Drivers of nematode community structure and of nematode microbiomes on an estuarine intertidal flat

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Table of Contents

List of abbrevations 11

Summary 1

Samenvatting 2

Chapter 1 General Introduction 1

1.1 Estuaries, their importance and different habitats 1

1.2 Intertidal flat habitat 2

1.2.1 Environmental characteristics 3

1.2.2 Intertidal flat MPB and benthos 6

1.2.2.1 Microphytobenthos (MPB) 6

1.2.2.2 Benthos 7

1.2.3 The interplay among biota and among biota and abiotic factors 7

1.2.3.1 Relationships between organisms (food web) 7

1.2.3.2 Influence of environmental heterogeneity on intertidal flat organisms 8

1.3 Nematodes 11

1.3.1 Nematodes' roles in ecosystems 11

1.3.1.1 Trophic position of tidal flat nematodes 12

1.3.1.2 Functional roles played by nematodes 15

1.3.2 Nematodes' distribution 16

1.3.2.1 Patterns 16

1.3.2.2 Drivers 16

1.4 Microbiomes 18

1.4.1 Host associated microbiomes and environmental microbiomes 19

1.4.2 Nematode associated microbiomes (NAM) 19

1.5 Study objectives and outline of this PhD thesis 21

1.5.1 Study objectives and outline of this thesis 21

1.5.2 Study area and model species 23

Chapter 2 Environmental drivers of spatial patterns of nematode abundance and genus composition at two spatial scales on an estuarine intertidal flat 25

Abstract 31

2.1 Introduction 31

2.2 Material and Methods 34

2.2.1 Study area and sampling 34

2.2.2 Sample processing and analytical procedures 35

2.2.2.1 Environmental variables 35

2.2.2.2 Nematode community analysis 36
2.2.3 Data processing and statistical analysis 37
2.3 Results 38
2.3.1 Sediment characteristics 38
2.3.2 Nematode densities 40
2.3.2.1 Mesoscale 40
2.3.2.2 Microscale 40
2.3.3 Nematode community 40
2.3.3.1 Mesoscale 40
2.3.3.2 Microscale 42
2.3.4 Environmental drivers of nematode density 45
2.3.4.1 Mesoscale 45
2.3.4.2 Microscale 45
2.3.5 Environmental drivers of nematode genus composition 45
2.3.5.1 Mesoscale 45
2.3.5.2 Microscale 47
2.4 Discussion 48
2.4.1 Environmental drivers at mesoscale 48
2.4.2 Environmental drivers at microscale 52
2.5 Conclusion 53
Acknowledgements 53
Supplementary information of Chapter 2 54
Chapter 3 Natural stable isotope ratios and fatty acid profiles of estuarine tidal flat nematodes reveal very limited niche overlap among co-occurring species 73
Abstract 75
3.1 Introduction 75
3.2 Materials and Methods 79
3.2.1 Sampling site, sampling procedure and collection of nematodes 79
3.2.2 Selection of nematode taxa for stable-isotope and fatty-acid analyses 80
3.2.3 Preparation of nematode samples for stable-isotope and fatty-acid analyses 82
3.2.4 Stable isotope analysis 83
3.2.5 Fatty acid analysis 83
3.2.6 Fatty acid biomarkers 84
3.2.7 Data analysis 84
3.2.7.1 Dual stable isotope data 84
3.2.7.2 Fatty acid composition and biomarker concentrations 86

3.3 Results ... 87
3.3.1 Trophic level and resources of nematodes based on SIA ... 87
3.3.2 Fatty acid composition ... 89
3.3.3 Fatty acid markers of nematode diet ... 91
3.4 Discussion ... 92
3.4.1 Carbon sources of tidal flat nematodes ... 92
3.4.2 The nematode part of the benthic food web comprises more than two trophic levels and a substantial degree of omnivory ... 95
3.4.3 Resource differentiation among nematode species is prominent ... 97
Acknowledgements ... 100
Supplementary information of Chapter 3 ... 101
Chapter 4 Characterization of marine nematode associated microbiomes by high-throughput sequencing ... 113
Abstract ... 115
4.1 Introduction ... 115
4.2 Material and Methods ... 118
4.2.1 Sampling and pre-treatment of samples ... 118
4.2.2 Analysis of environmental samples ... 119
4.2.2.1 Measurements ... 119
4.2.2.2 Data analysis ... 120
4.2.3 Collection of nematodes for microbiome analysis ... 120
4.2.4 DNA extraction, amplification, and sequencing ... 121
4.2.5. Bioinformatics ... 122
4.2.5.1 Sequence processing ... 122
4.2.5.2 Downstream data analysis and statistics ... 122
4.3 Results ... 124
4.3.1 Environmental variables ... 124
4.3.2 Microbiomes ... 125
4.3.2.1 Taxonomic profile ... 125
4.3.2.2 Nematode and sediment microbiomes ... 129
4.3.3 Core microbiomes ... 131
4.3.4 Relation of environmental variables with microbiomes ... 133
4.4 Discussion ... 134
4.4.1 Nematode microbiomes differ from the microbiomes of the sediments they inhabit ... 134
4.4.2 Nematode microbiomes are species-specific ... 137
4.4.3 Nematode microbiomes are context-dependent ... 139

