The effect of urbanization on meiofauna in sandy beaches of the southern North Sea

Michael Lemke, Department of Earth Sciences, Utrecht University (Marine Sciences)

1st Supervisor: Dr. Francesca Sangiorgi – Department of Geosciences, Utrecht University

2nd Supervisor: Dr. Jan-Niklas Macher -- Institute of Environmental Sciences (CML), University Leiden

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1 Abstract

The effect of urbanization on sandy beach meiofauna is poorly understood. To understand how intertidal meiofauna communities will respond to increasing urbanization pressures, their relationship to urbanization factors were investigated using environmental DNA metabarcoding with a COI marker. Eleven beaches were sampled in the southern North Sea between Scheveningen and Zandvoort and categorized based on a paper by Gonzalez et al. (2014) to determine their degree of urbanization. Generalized linear mixed modelling and nonmetric dimensional scaling statistical methods were used to assess alpha and beta diversity of the meiofauna communities at sampling location. The results showed that the lower intertidal zone were overwhelmingly dominated by polychaeta, followed by chromadorea and hydrozoan while in the upper intertidal zone the most abundant taxa were clitellata, chromadorea, and polychaeta. The urbanization factors showed no impact on OTU richness in the lower intertidal meiofauna, though the GLMM model showed a negative effect on OTU richness in the upper intertidal meiofauna associated with vehicle traffic, buildings on the sand, and poor dune condition. The nonmetric multidimensional scaling results presented a variety of factors that affected community composition. In the lower intertidal zone these factors were SQM, beach slope, dune condition, and UI, and SQM, beach slope, vehicle traffic, and beach cleaning. A GDM model revealed that geographic distance and beach slope greatly influenced the meiofauna compositional turnover, and that dune condition, vehicle traffic, and beach cleaning also had a smaller impact on the meiofauna beta diversity. These results suggest that beach meiofauna communities are influenced by urbanization and warrants

further studies to unravel these links., We emphasize the importance of study design that is appropriate for your research question and recommend using a combination of methods to avoid biases.

2 Introduction

Sandy beaches are the most common type of shoreline along the European coast and offer valuable economic, social, and ecological services. They are a hotspot for tourism, recreation, and coastal developments and provide essential functions such as buffering against storms and waves, water filtration and purification, nutrient recycling, sediment storage and transport, nesting sites for turtles and shorebirds, and many more (Defeo et al., 2009). Urbanization has led to intense coastal developments with consequences to sandy beach ecosystems. With anthropogenic changes predicted to intensify and with the advent of climate change it is critical to understand how sandy beach ecosystems are affected by this environmental change and to develop methods to gauge ecosystem health in order to protect these coastal environments (Defeo & Elliott, 2021; Lansu et al., 2024). This seemingly uninhabited terrain is in fact home to a rich and biodiverse community of organisms that occupy the interstitial space between sediment particles.

Meiofauna (also called meiobenthos) are small invertebrates living in the sediment or benthos of marine or freshwater environments, including nematodes, copepods, rotifers, platyhelminths, tardigrades, annelids, and ostracods. Meiofauna are often classified by size as those that will pass through a 1.0 mm mesh but are retained by a 44 µm mesh (Giere, 2009). Meiofauna that live on fine sandy beaches are typically relegated to the top 10 cm layer of the sand due to the availability of oxygen in the redox potential discontinuity (RPD) zone, with nematodes becoming more important in oxygen-depleted sediments with depth as they tend to be less sensitive to oxygen deficiency (Kotwicki et al., 2005). Meiofauna are adapted to the interstitial environment between the sand grains and

commonly have slender bodies, small size, contractibility, anchoring mechanisms, and protection against abrasion (Giere, 2009).

Meiofauna are important because they serve three key ecosystem roles. First, they play an integral role in marine food webs. Predators, including juvenile fish, enjoy meiofauna as a preferred food source. Copepods, in particular, are highly nutritional owing to their high fatty acid content (McLachlan & Defeo, 2018). The meiofauna themselves feed on primary producers and help transfer their energy to higher trophic levels. Second, meiofauna play a vital role in the breakdown and turnover of organic material in sand and sediments (Schratzberger & Ingels, 2018). Dissolved and particulate organic matter washed into the sand is recycled by the interstitial community and can be seen as a biological filter to cleanse coastal waters (Pearse et al., 1942). Lastly, because of their high turnover rate and sensitivity to anthropogenic inputs, they can be used as indicators for pollutants and in biomonitoring programmes (Schratzberger et al., 2000). Nematodes, in particular, have been the focus of several studies due to their abundance, biological and physical characteristics, and survivability under extreme conditions. One study of the Egyptian Mediterranean coast suggested that nematode community structure and functional traits could be used in biomonitoring and coastal restoration programs (Mitwally, 2022). Another study by Schratzberger et al. found trace metals composition and sediment grain size contributed to meiofauna assemblages and could help monitoring programs identify the extent of anthropogenic impacts (Schratzberger et al., 2000).

Beaches represent an economic and touristic incentive for governments and municipalities and thus maintenance and public perception of their beaches is a priority. In the Netherlands, approximately €10.4 million is spent every year on removing beach

litter (Mouat, J., Lozano, R.L., Bateson, H., 2010). The process of removing wrack and other debris from beaches may be necessary as an economic and public health perspective but is potentially deleterious to beach fauna and flora. Wrack removal has been shown to impact plant communities in dune ecosystems (Del Vecchio et al., 2017), and wrack also supports both macro- and meiofauna that use it as a habitat and food source. A review of beach cleaning by S. Zielinski et al. (2019) recommended less frequent/intensive cleaning methods as several studies have reported higher abundance and species diversity on beaches that are manually cleaned (Morton et al., 2015), (Stelling-Wood et al., 2016). There is evidence that macrofauna on sandy beach ecosystems are impacted over time by urbanization pressures such as beach cleaning, frequency of visitors, and proximity to urban centers (Augusto et al., 2023). Meiofauna have received less attention in the literature, though one study investigated the effects of a one-off machine cleaning event to nematode community structure and abundance (Gheskiere et al., 2006). While immediately after the event there was a decrease in both abundance and community structure, numbers recovered within 24 hours likely due to vertical migration forced from the rising tide. There is to this date there are very few studies investigating the effect of beach cleaning and/or other urbanization parameters on sandy beach meiofauna communities (Felix et al., 2016) . There is thus a need for larger-scale experiments to compare meiofauna communities and elucidate these urbanization effects.

Identification of meiofauna through the traditional morphology-based methods is difficult and time-consuming due to their small size and diversity. Environmental DNA metabarcoding has become a widely used and accepted method in many environments

thanks to high sample throughput, accuracy, and ease of use (Hering et al., 2018; Keck et al., 2022). For example, eDNA has been used for biodiversity biomonitoring campaigns (Deiner et al., 2017) and to assess threatened or invasive species (Rapp et al., 2021; Von Ammon et al., 2019). Metabarcoding has been successfully used to help develop the taxonomic resolution of meiofauna communities (Atienza et al., 2020; Steyaert et al., 2020) and less commonly to explore the relationships between meiofauna communities and environmental variables. Of the latter studies, the focus has been on physical parameters including salinity and granulometry (Bellisario et al., 2021) (Fais et al., 2020).

Using sequence data obtained through environmental DNA metabarcoding, this thesis investigates the impact of urbanization effects such as beach cleaning on the alpha and beta diversity of meiofauna communities in the Southern North Sea using generalized linear mixed modelling (GLMM), nonmetric multidimensional scaling (NMDS) statistics, and generalized dissimilarity modelling (GDM). The aim of this research is to identify factors that contribute to meiofauna biodiversity and community structure that will help identify species that could be used as bioindicators to be used in impact assessments that could ultimately inform policy and help protect coastal ecosystems.

3 Materials & Methods

3.1 Study area

Field sampling took place between July 25, 2023 and August 22, 2023. Samples were collected at eleven public, dissipative sandy beaches covering a span of 35 kilometers along the Southern North Sea in North and South Holland. (Figure 1). Beaches were chosen to achieve a gradient of high-density, machine-cleaned beaches like Scheveningen to beaches with high density at access points (Wassenaar), to natural conservation zones such as Noordwijkerhout.

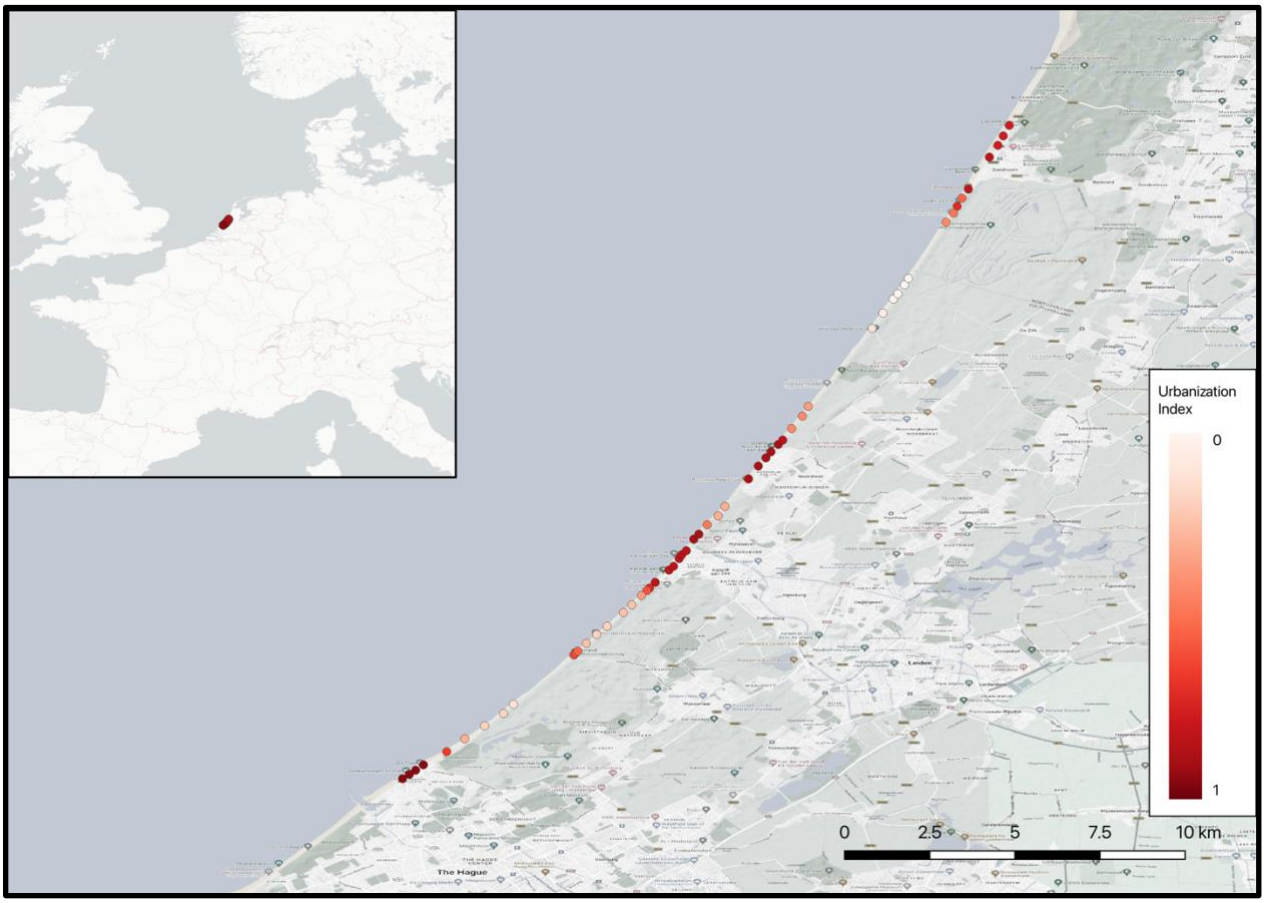

Figure 1. Sampling locations in the southern Dutch North Sea. Each circle represents a transect location, where six samples were taken between high and low tide. Darker colour points represent a higher level of urbanization.

Table 1. Sampling locations and samples collected. See supplementary material for all transects.

Location	Station	Latitude	Longitude	Transects	Sampling Date	Total Samples
Katwijk	T	52.1998041	4.3892825	5	25-7-2023	30
Katwijk South	KS	52.1945625	4.3833702	6	27-7-2023	36
Wassenaar	WA	52.163483	4.348504	6	31-7-2023	36
Noordwijkerhout	NO	52.303286	4.476266	6	2-8-2023	36
Katwijk North	KN	52.213045	4.3998987	5	4-8-2023	30
Scheveningen North	Schn	52.122064	4.294074	5	8-8-2023	30
Noordwijk North	NoN	52.253575	4.436104	5	10-8-2023	30
Zandvoort South	ZS	52.362852	4.517494	5	14-8-2023	30
Zandvoort City	ZC	52.3901373	4.53502	4	17-8-2023	24
Noordwijk City	NoC	52.238829	4.4233392	4	21-8-2023	24
Scheveningen City	SchC	52.1164457	4.2840893	4	22-8-2023	24

3.2 Sample collection

At each beach, 4-6 transects were sampled. The transects formed a gradient from the busiest areas (access points with restaurants and amenities) to lower traffic areas away from the main recreation zones. Within each transect, six equidistant samples were taken from the high tide line (sample B1) to the low tide line (sample B6) across the intertidal zone of the beach, with the lowest sample at the low tide line and the highest sample at the high tide line. Samples were collected as close to low tide as possible. Samples were taken using plastic 20-mL syringes with the end cut off. The syringes were inserted into the sand to the 10-mL mark and the sand was then transferred to a 50 mL falcon tube. The sample weight varied due to water volume and sand compression but typically measured between 13-17g. After sampling, the tubes were returned to the lab and stored at -20C until processed. In total, 330 samples were collected among 55 transects at 11 locations (**Table 1, Table S5)**. At each transect the beach slope was measured (in degrees), the intertidal width between the high tide and low tide lines, latitude and

longitude, and qualitative indicators of urbanization: proximity to urban centre, buildings on sand, beach cleaning, solid waste, vehicle traffic, quality of night sky, frequency of visitors, and dune condition. The qualitative indicators were used to determine the degree of urbanization at each transect (see section 3.8).

