen limitation (Karlson et al. 2015).

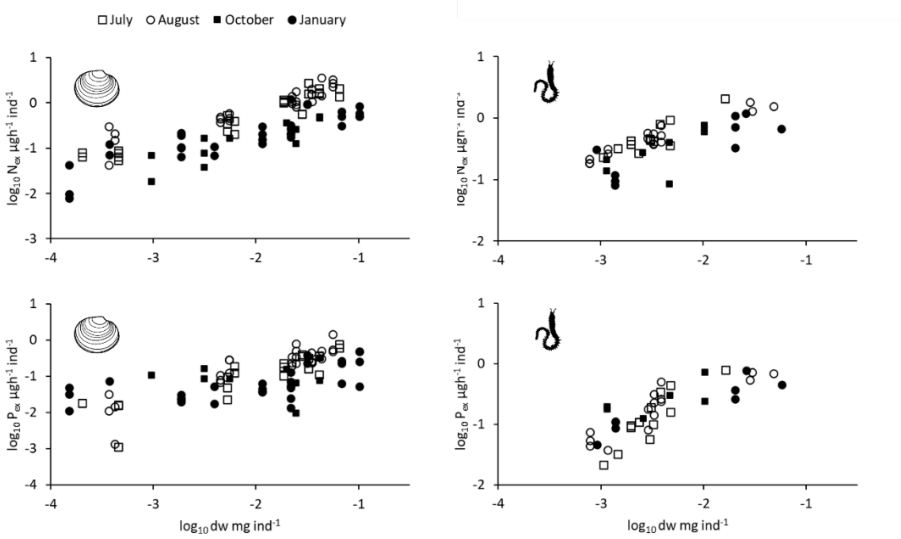


**Figure 7.** Benthic faunal N and P excretion rates were species-specific and size-dependent and showed seasonal variation in response to variation in water temperature.

### 3.2.2 Spatial variation in benthic community respiration and secondary production rates

The benthic community respiration and secondary production rates followed the variation in community biomass (chapter 3.1.4). As the community biomass was higher at sandy sites compared to muddy sites, also the respiration and secondary production rates were higher at shallow sandy compared to aphotic muddy sites. In addition, the respiration and secondary production rates increased across the land-to-sea gradient in response to increasing food quality at both habitat types. The statistical analyses of the study III showed that at the muddy sites, primary production estimates referring to food quality were the most important factors increasing the benthic community carbon stocks, respiration, and secondary production rates.

At shallow sandy sites with close benthic-pelagic coupling, the biological factors such as decreasing biomass dominance of *M. balthica* and increasing species richness explained over 50 % of the variation in the community carbon stocks, respiration, and secondary production rates. Probably, the better food quality towards the open sea could sustains high

benthic community biomass, which in turn directs higher carbon stocks and process rates. In addition, the increasing number of species and decreasing *M. balthica* biomass dominance towards the outer archipelago highlights the importance of diversity for the community secondary production. Higher species richness could lead to higher trait diversity and to better resource partitioning between the species (Karlson et al. 2010), which would in turn increase the community biomass and related processes such as respiration and secondary production. The results emphasize the importance of sustaining diverse benthic macrofaunal communities for maintaining the carbon stocks, respiration, and secondary production rates, i.e. processes essential for carbon turnover in coastal habitats.

## 3.3 Turnover of the benthic community carbon and nutrient stocks

As the degradation processes of organic matter mainly occur in the seafloor, benthic macrofauna has a central role in directing the mineralization processes (Josefson and Rasmussen 2000, Snelgrove et al. 2014, Allgeier et al. 2017). Simulated carbon and nutrient flows have shown that benthic fauna can take up 80 % and mineralize 40 % of the sinking organic material, thus the contribution of benthic fauna to organic matter processing is substantial in the Baltic Sea, and mainly driven by food availability (Ehrnsten et al. 2019, 2022). Species diversity together with trait diversity are known to increase biological productivity, and enhance ecosystem function (Cardinale et al. 2009, Snelgrove et al. 2014). As all species have a unique set of traits, they contribute differently to carbon and nutrient retention processes (Snelgrove et al. 2018). Therefore, shifts that simplify the benthic community composition, also reduce its trait distribution, and might decrease the benthic community carbon and nutrient stocks and accelerate their turnover rates (Sobral et al. 2023). For example, long-living animals with a large body size form a relatively stable carbon and nutrient stock with long turnover times, whereas small-sized species with short lifespan have traits that support fast carbon and nutrient turnover (Sobral et al. 2023).