4.5 Conclusions 141
Acknowledgements 141
Supplementary information of Chapter 4 142
Chapter 5 General Discussion 155
5.1 Introduction 157
5.2 Drivers of nematode community structure: what can we learn from a combination of a field survey, food-web biomarkers and nematode-associated microbiomes? 159
5.3 The (un)importance of bacteria as a resource for tidal flat nematodes? Lessons learnt from chapters 3 and 4 162
5.4 Trophic positioning and roles of marine nematodes: a tale of more than just primary and secondary consumers 167
5.5 Dedicated experiments do not always provide straightforward answers: The example of *Metachromadora remanei* 171
5.6 Future perspectives 172
5.6.1. Technological opportunities to improve the 'performance' of nematode community ecology 172
5.6.2. Expanding the microbiome approach to elucidate nematode diets 174
5.6.3. Getting more out of our microbiome analyses 175
5.6.4. A plea for more species-level and individual-based research 175
Acknowledgements 176
References list 177

List of abbrevations

Abbreviation	full name
A. fuscus	*Adoncholaimus fuscus*
a-car	α-Carotene
ANOSIM	analysis of similarity
ARA	arachidonic acid
ASW	artifical sea water
b-car	β-Carotene
c1c2	Chlorophyll c1,c2
chl *a*	Chlorophyll *a*
chl *b*	Chlorophyll *b*
D. hirsutum	*Daptonema hirsutum*
DF	Deposit feeder
DHA	Docosohexaenoic acid
diadino	Diadinoxanthin
diato	Diatoxanthin
DistLM	Distance-based linear model (analysis)
E. longispiculosus	*Enoploides longispiculosus*
E.brevis	*Enoplus brevis*
EF	Epigrowth feeder
EPA	eicosapentaenoic acid
FA	fatty acid
FAMEs	Fatty Acid Methyl Esters
FF	the nitrogen fractionation factor
FP	Facultative predator
fuco	Fucoxanthin
HUFA	highly unsaturated fatty acid
Lefse	Linear discriminant analysis effect size
lutein	Lutein
M. remanei	*Metachromadora remanei*
MGS	median grain size
MPB	microphytobenthos
MUFA	mono-unsaturated fatty acid
NAM	nematode associated microbiomes

Abbrevation	**Full name**
nMDS	non-metric multidimensional scaling
O. oxyuris	*Oncholaimus oxyuris*
O. setosus	*Odontophora setosus*
P	Predator
P. punctatus	*Praeacanthonchus punctatus*
PCA	Principal component analysis
PCoA	Principal Coordinates Analysis
PCR	polymerase chain reaction
PEAR	Paired-End reAd mergeR
peri	Peridinin
PERMANOVA	permutational multivariate analysis of variance
pheo	Pheophytin *a*
PUFA	polyunsatured fatty acid
pyro	Pyropheophytin *a*
QIIME	Quantitative Insights Into Microbial Ecology
S1	supplementary 1
SDs	Standard deviations
SEA	Standard ellipse area
SFA	saturated fatty acid
SIBER	Stable Isotope Bayesian Ellipses
SIMPER	similarity of percentages (analysis)
st1	station 1
T. acer	*Theristus acer*
TFA	total amount of fatty acid
TL	Trophic level
TOM	total organic matter
WLB	worm lysis buffer
zea	Zeaxanthin

Summary

Estuarine tidal flat sediments are highly productive and biologically rich ecosystems. Their secondary production provides nutrition to large numbers of migratory bird populations and to commercially relevant shellfish and fish stocks. This high productivity can be driven by a range of organic matter subsidies, including deposited phytoplankton and detritus of both terrestrial and marine origin, macroalgae, seagrasses and/or salt marsh vegetation. In most cases, however, the *in situ* productivity of microbial biofilms fuels a major part of the secondary production on estuarine intertidal flats. These biofilms are complex consortia of benthic microalgae and heterotrophs embedded in a biogenic polymer matrix. They play key roles in a range of important ecosystem functions, such as sediment stabilization and water quality improvement. Nevertheless, several unknowns still exist about the complex interplay between microphytobenthos (MPB), prokaryotes and benthic invertebrates in microbial biofilms on tidal flats.

Nematodes are by far the most abundant metazoans, and are also among the most species-rich taxa in estuarine and marine soft substrates and their biofilms. Their high abundances and generally high biomass turnover rates have caused speculation about their importance in tidal flat sediments. Their grazing and non-trophic interactions with biofilm-forming organisms may affect the activity and community structure of both MPB and of sediment bacteria, and thus probably also affect some of the ecosystem processes mediated by these micro-organisms. In addition, nematodes can be an important food source for higher trophic levels. Thus, nematodes may represent an important trophic link between biofilm-forming organisms and higher trophic levels. Moreover, the high local-scale species diversity of nematodes has puzzled ecologists for decades. Differential resource use is often invoked as a basis for niche differentiation among species, yet the vast majority of studies demonstrating that this would be prominent in marine nematodes are based on laboratory experiments on single species or on highly simplified assemblages, leaving the issue of resource differentiation under natural conditions rather understudied until present.