3.3 Reference database

In addition to the samples taken for metabarcoding, larger samples were taken at various locations to collect individual specimens. These large samples (a bucket) were taken to the lab and extracted from the sand with a magnesium chloride solution (MgCl concentration was adjusted to match the salinity of the seawater at the location sampled). Subsamples from the buckets were shaken vigorously with the MgCl solution and left for 10 minutes to detach from the sand particles, then shaken once more after the 10 minutes. The supernatant was then decanted through a 1 mm and 44 µm sieve cascade to isolate meiofauna and washed with seawater into a petri dish. Individual specimens were visualized through a stereomicroscope, then picked with Pasteur pipettes or pincers onto a glass slide and imaged using light microscopy with camera attached. The individual specimens were placed in individual wells in a 96-well PCR plate with DNA-preserving buffer. The DNA was extracted using a KingFisher robotic platform and then sequenced using NanoPore technology following a cleanup procedure. These sequences, along with the images, could then be used to annotate the metabarcoding data for the community samples. Reference barcoding complemented the sequencing results to this project but was not a focus. A more detailed protocol can be found in the supplementary material.

3.4 Metabarcoding

3.4.1 DNA extraction

The DNA extraction protocol was adapted from the soil DNA extraction (SDE) workflow by Bollmann-Giolai et al (2020). Each sample was lysed in the 50-mL falcon tube. To each sample, 17.5-20g of 1mm plastic beads were added, followed by 25 mL of lysis buffer (121mM guanidine thiocyanate, 181mM trisodium phosphate) and 3.6 mL of lysis additive buffer (150mM NaCl, 0.8% SDS, 0.5M Tris pH 7). The samples were then inverted and shaken by hand to mix the solutions and disperse the sand particles from the bottom of the tube. The sample was then vortexed for 30-45 seconds, and laid horizontally overnight to improve cell lysis.

The following day, samples were vortexed briefly then centrifuged at 2,250 x g for 5 minutes. 450 uL was taken from the supernatant and transferred to a new tube. For inhibitor removal, 250 uL of 133mM ammonium acetate was added. The mixture was left to incubate in the fridge for 10 minutes, and then centrifuged at 17,000 x g for 3 minutes. After centrifuging, 500 uL of the supernatant was transferred to a new 1.5 mL Eppendorf tube. In the new tube, 200 mL of 60mM aluminium ammonium sulfate dodecahydrate was added. Following this, the sample was again incubated for 10 minutes in the fridge, and then centrifuged for 10 minutes at 17,000 x g. The magnetic beads (SeraMag/Magnetic Carboxylate) were prepared by washing twice with a large volume of 1% Tween-20. To each sample, 20 uL of the beads and 420 uL of isopropanol were added then briefly vortexed and centrifuged. The samples were then left for 10 minutes for DNA to adhere to the beads. The samples were then placed on a magnetic rack and the solution was

removed from the sample tubes. The beads were then washed twice with 500uL of 80% ethanol. After washing, the ethanol was completely removed and the beads were left to air dry for 5 minutes. 50 uL of elution buffer (0.001M EDTA, pH 8, 0.01M Tris-HCl, pH 8) was then added to the samples and let to elute for 10 minutes. The samples were again placed on a magnetic rack and the elution was removed to a new Lo-Bind 1.5 mL Eppendorf tube and placed in the fridge for storage.

3.4.2 Library preparation

The metabarcoding library prep entails two main steps: an initial PCR reaction that amplifies the target region of DNA and a second PCR that labels each library with a unique index combination. During the first PCR round, the target DNA is amplified during 35 cycles with IDT10-tailed primers that are placed at the 5' position of the target specific part of the primer. The primer set used was chosen to target the universal cytochrome c oxidase subunit I gene (COI) and developed by Leray et al., 2013. The forward and reverse primers and their sequences with the attached IDT10-tails were IDT_mlCOIintF 5'CACTCTTTCCCTACACGACGCTCTTCCGATCT**GGWACWGGWTGAACWGTWTAYCCYCC**'3 (Leray et al., 2013) and IDT_HCO2198 5'GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT**TAAACTTCAGGGTGACCAAAAAATCA**'3 (Black et al., 1994), respectively. Post-PCR, all samples were checked on a 1.0% agarose gel with electrophoresis to ensure presence of DNA around ~400 bp. Once all the samples were processed, a bead-cleanup was performed with a Cytena C.Wash device using a 0.9:1 ratio of NucleoMag™ NGS Beads to amplified DNA.

Following the bead clean-up, a second PCR was performed to add a unique IDT10 index label to each sample. The PCR conditions were the same as the first PCR, except the number of cycles was reduced to 5. To ensure the dsDNA fragments were of the right size, the product of the second PCR reaction was tested with the Fragment Analyzer™ automated electrophoresis system using the DNA Kit DNF-910 (**Figure S10)**. Subsequently, a QiAgility liquid handling station was used to normalize all samples into new 96-well plates so that they could be equimolarly pooled. Each plate then had all of its samples pooled into an Eppendorf tube and the resulting end pool was then cleaned using using a 0.9:1 ratio of NucleoMag™ NGS Beads to amplified DNA. The 4150 TapeStation System was then used to measure the purity and concentration of the DNA in the end pools, and an equimolar subpool of each was then combined and sent for MiSeq sequencing.

3.4.3 Community metabarcoding bioinformatics

To analyze the raw reads from the sequencing data, a standardised workflow (Beentjes et al., 2019) was followed using the Galaxy platform (v23.0) (Afgan et al., 2018). This workflow consisted of firstly a FLASH merger (Magoč & Salzberg, 2011) to combine reads that have a minimum overlap of 50bp, a maximum overlap of 300bp, a maximum mismatch ratio of 0,2, and discarding non-merged reads. Next, the CutAdapt tool (Martin, 2011) was used to trim the primers with the settings requiring both primers to be present, 10 minimum matching bases, an error rate of 0.2, and a minimum read length of 10. Subsequently, PrinSeq (Schmieder & Edwards, 2011) was used to trim and filter the sequences to 310-316 bp in order to remove sequencing artifacts or errors caused by

sequence contamination. In order to generate a table of operational taxonomic units (OTUs), sequences were clustered with UPARSE (Edgar, 2013). UPARSE performs clustering with sequences that have 97% similarity, and the minimum accepted abundance before clustering chosen was 10. Further, OTUs were manually removed that received <0.03% of the reads per sample to remove potential low-level contaminations. OTUs were annotated with taxonomy utilizing the NCBI GenBank data (Benson et al., 2012). Taxonomic identification was enhanced using reference barcode data from the Folmer COI region (Black et al., 1994) of 941 specimens collected, identified, and sequenced during two workshops with taxonomic experts organized by Leiden University in May 2022 and June 2023. I participated in the June 2023 workshop and contributed to the reference barcodes that were generated using the protocol in section 3.3. For taxonomic annotation, the following thresholds were used to assign taxonomic ranks: species - 98%, genus - 95%, family - 90%, order - 85%, and class - 80%. Finally, to retain only OTUs pertaining to the meiofauna community, OTUs assigned to macrofauna and microbial eukaryotes, such as fungi and diatoms, were manually removed.

3.5 Tidal level variability on meiofaunal OTU diversity

Analysis using nonmetric multidimensional scaling (NMDS) revealed two distinct community groupings within a given transect, those from the upper intertidal zone of the beach and those from the lower intertidal zone **(Figure 2)**. The samples from the upper intertidal zone in the NMDS clustered into two larger circles while the lower intertidal zone samples clustered into more densely-packed overlapping ovals. To eliminate the intertidal variance between each transect and to elucidate the effect of environmental variables on the meiofaunal community, these two groupings were then later each analyzed

independently. Explicitly, the lower upper tidal and upper tidal samples were pooled and combined to ‘Upper Intertidal’, and the lower tidal, upper lower tidal, and lower intertidal were pooled and combined to ‘Lower Intertidal’. Samples from the ‘Intertidal’ area were excluded for these analyses.

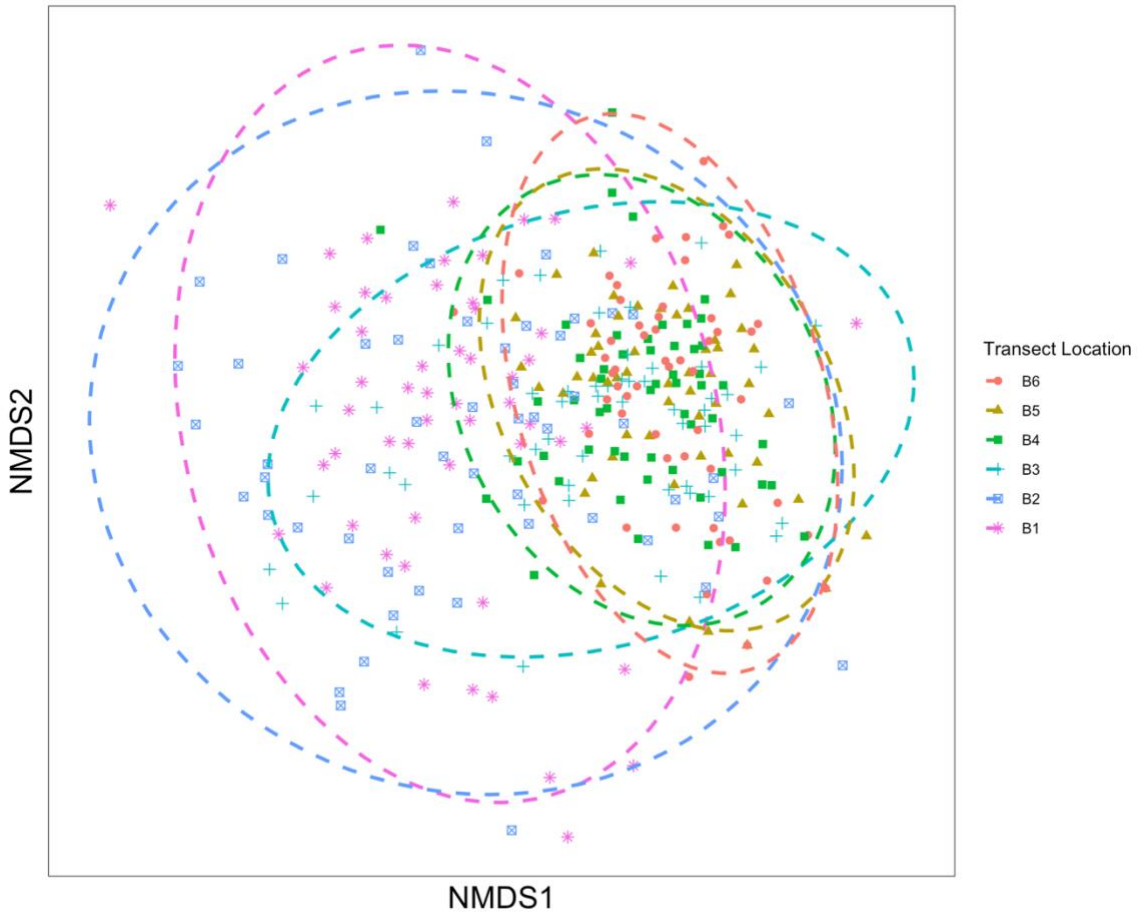

Figure 2. NMDS plot showing variability within transect. Hashed lines represent 95% confidence interval. Transect B1 = lowest intertidal point. Transect B6 = highest intertidal point.

3.6 Urbanization influence on meiofauna OTU richness

The effect of environmental urbanization variables was examined using generalized linear mixed model (GLMM) approach. The ‘glmmTMB’ function within the ‘glmmTMB’ package (Brooks et al., 2017) in R were utilized to develop the models for three datasets: all meiofauna, upper-tidal meiofauna, and lower-tidal meiofauna. A collinearity check found the urbanization index (UI) to be collinear with several variables (Variance Inflation Factor (VIF) >30) and so was excluded. Before assessing the models, all fixed effect variables were standardised to have a mean of zero and standard deviation of one. The strongest model fits based on Akaike's Information Criterion (AIC) scores were those that included Beach Cleaning, Buildings on Sand, Beach Slope, Vehicle Traffic, SQM, and Dune Condition as fixed effect variables, and no random effects (**supplementary materials, Table S6)**. A Poisson distribution was found to be the best fit for the ‘all meiofauna’ and ‘upper-tidal meiofauna’ groups, while a negative binomial distribution fit the lower-tidal meiofauna group the best. Finally, significant effects were assessed by p-values, where <0.001 indicates highly significant, <0.01 significant, and <0.5 marginally significant.