The results of this thesis indicated that the stability of the benthic community carbon and nutrient stocks, i.e. the amount of carbon and nutrients in the form of animal biomass, varied over temporal and spatial scales. Study I showed that when benthic communities were dominated by long-living bivalves, the carbon and nutrient stocks were stable over time

(Fig. 5E and 8). In contrast, if the communities were dominated by short-living species, the benthic community carbon and nutrient stocks would collapse seasonally (Fig. 5F). As the increase in the faunal biomass, stoichiometry and excretion rates showed strong seasonal patterns in the study system, it seems that the role of the benthic communities as sinks or sources of carbon and nutrients varies within a year.

In study III, the deep muddy sites had higher P:B compared to shallow sandy sites, indicating that the benthic communities in muddy sediments have faster growth rate, smaller body size, shorter lifespan, and consequently faster turnover of carbon (Wetzel 2001). In muddy sites, the P:B ratio also decreased across the land-to-sea gradient. Most likely, the benthic communities closest to land were affected by eutrophication and consisted of smaller fast-growing individuals with traits that enhance the nutrient turnover but decrease the carbon and nutrient retention capacity of the community (Fig. 8). In contrast, towards the open sea the benthic communities were more diverse and dominated by long-living large individuals, which form more stable C, N and P stock and have higher contribution to the temporal carbon and nutrient retention in the sediments (Fig. 8). Similar shifts in benthic community composition have observed across the coastal areas of the Baltic Sea (Villnäs et al. 2019, Carstensen et al. 2020, Sobral et al. 2023).

# 4. Conclusions and implications for future research

Productive coastal ecosystems are globally important for organic matter mineralization, and carbon and nutrient cycling (Snelgrove et al. 2014, 2018). Therefore, studying the drivers, pathways, and rates of these biogeochemical processes is essential for understanding ecosystem functioning. This thesis explored the role of benthic macrofauna in coastal carbon and nutrient cycling by extensive field studies (I and III) and laboratory measurements (II). To summarize, benthic macrofaunal communities contained a significant amount of carbon and nutrients compared to the surrounding environment, thus, benthic fauna can function as an important carbon and nutrient pool in the soft-sediment habitats of the coastal Baltic Sea. The turnover of the carbon and nutrients bound in faunal biomass is directed by the storage and recycling rates of these animals (Fig. 8). Firstly, the amount of carbon and nutrients stored in the benthic biomass was species-specific and followed the population dynamics of each species. On intraspecific level, the faunal elemental content varied with size and reproductive stage. The faunal stoichiometry also varied seasonally in response to food availability, as the body carbon content decreased from spring towards winter. However, as the community biomass was dominated by large and long-living bivalves, the carbon and nutrient stocks remained overall relatively stable (Fig. 8). Seasonal variation in the benthic faunal process rates was mainly driven by temperature, which directed the metabolic activity (Fig. 8). Furthermore, the benthic faunal excretion rates were species-specific and size-dependent, and the benthic community composition regulated the recycling rates of nutrients. The benthic community composition was also affected by spatial gradients in food quality, as the innermost sites with high terrestrial organic matter inputs had lower community abundance, biomass, C, N, P stocks, respiration and secondary production rates compared to the outer archipelago sites (Fig. 8).

This thesis manages to establish a baseline for the stoichiometric traits and variation in the stoichiometry of Baltic benthic fauna, but future research is still needed especially regarding the process rates of these species. To date, measurements of food uptake, assimilation, ingestion, egestion rates are still largely pending. In addition, information regarding the mineralization of the carcasses of benthic species would be important in establishing whether the benthic fauna functions as net sink or source of

carbon and nutrients (Vanni et al. 2013). Moreover, this thesis shows that benthic communities respond to variation in food sources, but it would be interesting to explore how the shift in phytoplankton community composition would alter the benthic community stoichiometry, and how that is reflected in the stoichiometric balance at higher trophic levels (Atkinson et al. 2017).