In order to improve our understanding of the functional roles of nematodes in tidal flat sediments, it is crucial that both trophic and non-trophic interactions among nematodes, and between nematodes and biofilm-forming organisms, are documented and understood. The overarching goal of this PhD was therefore to elucidate some of the interrelationships between microphytobenthos, bacteria and nematodes on an intertidal flat in the polyhaline reach of the Schelde estuary, SW Netherlands.

Because one needs to know patterns before one can understand the underlying processes, we first set out to describe the horizontal and vertical spatial distribution of nematode communities within an intertidal flat in relation to a number of potential drivers, including sediment granulometry and

intertidal position, but with a major focus on food/biofilm-related drivers (chapter 2). We subsequently selected nine common nematode species from this intertidal flat and used natural stable isotope ratios of carbon and nitrogen, as well as fatty acid profiles, to elucidate resource use, resource partitioning and trophic structure of these nine species (chapter 3). Because these approaches do not adequately address trophic relationships between nematodes and bacteria, and because recently booming interest in microbiomes has revealed the involvement of bacteria at all levels of MPB biofilm formation, as well as in the 'fitness' of a wide variety of animals, we analysed the microbiomes of the three nematode species which in chapter 3 proved most dependent on microphytobenthos. We investigated whether nematode microbiomes were a random subset of the microbiomes of the environment they inhabit, or rather indicated specific nematode-bacteria relationships. We assessed whether the nematode microbiomes were species-specific, and whether and to what extent they varied spatially and temporally (chapter 4).

These aims were addressed in three topical research chapters.

In chapter two, we studied spatial patterns and drivers of nematode density and genus composition at two different spatial scales (i.e. meso- and microscale), with drivers including sediment granulometry (median grain size, % silt), inundation period and food quality/quantity as indicated by various phytopigments. The mesoscale included 10 stations covering three different intertidal positions, while the microscale included 5 stations at one intertidal level with interdistances of < 50 m. Our results revealed mesoscale zonation and microscale patchiness patterns. These patterns were more pronounced in the surface layer than in deeper sediment layers. At the mesoscale, nematode communities differed mostly between the low-tide level, with highest densities and a different genus composition, and the high- and mid-tide levels. Nematode density in the top 0-2 cm layer was higher than in the lower two layers, while genus composition separated the low-tide from the mid- and high-tide stations, except in the depth layer of 4-6 cm. Similar trends were observed at the microscale, but here, differences in density and genus composition between stations showed rather inconsistent patterns with depth. Despite these inconsistencies, ANOSIM indicated that nematode communities were more dissimilar with depth than horizontally, irrespective of the scale of our study (meso- vs microscale). Nematode abundance and community composition were significantly affected by a range of food-related drivers as well as by sediment granulometry and inundation period, but the best combinations of explanatory variables differed both between depth layers and between scales of observation. The amount of explained variation in nematode abundance in the surface layer was, however, considerably higher at the microscale (46 vs < 10 %), whereas the opposite was true for the 2-4 cm layer (23 vs 63 %). In terms of genus composition, different combinations of food-related drivers and silt content or tidal level explained between 20 (at a depth of 4-6 cm) and 40 % (at the

surface layer) of the observed variation at the mesoscale. Surprisingly, single food-related drivers (α or β-carotene, depending on sediment depth) were the best predictors of variation in community composition at the microscale and explained considerably less variation (9-30 %) than at the mesoscale. Our study indicates that food availability is an important driver of nematode abundance and community structure; to some extent, this also holds for silt content and intertidal position. However, the expected larger importance of sediment granulometry and tidal level at the mesoscale, and of food availability at the microscale, did not occur.

In chapter 3, we used natural stable-isotope ratios of carbon and nitrogen, as well as fatty-acid profiles, to assess differential resource use, trophic level and degree of omnivory in nine abundant estuarine tidal flat nematode species, comprising different presumed feeding modes (deposit feeders, epistratum feeders, predators,…) and resource guilds (herbivores, carnivores,…). The bivariate isotopic standard ellipse areas (SEAc) of nematode species showed very limited overlap: the SEAc of *Daptonema* overlapped with those of *Metachromadora* and *Adoncholaimus*, while all other pairs of species had non-overlapping SEAc. Similarly, an ordination of the fatty acid (FA) composition showed very little overlap between species (only, and to a limited extent, between *Praeacanthonchus* and *Metachromadora*, and between *Daptonema* and *Theristus*). These results demonstrate that resource differentiation is pronounced among as well as within feeding modes and resource guilds. Nematodes comprised up to three different trophic levels (from primary to tertiary consumers, or from herbivores over mesopredators to predators), yet with the exception of some herbivores (i.e. *Metachromadora, Praeacanthonchus, Theristus*), omnivory was prominent. It occurred both in feeding modes where this could be expected (i.e. facultative predators and predators) and in feeding modes where this was much less expected (i.e. the supposedly herbivorous *Daptonema*). As a consequence, there was no clear separation in trophic level between presumed primary consumers and presumed carnivores, but rather a range of values spanning from trophic level 2 to almost trophic level 4. The FA composition of nematodes, by contrast, did largely separate carnivores from herbivores. Bivariate isotopic niche spaces were of similar size among most species, irrespective of their trophic level. That is surprising, since in early feeding-type classifications of marine nematodes, it was assumed that feeding types were ‘additive’ in terms of the resources that can be utilized. For example, a predator could eat herbivores but also the food of herbivores. That early view was supported by our data on trophic level and on FA composition, but it would then be expected to yield a larger resource niche for higher trophic levels. This was not the case, suggesting that even herbivores have flexible feeding strategies which allow them to utilize a range of resources, albeit mostly primary producers. Herbivory mainly targeted diatoms in some species (e.g. *Metachromadora, Praeacanthonchus, Adoncholaimus*), yet prominently included dinoflagellates in others (e.g. *Theristus, Enoploides, Daptonema*). Bacteria, in