3.7 Urbanization influence on community similarity among beaches

Beta diversity analyses were performed on three groups: all meiofauna, upper intertidal meiofauna, and lower intertidal meiofauna using Bray-Curtis dissimilarity calculated with the ‘vegan’ R package. The function ‘envfit’ was used to fit the environmental vectors onto the NMDS ordination.

3.8 Qualitative indicators, urbanization index (UI) and SQM

González et al., 2014 developed an index based on qualitative indicators of human intervention in order estimate the level of urbanization on a given beach. Each parameter was given a score between 0-5 based on qualitative characteristics of the beach. For example, the indicator 'Buildings on Sand' is given a score of 0-1 if there are no nearby buildings appreciable, a score of 2-3 if there are buildings close on the beach but not on the sand or the dunes, and a score of 4-5 if there are buildings that occupy the space at the beach or in the dunes. The other indicators that make up the UI are proximity to urban centers (1), cleaning of the beach (2), solid waste in the sand (3), vehicle traffic on the sand (4), quality of the night sky (5), frequency of visitors (6), and buildings on the sand (7). Additionally, MacLachlan et al (2013), developed an index that highlights the importance of dune condition on beach biodiversity. Here, we use the scoring for each qualitative indicator as specified in the aforementioned papers. The scores from the indicators in González et al., 2014 are added together and divided by 35 (# of indicators * max score (5)) to achieve the urbanization index (UI). A higher UI, thus, represents a higher level of beach urbanization. A Sky Quality Meter (SQM) value was obtained for each transect by entering the GPS coordinates into the mapping application at www.lightpollutionmap.info (v2.8.24) (Falchi et al., 2016).

3.9 Generalized dissimilarity modelling (GDM) on community similarity

A generalized dissimilarity model (GDM) was used to test the account for spatial effects and non-linear relationships in beta diversity (Ferrier et al., 2007). The GDM was created

using the package ‘gdm’ in R. All models were calculated using Bray-Curtis dissimilarity after performing a square root transformation on the read data. Jaccard dissimilarity, based on presence-absence rather than abundance, was also tested but discarded as the differences between Jaccard and Bray-Curtis were negligible. After checking for collinearity, the factors that were used in the model were beach slope, beach cleaning, transect length, dune condition, vehicle traffic, longitude, latitude, and SQM.

4 Results

4.1 Beach urbanization

The most urbanized beach was Scheveningen, with an urbanization index of 0.8857 for all four transects (**Table 2**). Every urbanization indicator had the highest score of 5, except for solid waste (3), vehicle traffic (4) and quality of night sky (4). The dunes in Scheveningen are also severely degraded or non-existent. The lowest urbanization index was in Noordwijkerhout, where the transects ranged from 0.3429 near the beach access point to 0.1714 in the protected area which is still open to the public. City beaches had the highest values (Katwijk – 0.7714, Noordwijk City – 0.8, Zandvoort City -0.7429 – 0.7714) and other beaches had urbanization indexes that decreased with distance from the access points.

Table 2. Highest and lowest urbanization indexes among the transects for each sampled beach.

Beach	Highest UI	Lowest UI
Katwijk	0.7714	0.7714
Katwijk South	0.7714	0.4
Wassenaar	0.6571	0.3714
Noordwijkerhout	0.3429	0.1714
Katwijk North	0.8	0.4857
Scheveningen North	0.6857	0.3429
Noordwijk North	0.8	0.514
Zandvoort South	0.7714	0.5429
Zandvoort City	0.7714	0.7429
Noordwijk City	0.8	0.8
Scheveningen City	0.8857	0.8857

4.2 Bioinformatics and taxonomy

The sequence data was processed using Galaxy (v23.0). Following this process, 7,327,586 reads were retained among 4,894 OTUs for the 334 samples. The four negative controls combined had a total of 930 reads. After removing non-meiofaunal OTUs and OTUs with less than 0.03% of the total reads per sample, 2,502,735 reads among 232 OTUs remained. The average number of reads from the upper intertidal area was relatively low (8,350) compared to the lower intertidal area (28382).

For all meiofauna, Polychaeta (phylum Annelida) was the most dominant taxa which represented 50.8% of the sample reads on average, followed by Chromadorea (6.8%, phylum Nematoda) and Copepoda (6.0%, of the Phylum Arthropoda) **(Figure 3)**. In the upper intertidal area, Clitellata (22.6%, phylum Annelida), Chromadorea (13.3%) and Polychaeta (10.4%) were the most dominant taxa. In the lower intertidal area, Polychaeta (53.7%), Chromadorea (7.6%) and Hydrazoa (5.8%, phylum Cnidaria) were the three most abundant taxa. The upper intertidal group was dominated by Clitellata in Scheveningen North, Katwijk, and Katwijk North, but overall the taxa were relatively evenly distributed compared to the lower intertidal zone. In the lower intertidal zone, which accounted for the majority of the reads, Polychaeta was >50% of the reads in 38/55 samples, even reaching >75% abundance in 18 samples.

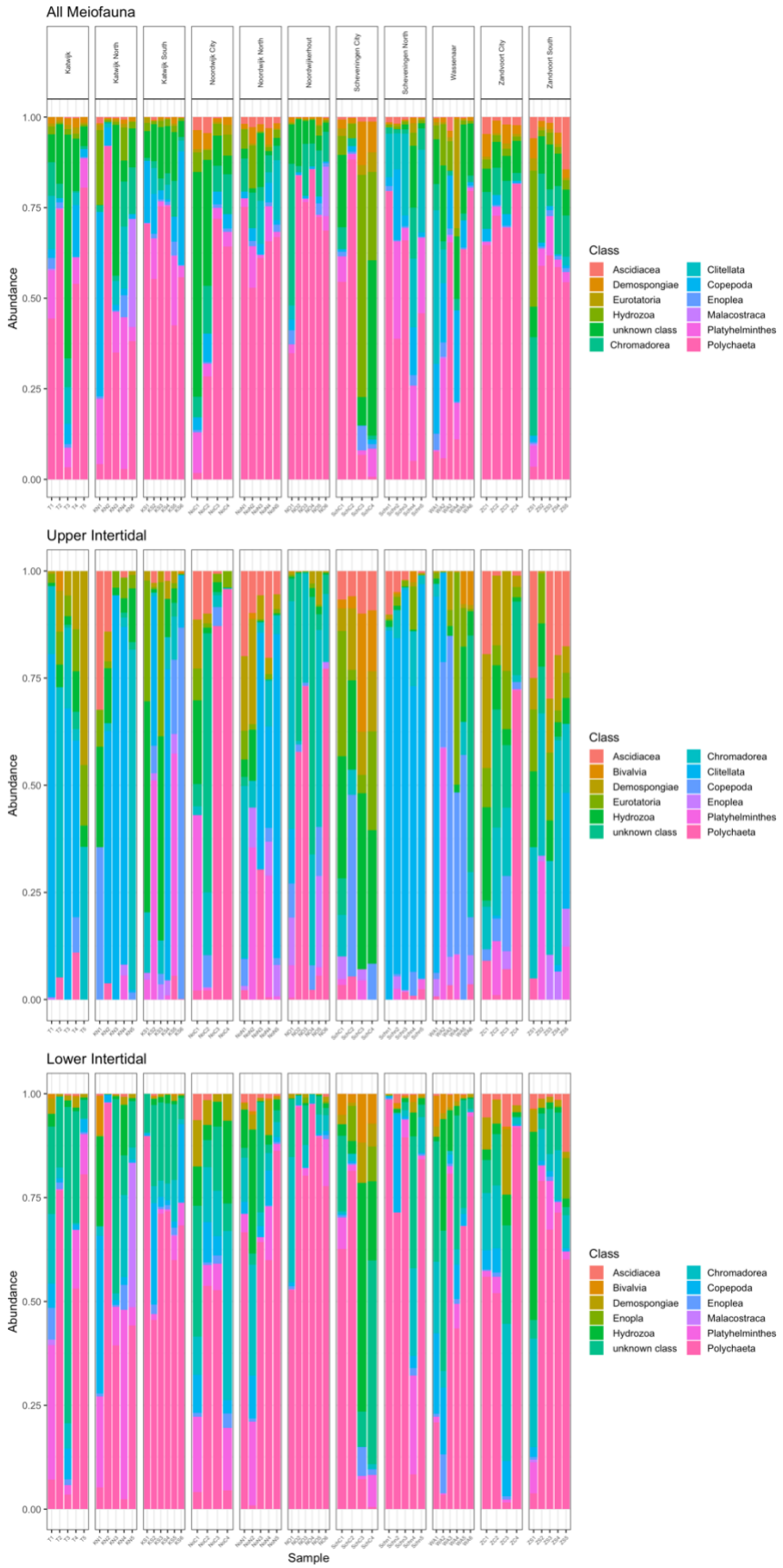
All Meiofauna
Upper Intertidal
Lower Intertidal
Katwijk
Katwijk North
Katwijk South
Noordwijk City
Noordwijk North
Noordwijkerhout
Scheveningen City
Scheveningen North
Wassenaar
Zandvoort City
Zandvoort South
Abundance
1.00
0.75
0.50
0.25
0.00
Sample
Class
Ascidiacea
Bivalvia
Demospongiae
Enopla
Eurotatoria
Hydrozoa
unknown class
Chromadorea
Clitellata
Copepoda
Enoplea
Malacostraca
Platyhelminthes
Polychaeta

Figure 3. Community composition and relative abundance of meiofauna at different tidal levels and grouped by sampling location. Note, that due to the relatively lower abundance of reads in the upper intertidal area, the lower intertidal community composition is disproportionately reflected in the overall meiofauna composition.

4.3 The impact of urbanization factors on OTU richness

From the GLMM analysis, a number of urbanization factors were found to have a significant influence on OTU richness for all meiofauna and in the upper intertidal zone **(Figure 4)**. Considering all meiofauna, the highest significance ($p < 0.01$ = **) was attributed to buildings on the sand (est = -0.05), which had a significant negative effect on OTU richness. A smaller but still significant positive effect ($p < 0.05$ = *) was attributed to beach cleaning (est = 0.09) and beach slope (est = 0.07). In the upper intertidal zone, a significant negative effect was found with vehicle traffic (est = -0.24, $p < 0.001$ = ***) and dune condition (est = -0.21, $p < 0.001$ = ***). Buildings on sand was also found to have a significant negative effect (est = -0.08, $p < 0.01$ = **), though less than vehicle traffic and dune condition. In the lower intertidal meiofauna community none of the urbanization factors were found to have any positive or negative significant effect on OTU richness. Overall, SQM did not show any effect in any of the groups, while beach cleaning and beach slope also had relatively low contributions to OTU richness.

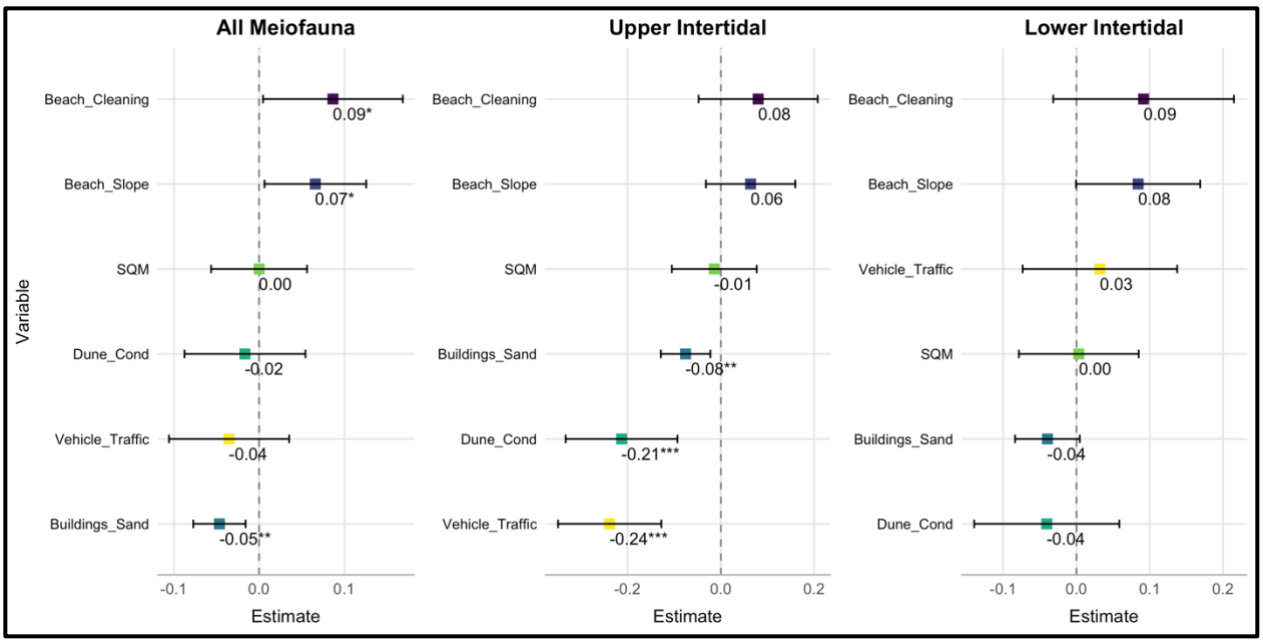

Figure 4. Effect of urbanization factors on OTU richness using GLMMs. The error bars around the square are the 95% confidence interval. p < 0.001 = ***: p < 0.01 = **: p < 0.05 = *. The estimate is the estimated change in the response variable for changes in the predictor variable.