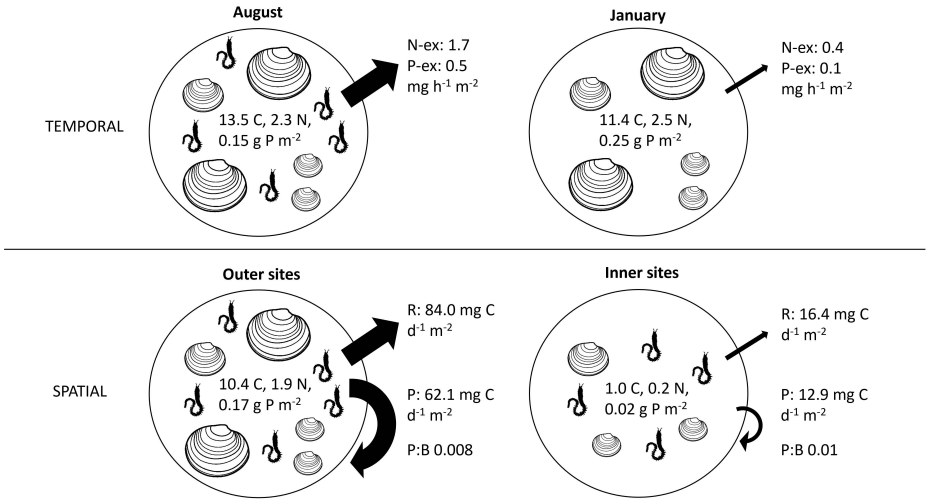


**Figure 8.** Variation of muddy sediment benthic faunal carbon and nutrient pools over temporal and spatial scales. Temporal variation: the community biomass, C, N, P stock, and excretion rates were highest in August, while in January the nutrient excretion rates decreased. The C, N, P stocks remained relatively stable, as the community biomass was dominated by large and long-lived bivalves. Spatial variation: In the outer archipelago, high food availability and quality supported high benthic community biomass, C, N P stocks, respiration, and secondary production rates. In contrast, at the inner archipelago with high inputs of terrestrial organic matter, the food availability and quality were lower, decreasing the community biomass, C, N, P stocks, and process rates. The higher P:B ratio at the inner sites indicated faster community carbon and nutrient turnover compared to outer sites. (C: carbon stock, N: nitrogen stock, P g m-2: phosphorus stock, N-ex: nitrogen excretion rate, P-ex: phosphorus excretion rate, R: respiration rate, P mg d-1 m-2: secondary production rate, P:B: secondary production to biomass ratio. Thickness of the arrows describes the volume of a process rate.)

Although the variation in benthic faunal stoichiometry occurred on relatively small scale, the animals clearly responded to changes in environmental conditions. Hence, long-term disturbances in resource availability or quality are likely to affect the body composition and metabolic rates of the benthic consumers, consequently changing the ability of the benthic communities to store and recycle carbon and nutrients. Increasing seawater temperatures due to global warming can boost the biochemical cycling, as the metabolic activity of macrofauna and microbes increases, but prolonged intense marine heatwaves can cause oxygen depletion, leading to decrease in biochemical and biological process rates (Kauppi and Villnäs 2022). In addition, eutrophication and increased land-use can cause excessive organic matter and nutrient inputs to the coastal Baltic Sea, which affect both the benthic habitats and food sources for the benthic consumers. Most likely, eutrophication together with seawater warming will reduce macrofaunal community biomass, and concurrently decrease the consumer bound carbon and nutrient stocks, and process rates, largely declining the processing of organic matter. Also, introductions of invasive species could rapidly alter the benthic community composition and function (Norkko et al. 2012). Overall, the results of this thesis highlight the importance of maintaining diverse benthic communities to sustain their contribution to coastal carbon and nutrient cycling.

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