contrast, appeared to be of limited nutritional importance, since prokaryote-specific biomarkers usually comprised less than 10 % of total FA. *Odontophora setosus,* the feeding ecology of which was hitherto unknown, was identified as a predator/omnivore with a trophic level in between that of secondary and tertiary consumers.

In chapter 4, we characterized the spatial (i.e. two stations with contrasting sediment granulometry) and temporal (i.e. three consecutive seasons) variation in the microbiomes of three microphytobenthos biofilm-associated marine nematode species (*Metachromadora remanei, Praeacanthonchus punctatus, Theristus acer*) and compared these with the microbiomes of the nematodes' substrates. Only 5 % of the prokaryotic OTUs (operational taxonomic units) found in sediments were ever encountered in nematode microbiomes, and only up to 20 % of OTUs from nematode microbiomes were present in sediments. There was also no link between the proportional abundance of specific bacterial taxa in sediments and in nematodes, demonstrating that nematode microbiomes were distinct from those of sediments. Moreover, only just less than half of the OTUs that were shared between nematodes and sediments were also common to all three nematode species, suggesting selective relationships between nematode species and sediment bacteria. These relationships probably involve selective feeding; no clear indications were found for the presence of prominent species-specific nematode-bacteria symbioses. Differences in nematode microbiomes were mostly prominent between *M. remanei* on the one hand and *T. acer* and *P. punctatus* on the other, which likely reflects differences in their mode of feeding. The microbiomes of sediments and nematodes were strongly context-dependent, differing among stations as well as seasons. A substantial portion (61 %) of the variation in sediment microbiomes, but a much smaller portion of the variation in nematode-associated microbiomes (7-23 %), could be explained by the spatiatemporal variation in sediment granulometry and in biomass and composition of the microphytobenthos.

In chapter 5, we integrate some of the main findings of the different chapters, and provide an outlook to future research perspectives to further our understanding of the roles of nematodes in tidal-flat ecosystem functioning. We emphasize that our results confirm that MPB carbon is probably the main energy source fueling intertidal nematode communities, but that there are multiple pathways from MPB to nematodes, ranging from direct grazing to predation on herbivores and on predators of herbivores. Bacteria can also provide a route from MPB carbon to nematodes, but their quantitative importance as a food source appears to be small: ≤ 12 %, and in most cases ≤ 5 %. However, our microbiome study indicates that the network of 'indirect interactions' between nematodes and bacteria is even larger and much less 'one-way' than expected, leaving the importance of bacteria for marine nematodes insufficiently understood.

The observed inverse relationship in the relative contributions of the biomarker FA EPA and DHA strongly suggests that some nematode species have a clear preference for diatoms over dinoflagellates, while others have an opposite preference. This is one of the first evidences of the utilization of dinoflagellates by marine nematodes, a link which should be further investigated. In addition to the potential significance of dinoflagellates as a resource for tidal-flat nematodes, we provide first evidence that zooplankton faecal pellets as well as dead microzooplankton may significantly contribute to the nutrition of at least some intertidal nematode species.

Nevertheless, our data also demonstrate that we should be extremely cautious not to generalize findings from one habitat type to others, and that we should not make simple generalizations about the feeding ecology and principal resources of specific nematode genera, as these may differ across environments as well as over time. Flexible feeding strategies and a high prominence of omnivory in many nematode species imply that the use of nematode feeding types, which make generalized inferences on feeding ecology for very heterogeneous groupings of nematodes, further looses relevance. Finally, our data strongly support the idea that resource niche differentiation, particularly when combined with the patchy distribution of resources in space and time and with differential dispersal rates, may account for the coexistence of large numbers of species at a local scale.