4.4 The impact of urbanization factors on community similarity

An NMDS analysis was employed to investigate the relationship between urbanization factors and the community composition of the studied meiofauna. The envfit analysis showed a different result for each intertidal group. For the whole meiofauna community, the urbanization factor that showed the highest effect was dune condition (r^2 = 0.3896, p < 0.001 = ***). SQM, Buildings on Sand and UI were also significant but to a lesser degree. (r^2 = 0.1879 and p = 0.006, r^2 = 0.141and p = 0.014, and r^2 = 0.1138 and p = 0.038, respectively). For the upper intertidal meiofauna, the most significant urbanization factors were SQM (r^2 = 0.2844, p < 0.001 = ***) and Vehicle Traffic (r^2 = 0.2179, p < 0.001 = ***), followed by Beach Slope (r^2 = 0.2322, p = 0.003) and Beach Cleaning (r^2 = 0.1552, p = 0.016). Lastly, the most significant urbanization effects for the lower intertidal

meiofauna were seen with SQM (r^2 = 0.4383, p < 0.001 = ***) and Beach Slope (r^2 = 0.2429, p < 0.001 = ***), while Dune Condition (r^2 = 0.228, p = 0.003) and UI (r^2 = 0.1464, p = 0.02) also had significant effects. The direction of the envfit vector for dune condition often was opposed to the other urbanization factors, while vectors for UI, beach cleaning, and vehicle traffic tended to group in the same general direction. The vectors for beach slope and SQM were closely aligned for lower intertidal meiofauna and relatively close for all meiofauna and upper intertidal meiofauna.

Table 3:: **Envfit Analysis Results showing the influence of environmental factors on the community similarity of meiofauna, upper intertidal meiofauna, and lower intertidal meiofauna.** NMDS1 and NMDS2 are the vector coordinates of the environmental factor.

Tidal Range	Enviornmental Factor	NMDS1	NMDS2	r^2	p-value
All Meiofauna					
	Beach Slope	-0.20321	-0.97913	0.0879	0.09
	Beach Cleaning	-0.6033	0.79751	0.0745	0.141
	UI	**-0.78526**	**0.61917**	**0.1183**	**0.038**
	SQM	**-0.74209**	**-0.6703**	**0.1879**	**0.006**
	Dune Condition	**0.84458**	**-0.53544**	**0.3896**	**0.001**
	Vehicle Traffic	-0.71118	0.70301	0.0014	0.967
	Buildings on Sand	**-0.99052**	**0.13734**	**0.1417**	**0.014**
Upper Intertidal					
	Beach Slope	**-0.68459**	**-0.72893**	**0.2322**	**0.003**
	Beach Cleaning	**0.3591**	**-0.9333**	**0.1552**	**0.016**
	UI	0.16926	-0.98557	0.0756	0.133
	SQM	**-0.9622**	**-0.27235**	**0.2844**	**0.001**
	Dune Condition	0.99184	0.12746	0.0617	0.197
	Vehicle Traffic	**0.54492**	**-0.83849**	**0.2179**	**0.001**
	Buildings on Sand	-0.71485	-0.69928	0.0302	0.428
Lower Intertidal					
	Beach Slope	**0.64181**	**0.76686**	**0.2429**	**0.001**
	Beach Cleaning	-0.98554	0.16945	0.1046	0.071
	UI	**-0.98931**	**0.14579**	**0.1464**	**0.02**
	SQM	**0.67957**	**0.73361**	**0.4383**	**0.001**
	Dune Condition	**0.67007**	**-0.7423**	**0.228**	**0.003**
	Vehicle Traffic	-0.9872	-0.15951	0.0593	0.233
	Buildings on Sand	-0.97194	0.23522	0.088	0.086

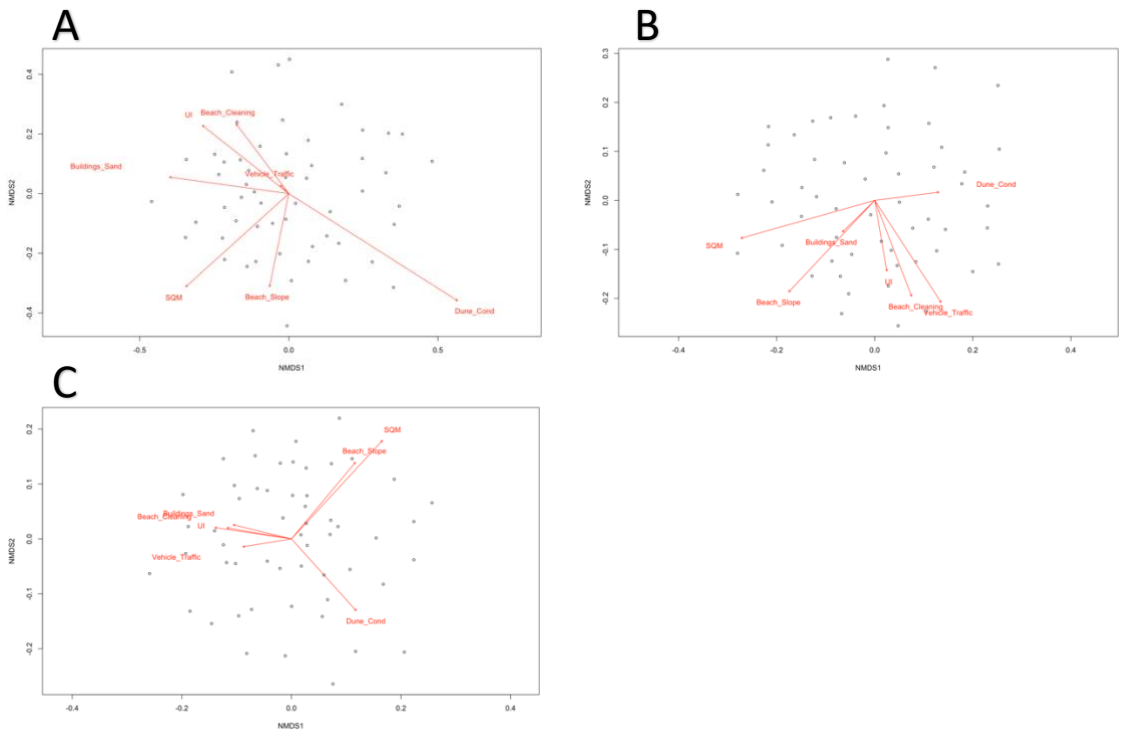

Figure 5. Non-metric multidimensional scaling (NMDS) plots showing community composition similarities and vectors indicating the influence of urbanization factors. A) Entire meiofauna community; B) Upper intertidal zone; C) Lower intertidal zone. The length and direction of the envfit vectors correlate with the strength of the influence and gradient of the urbanization factors, respectively.

4.5 Generalized dissimilarity model

The results of the generalized dissimilarity models revealed the strongest factors were geographic distance and beach slope **(Figures 6-8)**. Geographic distance was the most influential factor for all meiofauna and lower intertidal meiofauna followed by beach slope, while beach slope was the most influential for upper intertidal meiofauna. The I-splines of each factor generally followed similar patterns for each meiofauna group though the max height and shape tended to vary. Geographic distance differed linearly with geographic distance for both all meiofauna and lower intertidal meiofauna, but there was a smaller

community dissimilarity for upper intertidal meiofauna for geographic distance, which quickly plateaued.

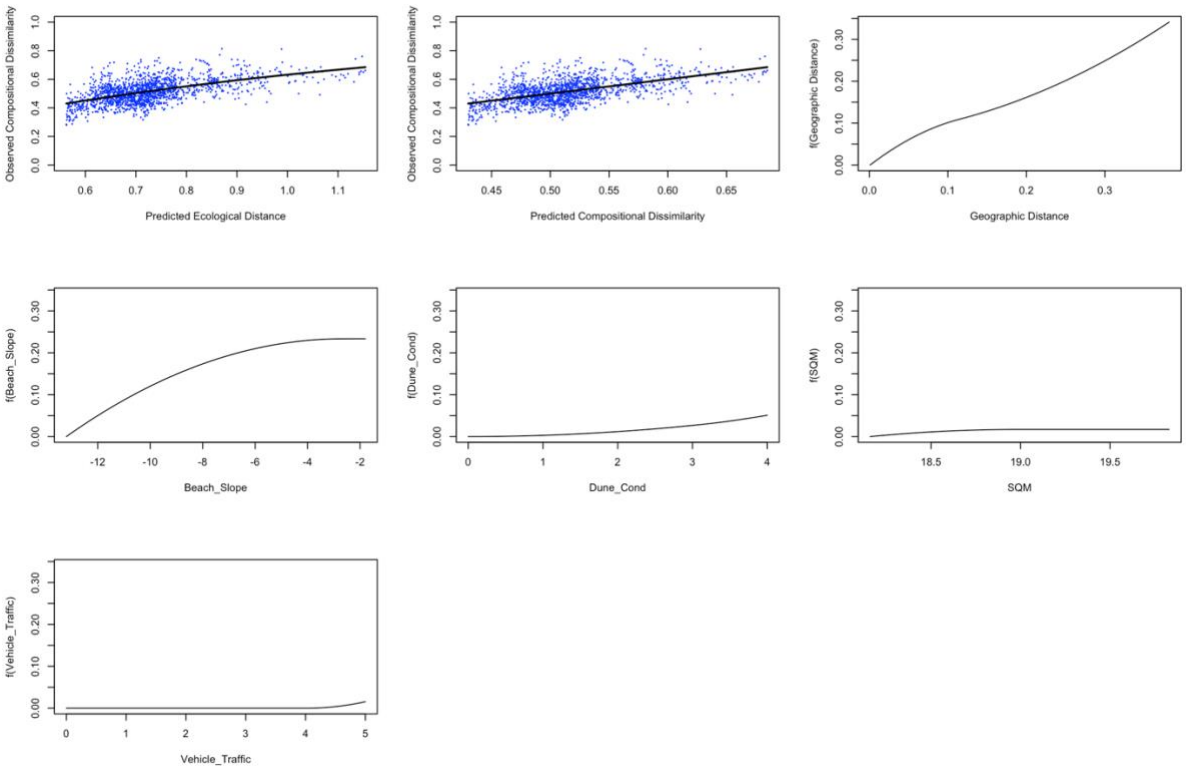

Figure 6. GDM-fitted I-splines for each urbanization factor and geographic distance for all meiofauna. The height of each curve represents the dissimilarity between sites associated with the variable and the steepness of the curves represents a higher rate of dissimilarity along the variable gradient.

Beach slope for all meiofauna and lower intertidal meiofauna showed rapid changes from -12 to -6, which then began to plateau, while the upper intertidal meiofauna showed higher compositional turnover at beach slopes towards -2. The maximum height of the I-splines for dune condition, SQM, vehicle traffic, and beach cleaning were generally much lower than for geographic distance and beach slope and gentler slopes. Dune condition had the greatest impact on dissimilarity for upper intertidal meiofauna, but only in the

highest section of the gradient, while the effect was more gradual for all meiofauna and lower intertidal meiofauna.

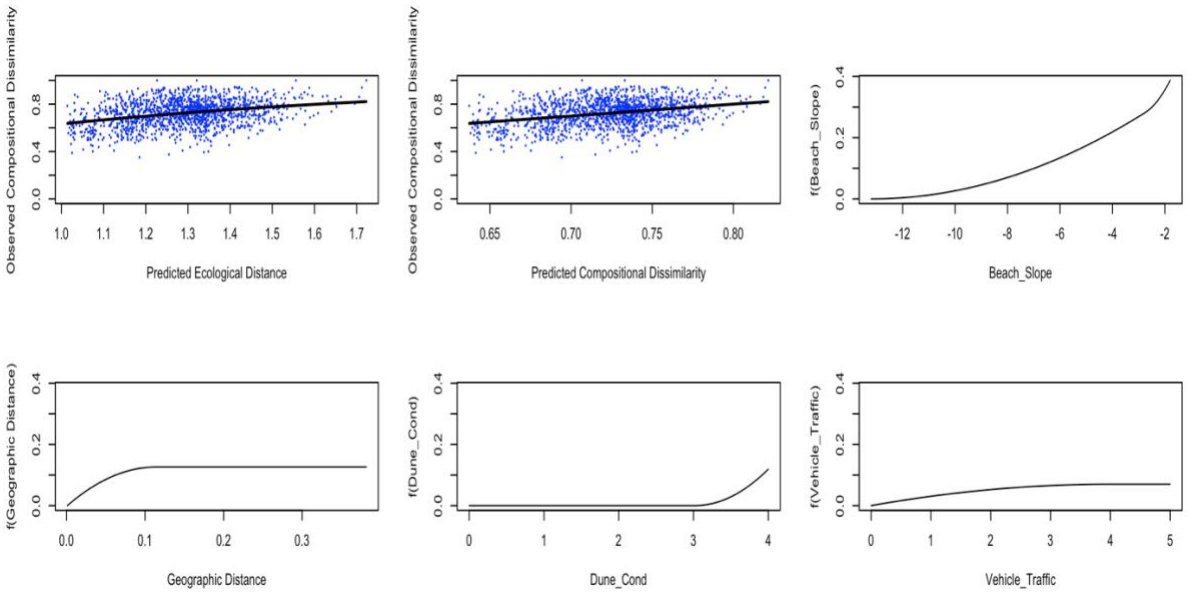

Figure 7. GDM-fitted I-splines for each urbanization factor and geographic distance for upper meiofauna. The height of each curve represents the dissimilarity between sites associated with the variable and the steepness of the curves represents a higher rate of dissimilarity along the variable gradient.