Samenvatting

Estuariene intergetijdengebieden staan bekend als zeer productieve ecosystemen met een hoge biodiversiteit. Secundaire productie in deze gebieden voorziet voedsel aan grote populaties trekvogels en commercieel belangrijke schelpdieren en vissoorten. Deze heterotrofe productie kan aangestuurd worden door een brede waaier aan organisch materiaal, onder meer van fytoplankton en detritus van zowel terrestrische als mariene oorsprong, macroalgen, zeegras en/of vegetatie van estuariene gebieden. Daarnaast is de in situ productie die plaatsvindt in microbiële biofilms zeer belangrijk voor de secundaire productie in estuariene getijdengebieden. Een biofilm is een complex systeem waar benthische microalgen en heterotrofen samenleven in een biogene polymere matrix. De biofilm speelt een belangrijke rol in bepaalde ecosysteemfuncties, zoals stabilisatie van het sediment en de verbetering van de waterkwaliteit. Toch zijn er nog steeds veel onduidelijkheden over de complexe interactie tussen microfytobenthos (MFB), prokaryoten en benthische ongewervelden in de microbiële biofilms in intergetijdengebieden.

Nematoden zijn het meest voorkomende fylum van de Metazoa en zijn op lokale schaal bovendien het meest soortenrijk in estuariene en mariene substraten en biofilms. De hoge abundantie en hoge turnover van biomassa van deze organismen hebben geleid tot speculaties over hun rol in intergetijdengebieden. De niet-trofische interacties tussen, en het begrazen van organismen die belangrijk zijn voor het vormen van een biofilm, kunnen de activiteit en gemeenschapsstructuur van zowel MFB als bacteriën in het sediment beïnvloeden. Dit laatste kan ook leiden tot het beïnvloeden van ecosysteemprocessen waarbij deze micro-organismen een belangrijke rol spelen. Bovendien kunnen nematoden een belangrijke voedselbron zijn voor hogere trofische niveaus. Nematoden kunnen dus een zeer belangrijke link zijn tussen organismen die biofilms vormen en hogere trofische niveaus. Bovendien zorgt de hoge diversiteit van nematoden op lokale schaal voor vele vragen bij ecologen. Deze hoge diversiteit wordt meestal verklaard door verschillen in gebruik van belangrijke (voedsel)bronnen, wat kan leiden tot nicheverschillen. Deze verschillen zijn enkel bewezen in experimenten in het laboratorium binnen 1 soort of in sterk vereenvoudigde gemeenschappen, wat ervoor zorgt dat het verschil in bronnengebruik onder natuurlijke condities tot op heden eerder onbekend terrein is.

Om de kennis van de functionele rol van nematoden in de intergetijdengebieden te vergroten, is het van cruciaal belang om zowel trofische als niet-trofische interacties tussen nematoden onderling, en tussen nematoden en andere organismen die de biofilm vormen, te bestuderen. Het overkoepelende doel van dit doctoraat is om de relaties tussen microfytobenthos, bacteriën en nematoden in intergetijdengebieden in het polyhaliene gebied van het Schelde-estuarium in het zuidwesten van Nederland te bestuderen.

Allereerst beschrijven we de horizontale en vertikale distributie van nematodengemeenschappen in een intergetijdengebied om meer over de natuurlijke patronen te weten te komen, een voorwaarde om de onderliggende processen beter te onderzoeken en begrijpen. De links tussen de nematodengemeenschappen en verschillende potentiële omgevingsvariabelen, zoals sedimentgranulometrie en de positie in het intergetijdengebied, worden onderzocht. Bovendien leggen we onze focus op drivers die het voorkomen en de abundantie en samenstelling van voedsel en biofilms reflecteren (hoofdstuk 2). We selecteerden negen veel voorkomende nematodensoorten van het intergetijdengebied, en gebruikten stabiele isotopen (koolstof en stikstof) en vetzuurprofielen om het gebruik van bronnen, de mogelijke differentiatie in brongebruik tussen soorten, en het trofische niveau van deze negen soorten te bepalen (hoofdstuk 3). Omdat deze technieken niet adequaat zijn om trofische relaties tussen nematoden en bacteriën te analyseren, en omdat recente microbioomanalyses aantonen dat bacteriën van belang zijn op alle niveaus bij het vormen van biofilms, werden de microbiomen van drie nematodensoorten, die in hoofdstuk 3 afhankelijk bleken van microfytobenthos, bestudeerd. We onderzochten of het microbioom van nematoden een random subset van het microbioom van de omgeving is, of dat er een specifieke relatie tussen nematoden en bacteriën kan aangetoond worden. Bovendien onderzochten we of de microbiomen van de nematoden soortspecifiek zijn en of er ruimtelijke en temporele verschillen zijn (hoofdstuk 4).