At the low end of the gradient, SQM showed a gentle increase which then plateaud for all meiofauna and lower intertidal meiofauna, while upper intertidal meiofauna showed no difference in dissimilarity. Beach cleaning was the most significant factor after geographic distance and beach slope for lower intertidal meiofauna, which showed a gradual increase all along the gradient. The GDM models showed no impact for all meiofauna and upper intertidal meiofauna with beach cleaning. Lastly, vehicle traffic had the highest impact on dissimilarity for upper intertidal meiofauna, with the max height of the I-spline almost as high as geographic distance and dune condition, with a gradual

increase along the gradient. All meiofauna and lower intertidal meiofauna saw a very slight response with vehicle traffic at the high end of the gradient.

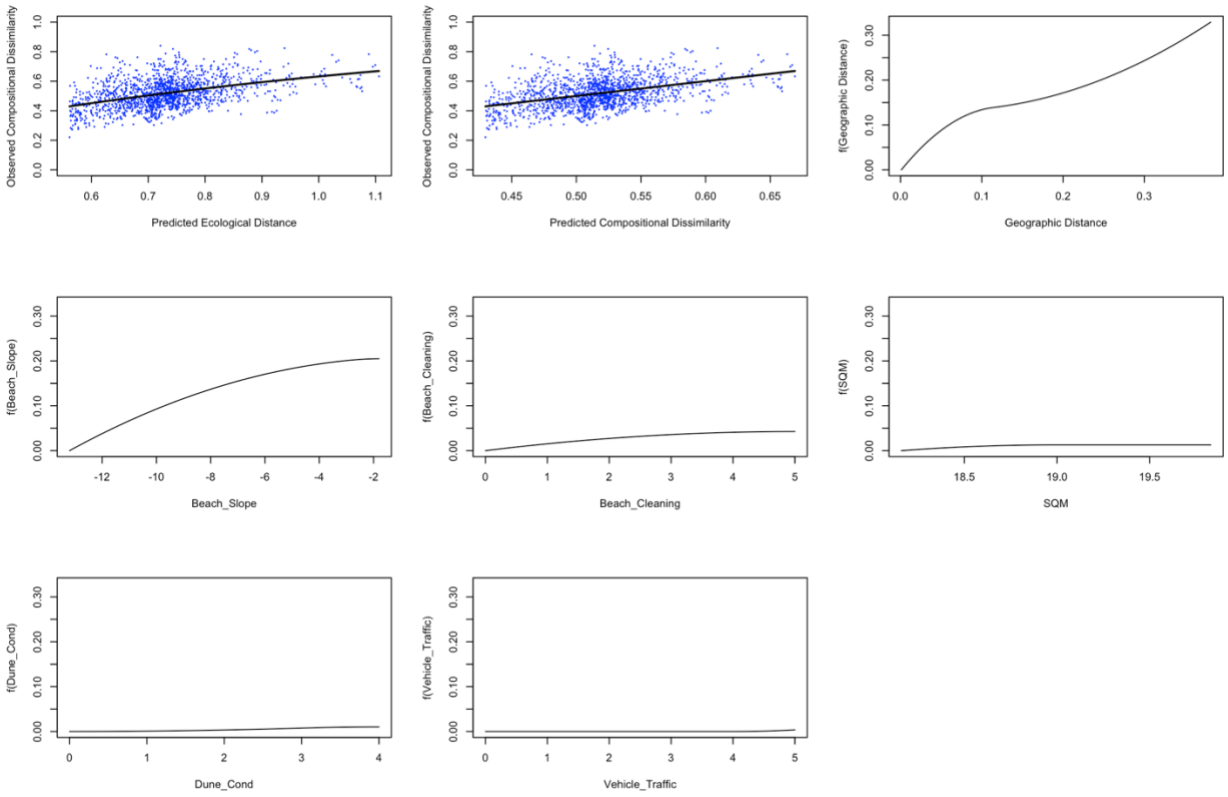

Figure 8. GDM-fitted I-splines for each urbanization factor and geographic distance for all lower meiofauna. The height of each curve represents the dissimilarity between sites associated with the variable and the steepness of the curves represents a higher rate of dissimilarity along the variable gradient.

5 Discussion

The importance of meiofauna to coastal ecosystems should not be understated and as such understanding the drivers that influence the communities and species there is necessary. Research investigating the effect of environmental factors have revealed that grain size and salinity have an effect on the composition of these communities, but that pollutants and trace metals can also play a role, opening the door for meiofauna assemblages to act as bioindicators (Schratzberger et al., 2000). Because of their high response time to environmental changes, meiofauna offer the opportunity to identify contaminant input and could act as a monitoring tool. Various studies have explored the effect of urbanization on macrofauna, but few studies have reported on the relationship between urbanization and meiofauna and those that exist are limited in scope. A better understanding of this relationship will help implement policy decisions to protect meiofauna, beach ecosystems, and in their practical development as bioindicators. The results have highlighted several urbanization factors that necessitate further discernment.

5.1 Community composition

The sequencing results obtained from this study showed a community dominated largely by the annelids, polychaeta and clitellata. Polychaeta was especially dominant in the lower intertidal area, while clitellata was the highest represented taxa in the upper intertidal area. This is at contrast with other studies that have found nematodes and copepods to be the most abundant groups through metabarcoding (Fais et al., 2020; Wang et al., 2023). This disparity likely is due to the extraction method used. In this study

we chose to extract DNA directly from a relatively large sediment sample (typically 13-17g). However, the choice of extraction method has been shown to play a large role in the inferred community composition as those samples obtained from first isolating the meiofauna through either centrifugation and flotation with Ludox or a $MgCl_2$ decantation led to a higher number of meiofauna taxa (Castro et al., 2021). The higher abundance of annelids likely reflects the relatively higher biomass of these individuals and shedding of cells as they crawl through the sediment. Further, we chose to use a low-cost, high throughput DNA extraction method known as the **s**oil **D**NA **e**xtraction workflow (SDE) (Bollmann-Giolai et al., 2020), as opposed to commercial soil DNA extraction kits like PowerSoil. The benefit of our adapted SDE protocol enabled larger samples sizes (~15g) to obtain a more representative community while limiting the price tag per extraction. The cost per PowerSoil Max extraction (max. 10g per sample) is approximately $25 USD, which would be prohibitive to extract DNA from 330 samples for a student project and even many professional researchers. While our method proved effective to obtain extracted DNA, it is possible that in optimizing our SDE protocol to larger volumes could have introduced a bias. Indeed, even after complete DNA extraction often samples remained a dark or light brown colour, potentially indicating the retention of humic substances which can inhibit PCR amplification (Matheson et al., 2010). While this colour was observed predominantly in dune samples and beach sand samples were typically clear, diluting samples was often necessary to overcome this inhibitive effect and to visualize DNA on an agarose gel. Diluting, however, reduces the efficiency and consistency of PCR amplification by lowering the DNA concentration. The choice of polymerase also can play a role, and humic acids have been shown to inhibit Phire HS

(the polymerase used in this study) even at relatively low concentrations (Matheson et al., 2010). In comparing the diluted vs non-diluted samples using NMDS, there seemed to be no clear difference between the communities.

Another important consideration is the choice of the targeted marker region. A review paper examining metabarcoding techniques for meiofauna found that when only one target marker was used, 44/45 studies used the nuclear 18S rRNA marker region (Gielings et al., 2021), with some studies citing sequencing length limitations of the Illumina platform (Brannock & Halanych, 2015). The advantage of the COI Folmer primers is the more variable region that allows it to discriminate better between species, however the lack of reference database sequences can lead to high levels of unidentified OTUs (Atienza et al., 2020). Another consideration is primer bias. One study from Svalbard of deep-sea sediment samples reported 33 different nematode species using 18S metabarcoding while only 1 nematode was found during COI metabarcoding (Van Den Heuvel-Greve et al., 2021). While the Folmer region of the COI gene is chosen for its broad and effective capability to identify metazoans (Hebert et al., 2003), there is evidence purporting challenges in amplifying marine nematodes (Derycke et al., 2010). In addition, the choice of database to annotate sequences can lead to a bias and some sequences may not be represented in certain databases. In this study, we were able to use a reference database of 941 meiofauna that were identified and sequenced for the Folmer COI region by taxonomists during two workshops. This helped to alleviate some of the challenges with database selection and species misidentification, however since many taxa could not be identified to the species level, the analysis must be done on an OTU-level.

In summary, several technical and experimental challenges and choices will lead to biases and could ultimately affect the interpretation of the results. Ideally, future studies should strive to eliminate these biases by, when possible, using a number of techniques. This includes but is not limited to using different extraction methods (sediment vs isolated meiofauna), target marker regions (18S vs COI), and choice of database (BOLD vs Genbank). These choices, along with the environmental factors all contribute to the community, and make reproducibility among studies difficult.

5.2 Urbanization factors

The consequence of population migration towards the coast has resulted in coastal modifications that threatens sandy beach ecosystems. Some of the direct threats involve beach cleaning, trampling, light and sound pollution, dune destruction, nourishment, and pollution (Defeo et al., 2009).

Many of the urbanisation factors examined in this study were naturally found to be correlated, and so were excluded from analysis. These factors include proximity to urban centre, solid waste, and frequency of visitors. The following section will discuss each qualitative indicator individually.

Beach Cleaning was found to have a statistically small positive effect when considering all meiofauna in OTU richness, and there was a statistical significance for community similarity in the upper intertidal zone. It is not immediately clear why beach cleaning could lead to higher OTU richness. The removal of wrack and other macroscopic items from the upper sediment layer sifts, replaces, and compresses sand and removes organic material, leading to deleterious effects on beach macrofauna (Dugan et al., 2003).

To date, only one study has looked at the effect of beach cleaning on meiofauna. Ghesekiere et al. (2006) found that while strandline meiofauna were immediately impacted in abundance and assemblage structure, rapid recolonization of meiofauna via passive vertical migration occurred within 24 hours of a cleaning event. It The results here indicate that long-term beach cleaning may affect these recolonization rates in the upper intertidal area that has led to slight community differences according to the non-metric multidimensional scaling analysis. OTU richness may be increased by complex food web interactions, where a loss of one or more species may positively influence the propagation of other species. Another possibility would consider the composition of this dataset. Ghesekiere's 2006 paper focused on meio-nematofaunal community structure, yet our dataset is highly influenced by the dominance of annelids. It is therefore plausible that beach cleaning affects these groups of meiofauna in contrasting ways, with nematodes being more susceptible and annelids benefitting from the sediment mixing.

Beach Slope also showed a small positive effect for OTU richness for all meiofauna, and a significant effect for both upper and lower intertidal zones in community differences. Global patterns of meiofauna demonstrate a loss of OTU richness with steeper beach slopes (Defeo & McLachlan, 2013). This is in contrast with the results here, but could be due to the dominance of annelids in this dataset. Because annelids are already involved with burrowing and sediment mixing (Van Regteren et al., 2017), they are likely more resistant to dynamic sediment conditions which could also account for the community differences in the upper and lower intertidal zones.

Buildings on Sand was found to have a negative significant effect on OTU richness in the upper intertidal zone and for all meiofauna, and accounted for community

differences for all meiofauna. Buildings on Sand likely has no direct impact, given that the transects never approached the buildings, but is rather a proxy for other urbanization effects such as frequency of visitors, beach cleaning, and vehicle traffic. Buildings will also affect aeolian patterns and affect the deposition of sand and morphodynamics of dunes (Poppema et al., 2022). The transformation of the beach environment by structures did show an impact, but it is overshadowed by other urbanization factors and likely to indicate other effects such as trampling or pollution.

Dune Condition was the factor most significant for all meiofauna in community difference and was negatively statistically significant for OTU richness in the upper intertidal zone. Gheskiere et al. (2005) described that in pristine beaches with unimpacted dunes, total organic matter (%TOM) increased from the upper intertidal area towards the dunes, but in beaches that have been modified and intensely used, there was a decrease of interstitial %TOM. Thus, the lower OTU richness in the upper intertidal area could be related to lower %TOM in areas with degraded dunes. Further, degraded dunes will be commonly in high tourist areas with a higher degree of trampling, and interruption of sub-terranean freshwater connections that may also affect the communities.

The sky quality meter, or **SQM**, did not show any statistical significance on OTU richness, but was statistically significant in community similarity for all meiofauna, upper intertidal meiofauna, and especially lower intertidal meiofauna ($r^2 = 0.4383$). If SQM affects the turnover and/or replacement of a species, it could affect beta diversity while not necessarily having any impact on OTU richness. In this case, one species in the community that cannot withstand high light pollution may be replaced by one that is more tolerant. Beach fauna such as amphipods, isopods, and insects, rely on diurnal cycles

and signals to return to burrows (Fallaci et al., 2002). The impact of light pollution on meiofauna has not been studied, but these results suggest an interaction between light pollution and the meiofauna communities. Although meiofauna are interstitial, they do exhibit diurnal vertical migration patterns (Dye, 1978). As the Netherlands has the highest light pollution in mainland Europe (Widmer et al., 2022), all SQM measurements were relatively low (indicating higher light pollution), ranging 18.16 to 19.83. This would imply that meiofauna communities are highly susceptible to even small changes in light pollution, or that higher light pollution is correlated with other environmental factors that affect meiofauna populations. Given that light is known to influence many physiological processes in a range of animals (Hussein et al., 2021) and the statistically significant result here, SQM might be a useful indicator to account for community differences in meiofauna or as a proxy for some underlying environmental effect associated with higher light pollution.

The concept of an urbanization index **(UI)** from Gonzalez et al. (2014) while effective in their own study, was not fruitful here. While Gonzalez et al. found a high correlation of night sky quality to urbanization index value ($r = -0.84$), the correlation between our sampled beaches was low ($r^2 = 0.1622$) (**Supplementary figure S9**). This discrepancy could represent a challenge with utilizing the index on smaller scales (transects) as opposed to an entire beach. Given that the Netherlands has the highest population density in mainland Europe and the degree to which its beaches have been modified, it is difficult to find a beach that has not been urbanized to some degree. Nonetheless, a small but significant effect of UI was found for all meiofauna and for lower intertidal meiofauna in regards to beta diversity.

Lastly, **Vehicle Traffic** played a significant role in OTU richness and community difference for upper intertidal meiofauna. It would seem logical that vehicle traffic impacts meiofauna in a similar way to beach cleaning, in that it "…physically disturbs the sediment, its micro-topography and its inhabitants, therefore creating a uniform habitat with a short durational stability" (Gheskiere et al., 2005). Given that the upper intertidal area is less dynamic, inhabitants of this zone are probably more susceptible to disturbances which cause "reduction in soil macro porosity, air/water permeability, changes in sediment topography and perturbs the sand almost continuously" (Gheskiere et al., 2005).

Overall, upper intertidal meiofauna seemed to be more affected by urbanization factors than lower intertidal meiofauna. The NMDS analysis showed the urbanization factors that contributed the most to community differences in the upper intertidal area were press disturbances (beach cleaning, vehicle traffic), SQM, and beach slope. Lower intertidal meiofauna exhibited higher resilience as none of the investigated urbanization factors affected OTU richness, however SQM, dune condition, and beach slope all had significant effects on beta diversity. Considering the entire meiofauna community, dune condition was played the largest role in community differences. The dune and beach environments are not isolated from one another, and it is plausible that the degradation or lack of dunes will affect the composition of beach meiofauna. Insects can be blown towards the beach through aeolian transport (Quilter, 1987), dune birds may feed on beach fauna (Anton McLachlan, 1988), and groundwater flow through aquifers may be transport nitrogen and nutrients towards the surfzone (Johannes, 1980). The interaction or lack thereof between these elements could be responsible the shift in beta diversity.

5.3 Generalized dissimilarity model

GDMs are a powerful tool to help analyze spatial patterns in beta diversity. Geographic distance and beach slope had the most prominent influence on beta diversity based on the GDM. As these factors largely shape the geomorphology of the beach and the environment in which the meiofauna thrive, it is natural these would be responsible for the greatest dissimilarity between sites. In the models, some of the factors affected the community only at high levels (i.e. dune condition on upper intertidal meiofauna, or vehicle traffic on lower intertidal meiofauna) while other effects were more gradual (beach cleaning on lower intertidal meiofauna or vehicle traffic on upper intertidal meiofauna). These models show that there is an intricate relationship with each of the variables on each group of meiofauna. While one group of meiofauna may be affected even at lower levels of disturbances from urbanization, another may be only affected at higher levels, or not at all. As a result, certain meiofauna of the intertidal zone will be more resilient to different urbanization effects, which will also be dependent on which types of meiofauna dominate that region. Some of the results of the GDM are in contrast with those from the GLMM. For example, SQM appeared to play a large role in compositional turnover in the GLMM but were largely insignificant in the GDM. This could suggest the effect of SQM was spatial and not based on the effects of light pollution, since SQM generally increases with distance from urban centres. GDM is said to be more flexible as it can capture complex relationships by using a non-linear modelling approach, incorporates spatial patterns, and allows for interactive effects between variables (Mokany et al., 2022). Since the models are implemented with different assumptions (non-linear vs linear relationships between response variable and predictors, spatial factor), it is unwise to directly compare

the results. However, the models can be a valuable complement to each other and provide more information than one alone. The GDM results imply that there is indeed a spatial component to the data, and that dune condition, vehicle traffic, and beach cleaning are acting as stressors to meiofauna communities.

5.4 Challenges

Our method of DNA extraction was a novel approach that aimed to limit costs while at the same time increase the sample volume. A common method of DNA extraction Is to work with 0.25g samples that are compatible with a QIAGEN PowerSoil kit (Gielings et al., 2021). However, it is known that beach meiofauna tend to be heterogenous in space (Lee et al., 1977). Thus, we aimed to use larger samples in order to provide a more representative beach community. We managed to obtain on average ~43 unique OTUs for each transect, while another study commonly found upwards of 100 for similar locations along the North Sea (unpublished data). This fact, and the overabundance of annelids may indicate the DNA extraction was not entirely effective. Sequencing depth could also have contributed to this discrepancy. Many non-meiofaunal taxa reads were found, which compete with the meiofauna DNA during PCR and sequencing, leading to lower meiofauna reads. As mentioned previously, the choice of target marker region can also lead to bias and in ideal situations both 18S and COI should be used.

Another difficulty that may have affected the interpretation of the results was our location selection. Because there are hardly any natural environments left in the Netherlands, the sampling areas were skewed to oversampling highly urbanized beaches, which may have increased the difficulty in finding statistically significant results.

While it may be possible to find a location further away, we would then be introducing more variables that could cause community differences and strived to sample beaches along the same coastline that have similar characteristics.

In summary, the variance in OTU richness and community variability between transects was partly explained by urbanization factors. More and more studies are helping to unravel the complexities of meiofauna community structure by interpreting environmental data and patterns. These results show that there is yet more to the story. The numerous changes that urbanization inflicts to the coast indubitably affect this vital ecosystem and meiofauna acting as bioindicators have the potential to help us perform impact assessments and protect beaches from further degradation.

6 Conclusion

With the advent of urbanization and climate change, coastal ecosystems certainly are facing challenges. This study will add to the litany of evidence showing that the methods used in analyzing meiofauna communities with metabarcoding should be seriously considered. When possible, several approaches are recommended, such as using both 18S and COI as target gene markers and comparing different sequence databases. In this study there were several significant effects found associated with urbanization factors on meiofauna communities. To understand the effect that vehicle traffic has on upper intertidal meiofauna is straightforward, as the trampling force may crush the meiofauna, compact the sand, and ultimately disturb their interstitial environment. Other indicators are less straightforward, such as SQM that may simply be a proxy for more urbanization

and the consequences that come with it (pollution, trampling, etc.), or could light pollution have a physiological effect on meiofauna? To date, beach morpho-dynamics, salinity, grain size, and %TOM have all been studied to explain differences in meiofauna biodiversity. Here, I suggest that some urbanization factors may also play a role and should be further studied to understand their part in meiofauna community structure. This dataset was dominated by annelids, and it would be worth investigating similar factors with datasets that express the typical meiofauna community with higher abundances of arthropods and nematodes. Further, the GDM allowed a more nuanced view on the analysis of urbanization factors and was able to incorporate spatial data to help untangle the effects of urbanization.

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8 Supplementary Material

Table S4. Raw output from generalized linear mixed models for all meiofauna, upper intertidal meiofauna, and lower intertidal meiofauna, respectively.

	Poisson GLMM (All Meiofauna)		
Coeffcient	*Log-Mean*	*Conf. Int (95%)*	*P-value*
Intercept	3.87	3.78 – 3.95	<0.001
Beach Slope	0.07	0.01 – 0.13	0.030
Beach Cleaning	0.09	0.00 – 0.17	0.038
Vehicle Traffic	-0.04	-0.11 – 0.04	0.327
Buildings on Sand	-0.05	-0.08 – -0.02	0.003
SQM	0.00	-0.06 – 0.06	0.999
Dune Condition	-0.02	-0.09 – 0.05	0.647
Observations	55		
R^2 conditional / R^2 marginal	NA / 0.351		

	Poisson GLMM (Upper Intertidal)		
Coeffcient	*Log-Mean*	*Conf. Int (95%)*	*P-value*
Intercept	2.92	2.78 – 3.07	<0.001
Beach Slope	0.06	-0.03 – 0.16	0.192
Beach Cleaning	0.08	-0.05 – 0.21	0.219
SQM	-0.01	-0.11 – 0.08	0.762
Dune Condition	-0.21	-0.33 – -0.09	0.001
Vehicle Traffic	-0.24	-0.35 – -0.13	<0.001
Buildings on Sand	-0.08	-0.13 – -0.02	0.005
Observations	55		

R^2 conditional / R^2 marginal NA / 0.433

	nbinom GLMM (Lower Intertidal)		
Coeffcient	*Log-Mean*	*Conf. Int (95%)*	*P-value*
Intercept	3.48	3.35 – 3.60	<0.001
Beach Slope	0.08	-0.00 – 0.17	0.052
Beach Cleaning	0.09	-0.03 – 0.21	0.146
SQM	0.00	-0.08 – 0.08	0.940
Dune Condition	-0.04	-0.14 – 0.06	0.422
Vehicle Traffic	0.03	-0.07 – 0.14	0.552
Buildings on Sand	-0.04	-0.08 – 0.00	0.078
Observations	55		
R^2 conditional / R^2 marginal	NA / 0.024		

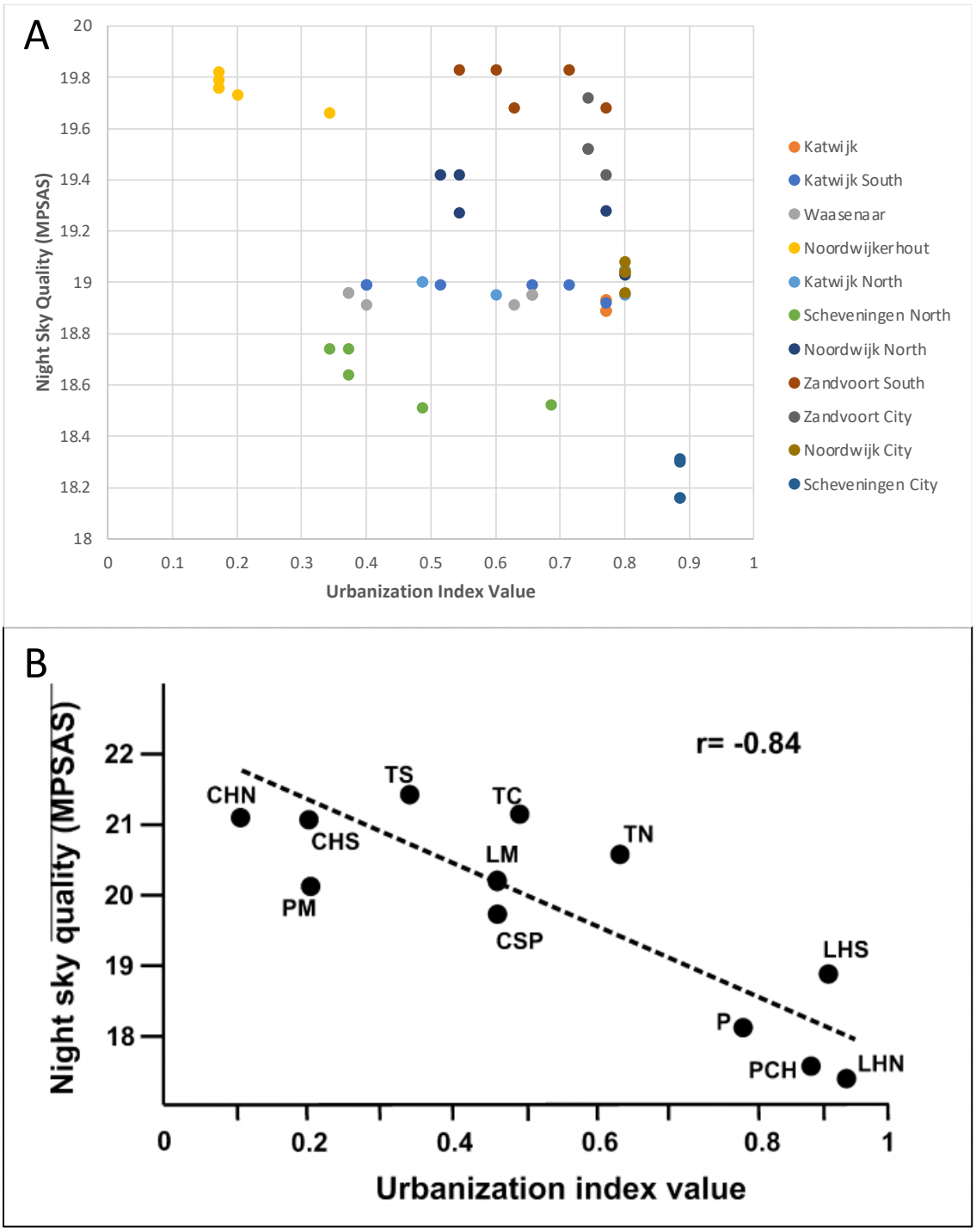


Figure S9. A) Relationship between urbanization index value and night sky quality for each transect in sandy beaches along the Southern North Sea B) Relationship between urbanization index value and night sky quality for several sandy beaches sampled along the northern Chilean coast from González et al. (2014).

Table S5. All transects and qualitative indicators of urbanization for each transect.