Deze doelstellingen werden benaderd in drie thematische onderzoekshoofdstukken. In hoofdstuk 2 bestudeerden we de ruimtelijke patronen en drivers van nematodendensiteit en genuscompositie op twee verschillende ruimtelijke schalen (meso- en microschaal). De drivers die hierbij in rekening gebracht werden waren sedimentgranulometrie (mediane korrelgrootte, % slib), overstromingstijd en voedselkwantiteit en –kwaliteit (aan de hand van verschillende fytopigmenten). Op mesoschaal werden 10 stations, die drie verschillende intertidale posities vertegenwoordigden, bestudeerd. Op microschaal werden 5 stations op slechts 1 intertidale positie bestudeerd, met afstanden tussen de verschillende stations kleiner dan 50 m. Onze resultaten toonden aan dat op mesoschaal zonatie en op microschaal het voorkomen van verschillende patches de belangrijkste patronen zijn. Deze patronen waren duidelijker in de bovenste sedimentlaag vergeleken met de diepere lagen. Op mesoschaal verschilden de nematodengemeenschappen het meest tussen laag-intertidaal gelegen locaties, waar ze een hogere densiteit bereikten en een verschillende genussamenstelling vergeleken met locaties in het hoog- en midden-intertidaal. Nematodendensiteit in de bovenste laag (0-2cm) was hoger dan in de twee diepere lagen. Genussamenstelling verschilde tussen de laaggelegen stations, vergeleken met de midden- en hooggelegen stations, behalve in de diepere laag van 4 tot 6 cm. Gelijkaardige trends werden gevonden op microschaal, maar hier waren de verschillen in densiteit en genuscompositie tussen de stations eerder inconsistent in relatie tot diepte. Desondanks toonde de

ANOSIM aan dat nematodengemeenschappen meer verschilden tussen dieptes (verschillende lagen) dan horizontaal (de verschillende stations), en dit ongeacht de schaal van de studie (meso- vs. microschaal). Abundanties van nematoden en de samenstelling van de gemeenschap werden significant beïnvloed door een waaier aan voedselgerelateerde drivers, evenals door sedimentgranulometrie en duur van overstroming, maar deels andere drivers waren belangrijk op meso- dan op microschaal. De hoeveelheid variatie in abundantie van nematoden in de bovenste sedimentlaag die door de gemeten variabelen verklaard kon worden was hoger op microschaal (46 *vs.* < 10%); in de 2-4 cm laag, daarentegen, zagen we het omgekeerde (23 vs. 63%). Voor genuscompositie verklaarden verschillende combinaties van voedselafhankelijke drivers en hoeveelheid slib of positie in het intergetijdengebied tussen de 20 (op een diepte van 4-6 cm) en 40% (in de bovenste sedimentlaag) van de variatie op mesoschaal. Verrassend was dat enkelvoudige voedselafhankelijke drivers (α of β-caroteen, afhankelijk van sedimentdiepte) de beste voorspellers waren voor de variatie in gemeenschapssamenstelling op microschaal én een kleiner deel van de variatie verklaarden (9 – 30%) dan op de mesoschaal. Onze studie toonde aan dat voedselbeschikbaarheid een belangrijke driver voor abundanties van nematoden en gemeenschapsstructuur is. Tot op zekere hoogte geldt dit ook voor de hoeveelheid slib en de locatie in het intergetijdengebied. Desondanks waren het belang van sedimentgranulometrie en locatie in het intergetijdengebied op mesoschaal, en van voedselbeschikbaarheid op microschaal, minder prominent aanwezig dan verwacht.

In hoofdstuk 3, werden stabiele-isotoopanalyses van koolstof en stikstof, evenals analyses van vetzuurprofielen, uitgevoerd om mogelijke verschillen in bronnengebruik, trofisch niveau en graad van omnivorie in negen veel voorkomende nematodensoorten uit het intergetijdengebied na te gaan. Deze negen soorten behoorden toe tot verschillende ‘voedingsgroepen’ (depositvoeders, epistratumvoeders, predatoren en een onbekende voedingsgroep) en ‘resouce guilds’ (herbivoren, carnivoren, en onbekend). De bivariate isotopische standaard ellipsoppervlaktes (SEAc) van de nematodensoorten toonden relatief weinig overlap: the SEAc van *Daptonema* overlapte met deze van *Metachromadora* en *Adoncholaimus*, terwijl alle andere soortenparen geen overlappende SEAc hadden. De ordinatie van de vetzuursamenstelling (FA) toonde ook zeer weinig overlap tussen de soorten (enkel een zeer kleine overlap tussen *Praeacanthonchus* en *Metachromadora*, en tussen *Daptonema* en *Theristus*). Deze resultaten tonen aan dat verschillen in bronnengebruik zowel binnen als tussen ‘voedingsgroepen’ en ‘resource guilds’ nadrukkelijk aanwezig zijn. De onderzochte nematoden namen samen een ketenlengte van ongeveer drie trofische niveaus in (van primaire tot tertiare consumenten, of van herbivoren over mesopredatoren tot predatoren). Met uitzondering van enkele herbivoren (i.e. *Metachromadora, Praeacanthonchus, Theristus*) was omnivorie prominent aanwezig, en dit zowel voor de beide voedingsgroepen waar we dit verwacht hadden (facultatieve

predatoren en predatoren) als in een voedingsgroep waar dit veel minder verwacht werd (i.e. de verwachte herbivoor *Daptonema*). Er was hierdoor geen duidelijke scheiding in trofisch niveau tussen sommige verwachte primaire consumenten en carnivoren terug te vinden, maar eerder een continuüm van trofisch niveau 2 tot niveau 4. Op basis van hun vetzuursamenstelling konden carnivore en herbivore nematoden wel duidelijk van elkaar onderscheiden worden. Bivariate isotopische nicheruimtes waren gelijkaardig van grootte voor de verschillende soorten, onafhankelijk van hun trofisch niveau. Dit is verwonderlijk aangezien in vroegere classificaties gebaseerd op voedingstype gesuggereerd werd dat de voedingstypes additief waren inzake de bronnen die gebruikt konden worden. Als voorbeeld kan een predator zich voeden op herbivoren, maar ook op het voedsel van de herbivoren. Dit inzicht werd gesteund door onze resultaten, maar er zou dan ook verwacht worden dat er een meer divers voedselbronnengebruik terug te vinden zou zijn in de hogere trofische niveaus. Dit bleek dus niet uit de SEAc, wat erop wijst dat ook nematoden op lagere trofische niveaus flexibele voedingsstrategieën hebben waardoor ze een vrij breed bereik aan bronnen kunnen gebruiken, zij het dan vooral primaire producenten. In de analyses werd voorts aangetoond dat herbivorie zich bij sommige soorten preferentieel op diatomeeën richt (b.v. *Metachromadora, Praeacanthonchus, Adoncholaimus*), maar bij andere (b.v. *Theristus, Enoploides, Daptonema*) meer op dinoflagellaten. Bacteriën bleken daarentegen van minder belang als voedsel, aangezien specifieke prokaryote biomarkers meestal ruim minder dan 10% van de totale vetzuurgehaltes vertegenwoordigden. Van *Odontophora setosus* was de voedingsecologie tot hiertoe niet gekend; deze soort werd nu geïdentificeerd als predator/omnivoor met een trofisch niveau tussen dat van een secundaire en tertiare consument.

In hoofdstuk 4 werden de ruimtelijke (twee stations met verschillende sediment granulometrie) en temporele variatie (drie opeenvolgende seizoenen) in het microbioom van drie mariene nematodensoorten, die geassocieerd leven met microfytobenthosbiofilms (*Metachromadora remanei, Praeacanthonchus punctatus, Theristus acer*), onderling vergeleken, alsook met het microbioom van het substraat waarin de nematoden. Slechts 5 % van de OTUs (‘operational taxonomic units’) afkomstig van de prokaryoten in het sediment werden ook teruggevonden in de microbiomen van de nematoden, en slechts 20 % van de OTUs van het microbioom van nematoden waren aanwezig in het sediment. Bovendien was er geen verband tussen de proportionele abundantie van specifieke bacteriële groepen in het sediment en in de microbiomen van de nematoden, wat duidelijk aantoont dat de microbiomen van nematoden verschillen van het microbioom van het sediment. Slechts minder dan de helft van de OTUs die zowel in de microbiomen van de nematoden als in het sediment voorkwamen, kwamen ook abundant voor in alle drie de nematodensoorten. Dit suggereert dat er een selectieve relatie bestaat tussen de nematodensoort en de bacteriën van het sediment. Deze

relatie kan mogelijk te maken hebben met selectieve voedingsstrategieën; anderzijds werden geen duidelijke indicaties gevonden voor de aanwezigheid van soortspecifieke symbioses tussen bacteriën en nematoden. Verschillen tussen microbiomen van nematoden werder gevonden bij *M. remanei* enerzijds en *T. acer* en *P. punctatus* anderzijds, wat mogelijk wijst op verschillen in voedingswijze. De microbiomen van het sediment en van de nematoden waren sterk afhankelijk van locatie en seizoen. Een groot deel van de variatie (61 %) in het microbioom van sedimenten, maar een kleiner deel bij het microbioom van de nematoden (7-23 %), kon verklaard worden door de spatiotemporele variatie in granulometrie van het sediment en door de biomassa en samenstelling van het microfytobenthos.

In hoofdstuk 5 integreren we enkele van de hoofdconclusies van de verschillende hoofdstukken en geven we een vooruitblik op mogelijk toekomstig onderzoek om onze kennis van de rol van nematoden in intergetijdenzones te vergroten. We concluderen dat onze resultaten bevestigen dat MFB koolstof wellicht de belangrijkste energiebron is voor intertidale nematodengemeenschappen, maar dat er verschillende 'pathways' zijn om aan deze koolstof te geraken, gaande van directe begrazing over predatie op herbivoren tot predatie op predatoren van herbivoren. Bacteriën kunnen hierbij ook een rol spelen, maar hun kwantiatieve belang als voedselbron lijkt minder groot : ≤ 12%, en zelfs meestal ≤ 5%. Desondanks toont onze microbioomstudie aan dat er een netwerk van indirecte interacties tussen bacteriën en nematoden bestaat dat omvattender en minder unidirectioneel is als eerst gedacht. Dit zorgt ervoor dat het belang van bacteriën voor mariene nematoden nog steeds niet voldoende gekend is.