OTU_ID	Sample	Location	Type	Date	Transect	Latitude	Longitude	Beach_Slope	Transect_Units	Transect_Length	Salinity	Prox_UC	Buildings_Sand	Beach_cleaning	Solid_Waste	Vehicle_Traffic	SDM	Quality_NightSky	Freq_Visitors	UI	Dune_Cond
T1	T1	Katwijk	Beach	25-7-2023	1	52.1998041	4.3892825	-5.5	75	57.9	33	4	5	5	1	4	18.93	4	4	**0.77142857**	2
T2	T2	Katwijk	Beach	25-7-2023	2	52.2014176	4.3912355	-6	50	38.6	33	4	5	5	1	4	18.93	4	4	**0.77142857**	2
T3	T3	Katwijk	Beach	25-7-2023	3	52.2047441	4.3936347	-6.2	55	42.46	33	4	5	5	1	4	18.89	4	4	**0.77142857**	2
T4	T4	Katwijk	Beach	25-7-2023	4	52.2063957	4.3947683	-5.7	52	40.144	33	4	5	5	1	4	18.89	4	4	**0.77142857**	2
T5	T5	Katwijk	Beach	25-7-2023	5	52.2080992	4.3967012	-5	78	60.216	33	4	5	5	1	4	18.89	4	4	**0.77142857**	2
KS1	KS1	Katwijk_South	Beach	27-7-2023	1	52.1945625	4.3833702	-4.2	58	44.776	31	4	5	5	1	4	18.92	4	4	**0.77142857**	3
KS2	KS2	Katwijk_South	Beach	27-7-2023	2	52.1921334	4.3809653	-4.1	52	40.144	31	4	4	5	1	4	18.99	4	3	**0.71428571**	3
KS3	KS3	Katwijk_South	Beach	27-7-2023	3	52.1911006	4.3798726	-3.1	96	74.112	31	3	4	5	1	4	18.99	4	2	**0.65714286**	3
KS4	KS4	Katwijk_South	Beach	27-7-2023	4	52.188894	4.377499	-3	51	39.372	31	2	0	5	1	4	18.99	4	2	**0.51428571**	4
KS5	KS5	Katwijk_South	Beach	27-7-2023	5	52.1849163	4.3733421	-7	50	38.6	31	1	0	2	1	4	18.99	4	2	**0.4**	4
KS6	KS6	Katwijk_South	Beach	27-7-2023	6	52.181656	4.369718	-6.5	60	46.32	31	1	0	2	1	4	18.99	4	2	**0.4**	4
WA1	WA1	Wassenaar	Beach	31-7-2023	1	52.163483	4.348504	-5.9	45	34.74	31	1	4	5	1	4	18.95	4	4	**0.65714286**	3
WA2	WA2	Wassenaar	Beach	31-7-2023	2	52.164201	4.349226	-4.8	58	44.776	31	1	4	5	1	4	18.95	4	4	**0.65714286**	4
WA3	WA3	Wassenaar	Beach	31-7-2023	3	52.165102	4.350182	-5.3	49	37.828	31	1	4	5	1	4	18.91	4	3	**0.62857143**	4
WA4	WA4	Wassenaar	Beach	31-7-2023	4	52.16847	4.353855	-5.1	70	54.04	31	1	0	2	1	4	18.91	4	2	**0.4**	4
WA5	WA5	Wassenaar	Beach	31-7-2023	5	52.172234	4.358272	-5.8	51	39.372	31	1	0	2	1	3	18.96	4	2	**0.37142857**	4
WA6	WA6	Wassenaar	Beach	31-7-2023	6	52.175778	4.362744	-3.2	75	57.9	31	1	0	2	1	3	18.96	4	2	**0.37142857**	4
NO1	NO1	Noordwijkerhout	Beach	2-8-2023	1	52.303286	4.476266	-2.5	69	53.268	30	1	0	4	1	0	19.66	3	3	**0.34285714**	4
NO2	NO2	Noordwijkerhout	Beach	2-8-2023	2	52.309827	4.480898	-2.8	82	63.304	30	1	0	0	1	0	19.73	3	2	**0.2**	4
NO3	NO3	Noordwijkerhout	Beach	2-8-2023	3	52.315799	4.485325	-2.2	90	69.48	30	1	0	0	1	0	19.76	3	1	**0.17142857**	4
NO4	NO4	Noordwijkerhout	Beach	2-8-2023	4	52.31809	4.487289	-2.3	83	64.076	30	1	0	0	1	0	19.76	3	1	**0.17142857**	4
NO5	NO5	Noordwijkerhout	Beach	2-8-2023	5	52.321896	4.490123	-1.9	120	92.64	30	1	0	0	1	0	19.82	3	1	**0.17142857**	4
NO6	NO6	Noordwijkerhout	Beach	2-8-2023	6	52.324642	4.491821	-1.8	92	71.024	30	1	0	0	1	0	19.79	3	1	**0.17142857**	4
KN1	KN1	Katwijk_North	Beach	4-8-2023	1	52.213045	4.3998987	-2.6	70	54.04	30.5	4	4	5	1	5	19.05	4	5	**0.8**	3
KN2	KN2	Katwijk_North	Beach	4-8-2023	2	52.2151726	4.4020518	-2.5	115	88.78	30.5	4	4	5	1	5	18.95	4	5	**0.8**	3
KN3	KN3	Katwijk_North	Beach	4-8-2023	3	52.219204	4.405636	-2.8	108	83.376	30.5	3	0	5	1	5	18.95	4	3	**0.6**	4
KN4	KN4	Katwijk_North	Beach	4-8-2023	4	52.2229965	4.4103254	-2.7	110	84.92	30.5	3	0	3	1	3	19	4	3	**0.48571429**	4
KN5	KN5	Katwijk_North	Beach	4-8-2023	5	52.227099	4.413233	-2.5	140	108.08	30.5	3	0	3	1	3	19	4	3	**0.48571429**	4
Schn1	Schn1	Scheveningen_North	Beach	8-8-2023	1	52.122064	4.294074	-5.2	73	56.356	30	2	4	5	1	4	18.52	4	4	**0.68571429**	3
Schn2	Schn2	Scheveningen_North	Beach	8-8-2023	2	52.127596	4.301802	-3.2	88	67.936	30	3	0	4	1	2	18.51	4	3	**0.48571429**	4
Schn3	Schn3	Scheveningen_North	Beach	8-8-2023	3	52.133127	4.31032	-3.3	110	84.92	30	2	0	2	1	2	18.64	4	2	**0.37142857**	4
Schn4	Schn4	Scheveningen_North	Beach	8-8-2023	4	52.138428	4.3184273	-3.5	102	78.744	30	2	0	2	1	2	18.74	4	2	**0.37142857**	4
Schn5	Schn5	Scheveningen_North	Beach	8-8-2023	5	52.142376	4.322669	-2.8	108	83.376	30	1	0	2	1	2	18.74	4	2	**0.34285714**	4
NoN1	NoN1	Noordwijk_North	Beach	10-8-2023	1	52.253575	4.436104	-2.4	122	94.184	31	4	4	5	1	5	19.03	4	5	**0.8**	1
NoN2	NoN2	Noordwijk_North	Beach	10-8-2023	2	52.255465	4.438009	-2.3	117	90.324	31	4	4	5	1	5	19.28	3	5	**0.77142857**	1
NoN3	NoN3	Noordwijk_North	Beach	10-8-2023	3	52.260385	4.441826	-2.4	142	109.624	31	2	0	5	1	5	19.27	3	3	**0.54285714**	3
NoN4	NoN4	Noordwijk_North	Beach	10-8-2023	4	52.265716	4.44642	-2.1	172	132.784	31	2	0	5	1	5	19.42	3	3	**0.54285714**	3
NoN5	NoN5	Noordwijk_North	Beach	10-8-2023	5	52.26986	4.44896	-3.5	97	74.884	31	2	0	5	1	4	19.42	3	3	**0.51428571**	3
ZS1	ZS1	Zandvoort_South	Beach	14-8-2023	1	52.362852	4.517494	-2.3	148	114.256	31	4	4	5	1	5	19.68	3	5	**0.77142857**	1
ZS2	ZS2	Zandvoort_South	Beach	14-8-2023	2	52.35898	4.514772	-2	140	108.08	31	4	0	5	1	5	19.68	3	4	**0.62857143**	3
ZS3	ZS3	Zandvoort_South	Beach	14-8-2023	3	52.355515	4.512712	-2.1	140	108.08	31	3	4	5	1	5	19.83	3	4	**0.71428571**	3
ZS4	ZS4	Zandvoort_South	Beach	14-8-2023	4	52.352587	4.511135	-2.2	120	92.64	31	3	0	5	1	5	19.83	3	4	**0.6**	3
ZS5	ZS5	Zandvoort_South	Beach	14-8-2023	5	52.348661	4.507955	-3	143	110.396	31	3	0	5	1	4	19.83	3	3	**0.54285714**	3
ZC1	ZC1	Zandvoort_City	Beach	17-8-2023	1	52.3901373	4.53502	-2.4	131	101.132	31	4	5	5	1	4	19.72	3	4	**0.74285714**	1
ZC2	ZC2	Zandvoort_City	Beach	17-8-2023	2	52.385621	4.53244	-2.5	125	96.5	31	4	5	5	1	4	19.52	3	4	**0.74285714**	1
ZC3	ZC3	Zandvoort_City	Beach	17-8-2023	3	52.381583	4.53013	-2.1	127	98.044	31	4	5	5	1	4	19.52	3	4	**0.74285714**	1
ZC4	ZC4	Zandvoort_City	Beach	17-8-2023	4	52.376543	4.526495	-2	126	97.272	31	4	5	5	1	4	19.42	3	5	**0.77142857**	1
NoC1	NoC1	Noordwijk_City	Beach	21-8-2023	1	52.238829	4.4233392	-2.5	95	73.34	31	4	5	5	1	4	19.08	4	5	**0.8**	2
NoC2	NoC2	Noordwijk_City	Beach	21-8-2023	2	52.2443177	4.4275234	-2.8	98	75.656	31	4	5	5	1	4	18.96	4	5	**0.8**	2
NoC3	NoC3	Noordwijk_City	Beach	21-8-2023	3	52.2478288	4.4307967	-2.1	108	83.376	31	4	5	5	1	4	19.04	4	5	**0.8**	2
NoC4	NoC4	Noordwijk_City	Beach	21-8-2023	4	52.2504197	4.4329425	-2.4	108	83.376	31	4	5	5	1	4	19.04	4	5	**0.8**	2
SchC1	SchC1	Scheveningen_City	Beach	22-8-2023	1	52.1164457	4.2840893	-3.5	71	54.812	31	5	5	5	3	4	18.3	4	5	**0.88571429**	1
SchC2	SchC2	Scheveningen_City	Beach	22-8-2023	2	52.1141547	4.2807647	-7.9	35	27.02	31	5	5	5	3	4	18.31	4	5	**0.88571429**	0
SchC3	SchC3	Scheveningen_City	Beach	22-8-2023	3	52.1123823	4.2780587	-11.2	25	19.3	31	5	5	5	3	4	18.16	4	5	**0.88571429**	0
SchC4	SchC4	Scheveningen_City	Beach	22-8-2023	4	52.1105191	4.2751277	-13.2	20	15.44	31	5	5	5	3	4	18.16	4	5	**0.88571429**	1

Protocol for reference barcoding

Sampling for reference barcoding

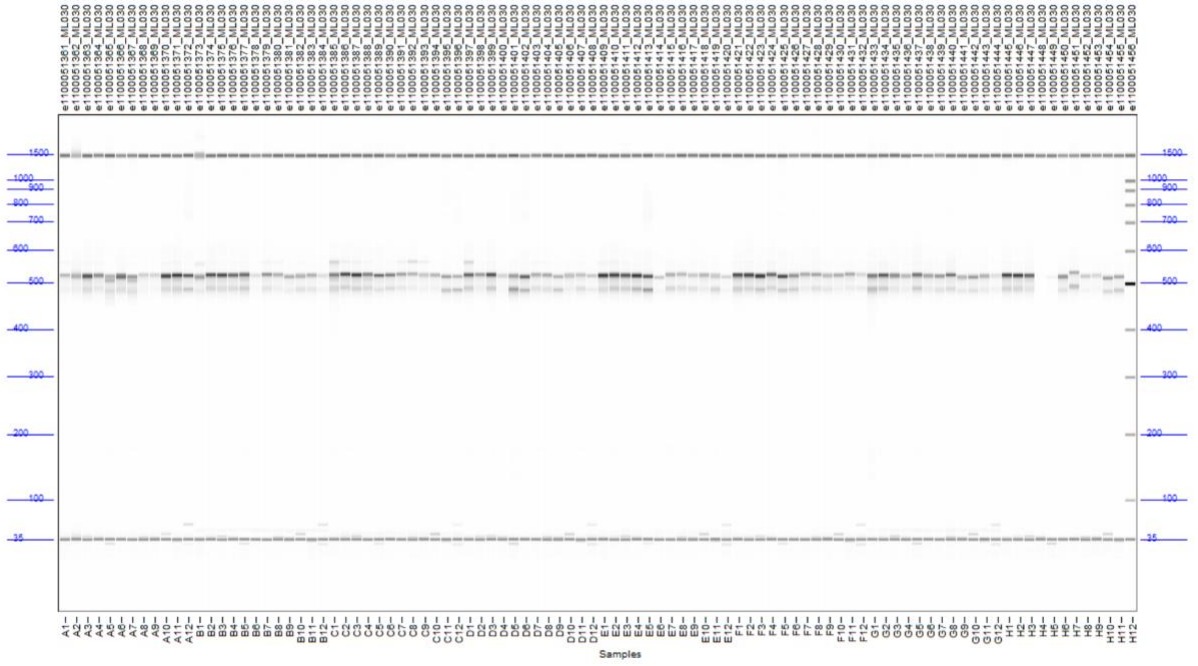
Meiofauna specimens were collected during two taxonomic workshops held at Naturalis Biodiversity Center in Leiden, the Netherlands, in May/June 2022 and July/August 2023. Samples were collected from 20 locations along the Dutch West Coast, by either taking sediment cores to a depth of 10cm, by filtering coastal groundwater and sand through dug holes, or by scraping hard substrates, depending on the targeted taxonomic group. The samples were transported to the Naturalis laboratory for meiofauna extraction using decantation through a 40µm sieve after anesthetization with isosmotic $MgCl_2$ (Somerfield & Warwick, 2013). For a detailed list of all sample locations and sample types, see supplementary table S1. After extraction, meiofauna specimens were identified to the lowest possible taxonomic rank using stereo and light microscopes. Specimens were transferred to PCR plates and submitted to the DNA extraction pipeline described below.

DNA extraction and amplification for reference barcoding

The DNA extraction process for meiofauna specimens was performed using the Macherey-Nagel (Düren, Germany) NucleoSpin tissue kit on the KingFisher (Waltham, USA) robotic platform, following the manufacturer's protocol. After extraction, PCRs were performed with Geller COI primers (Geller et al., 2013), targeting the 658 base-pair-long Folmer fragment of the mitochondrial cytochrome c oxidase I gene, which is the commonly used DNA barcode for animals (Folmer et al., 1994; Hebert et al., 2003). Samples were sequenced on the Oxford Nanopore GridION platform (Oxford Nanopore Technologies, Oxford, United Kingdom). The protocol was as follows:
Each PCR reaction contained 10.2 µl of MiliQ water, 4 µl of 5X PCR buffer (Qiagen; Hilden, Germany), 0.8 µl of 10 mg/ml BSA (Promega, Madison, Wisconsin, United States), 1 µl of 10 picomolar/µl primers, 0.4 µl of 2.5mM dNTPs, 0.4 µl of 5U/µl Phire II Taq polymerase (Thermo Fisher, Waltham, Massachusetts, United States), and 1 µl template DNA. PCR started with an initial denaturation of 30 seconds at 98 °C, followed by 35 cycles of 5 seconds denaturation at 98 °C, 10 seconds annealing at 50 °C, 15 seconds elongation at 72 °C, and a final extension of 5 minutes at 72 °C. Each PCR included a negative control using Milli-Q water (Merck; Rahway, New Jersey, United States) instead of template DNA. We cleaned the PCR products using AmPure magnetic beads (Brea, California, United States) with a ratio of 0.9:1. Subsequently, a second PCR was performed to individually label the samples, with 2.5 µl of ONT barcode primers, 5 µl of LongAmp Taq 2x master mix (New England Biolabs, Ipswich, Massachusetts, United States), and 2.5 µl of the PCR product as template. The PCR protocol for the second amplification was as follows: an initial denaturation of 3 minutes at 95 °C, followed by 15 cycles of 15 seconds denaturation at 95 °C, 15 seconds of annealing at 65 °C, 50 seconds of elongation, and a final extension step of 3 minutes at 65 °C. The success was checked using the TapeStation platform. The samples were then pooled at equimolar concentrations to achieve a final concentration of approximately 200 femtomolar. This was followed by a purification step using a 0.7:1 bead cleanup, targeting amplicons of 700 bp length. Finally, the purified DNA pools were eluted in 11 µl of nuclease-free water and their concentration was quantified using the Tape station (D5000 kit).

Sequencing and bioinformatics for reference barcoding

Sequencing was conducted using the Oxford Nanopore GridION sequencer on two FLO-MIN112 flow cells, with the SQK-NBD112.24 sequencing kit. The Basecalling was done with MinKNOW (v23.04.5), the run duration was set to 72H, and super accuracy basecalling was selected. The demultiplexing was performed with Guppy barcoder (v6.4.6). The consensus calling consisted of several steps combined together in a Snakemake (Mölder et al., 2021) pipeline: First, the reads (containing primers at both ends) were filtered by size (>=558, <= 758) and quality (>=10), and then reoriented with Cutadapt (v4.5, max error rate 20%, 80% coverage), which also removed flanking sequences. Then consensus sequences were generated using NGSpeciesID v0.3.0 (Sahlin et al., 2021) with Medaka polishing (v1.8.0, r104_e81_sup_g5015 model). A final round of primer sequence trimming was performed with Cutadapt. Following this, multi-fasta files containing consensus sequences were written by using a custom script. Quality control and visualisation of the processed FASTQ files was conducted using NanoPlot (De Coster et al., 2018) and MultiQC (Ewels et al., 2016). All resulting sequences underwent manual curation in Geneious Prime (version 2023.2) and were searched against existing references in NCBI GenBank using BLASTn (Ye et al., 2006). All scripts used for processing of Nanopore data are available on GitHub: https://gitlab.com/arise-biodiversity/sequencing/arise-barcoding-pipeline/-/tree/1a2fa544615be54dccb7136ca20d3664ba85d467.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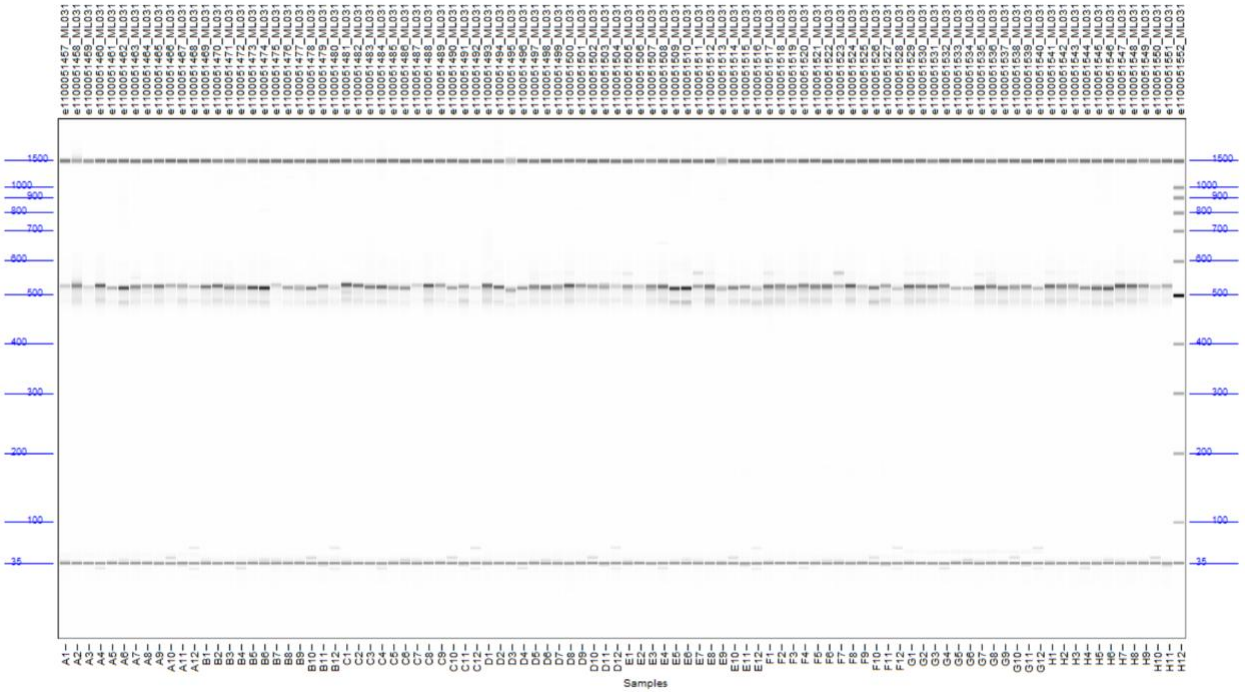
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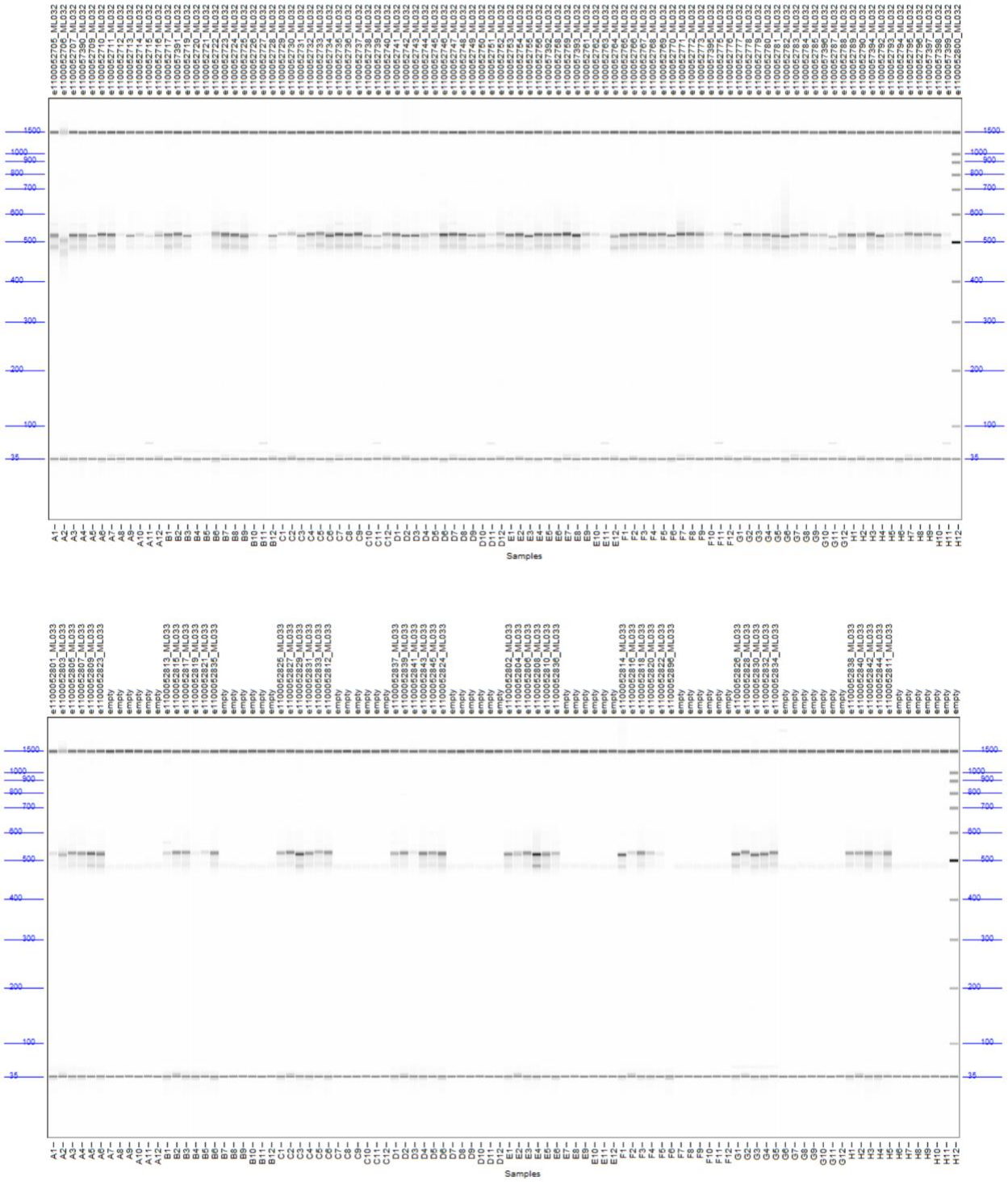

Figure S10. Fragment analyzer results.

Table S6. GLMM model output from 'glmmTMB' package in R. The bolded model was chosen as the best fit.BS = Beach Slope, BC = Beach Cleaning, SQM = sky quality meter, DC = Dune Condition, VT = Vehicle Traffic, Bsand = Buildings on Sand, FV = Frequency of Visitors, SW = Solid Waste, pUC = Proximity to Urban Centre.

	AIC score	Overdispersion	Factors Included	Model	Random effects?
All Meiofauna	**374.98**	**1.041**	**BS, BC, SQM, DC, VT, Bsand**	**Poisson**	**No**
	376.07	1.000	BS, BC, VT, Bsand	Poisson	No
	377.73	1.061	BS, BC, SQM, DC, VT, Bsand	Poisson	Yes
	378.82	1.167	BS, BC, SQM, DC	Negative Binomial	No
	381.16	1.095	BS, BC, SQM, DC	Poisson	No
Upper Intertidal	**329.85**	**1.325**	**BS, BC, SQM, DC, VT, Bsand**	**Poisson**	**No**
	332.16	1.197	BS, BC, SQM, DC, VT, Bsand	Negative Binomial	No
	334.92	1.053	BS, BC, SQM, DC, VT, Bsand	Poisson	Yes
	341.26	1.548	BS, VT, SQM, pUC, Bsand	Poisson	No
Lower Intertidal	**382.77**	**1.159**	**BS, BC, SQM, DC, VT, Bsand**	**Negative Binomial**	**No**
	383.52	1.613	BS, BC, SQM, DC, VT, Bsand	Poisson	No
	385.84	0.903	BS, BC, SQM, DC, VT, Bsand	Poisson	Yes
	390.70	0.827	BS, TL, SQM, DC, FV, SW	Poisson	Yes