De geobserveerde inverse relatie van de relatieve contributie van de biomerkervetzuren EPA en DHA toont aan dat sommige nematodensoorten een sterke voorkeur hebben voor diatomeën eerder dan voor dinoflagellaten, terwijl andere de omgekeerde voorkeur vertonen. Dit is één van de eerste onderzoeken die het gebruik van dinoflagellaten door mariene nematoden aantoont. Naast dit potentiële gebruik van dinoflagellaten als voedselbron bij mariene nematoden in intergetijdengebieden, konden we ook aantonen dat faecale pellets, alsook kadavers van dood microzoöplankton een belangrijke voedselbron kunnen zijn voor sommige nematodensoorten.

Onze data toont aan dat we zeer voorzichting moeten zijn om onze bevindingen door te trekken naar andere habitats, en dat we geen eenvoudige generalisaties over voedselecologie en belangrijke voedselbronnen kunnen maken, aangezien deze kunnen verschillen naargelang tijd en plaats. Flexibele voedingsstrategieën en het veelvuldig voorkomen van omnivorie maken dat het gebruik van voedingstypeclassificaties bij nematoden, die typisch generalisaties maken over de voedingsecologie van een zeer heterogene groep van nematoden, zijn relevantie verliest. Ten slotte tonen onze data aan dat nichedifferentiatie in voedselbronnen, voornamelijk wanneer dit gecombineerd is met een

spotsgewijze distributie van voedselbronnen en met verschillende dispersiesnelheden, kunnen leiden tot de coëxistentie van een groot aantal soorten op lokale schaal.

Chapter 1 General Introduction

Chapter 1 General introduction

1.1 Estuaries, their importance and different habitats

Estuaries are very diverse in appearance and main features; hence, many descriptions of what is an estuary have been proposed (see review, Elliott and McLusky, 2002). One definition states that estuaries are semi-enclosed bodies of water, within which seawater is measurably diluted with freshwater (Pritchard, 1967; Elliott and McLusky, 2002). Estuaries are generally shallow and easy to access, and therefore a close relationship between estuaries and humans has existed for centuries. Estuaries are often divided into three parts: upper, middle and lower (Fig. 1.1), which often correspond to broad salinity zones (oligohaline, mesohaline and poly/euhaline) (Elliott and McLusky, 2002; Kaiser et al., 2011). Moreover, each section is subject to different water movements (e.g. remarkable daily tidal action at the upper estuary), but also to different degrees of variation in environmental factors such as salinity and sediment particle size; salinity fluctuates on a daily basis with the tides, and the largest salinity fluctuations over a tidal cycle are typically found in the mesohaline zone (Flemming, 2011). The geomorphology, hydrodynamics and sedimentology of an estuary determine the extent of intertidal flats, resulting in often wide and muddy intertidal flats (see Fig. 1.2) in the meso- and polyhaline zones, particularly in coastal-plain type, funnel-shaped estuaries, which are wide at the mouth and have a characteristic V-shaped cross-section (Kaiser et al., 2011).

Nutrients and sediments both from land and sea are often transported and trapped by estuaries through several actions, such as water flow, waves and tidal movements (https://www.niwa.co.nz/education-and-training/schools/students/estuaries). This is one of the main reasons why estuaries are among the most heterogenous marine ecosystems. The concentrations of organic matter in estuarine waters are typically much higher than those in the open sea (100 mg l^{-1} vs 1-3 mg l^{-1}) (Kaiser et al., 2011), resulting in very high metabolic rates in waters and sediments. Because much of the organic matter in estuaries is allochtonous and derives either from land/freshwater or from the marine realm, estuaries are often net sinks of organic matter and net heterotrophic systems, in which respiration – at least in the oligo- and mesohaline zones – largely exceeds *in situ* primary production (Heip et al., 1995). This imbalance between respiration and in situ primary production essentially means that the primary production by phytoplankton and microphytobenthos in the estuary is by no means sufficient to support all the secondary production processes, hence other resources are also needed to fuel in *situ* secondary production.

Estuaries provide high ecological value, as well as several goods and services for humans. For example, they harbour a high diversity of habitats (which range from intertidal flats and salt marshes over seagrass beds, tidal pools and rocky reefs to subtidal ‘channels’. An example is the Schelde estuary, a

macrotidal temperate estuary which crosses the Dutch-Belgian border and is characterized by vast areas of intertidal flats and scattered salt marshes (Meire et al., 2005). Estuaries also harbor a high biodiversity of organisms, from prokaryotes over protists to higher plants and animals, including taxa with freshwater origin and taxa with marine origin; the rich benthos communities on intertidal flats provide food to large numbers of migratory birds and a variety of other vertebrate and invertebrate organisms (Herman et al., 1999; Ysebaert et al., 2005; Kaiser et al., 2011). Multiple valuable functions and services of estuaries at least in part depend on the highly diverse life; for instance, microbes decompose much of the organic matter load of estuaries, providing cleaner water. In addition, estuaries are used to transport goods from the sea to land, function as farming grounds for shell fish etc. Because of their intense use by men, and because of the often many inputs from freshwater tributaries as well as from terrestrial run-off, many estuaries are heavily loaded by anthropogenic pollution (McLusky, 1999; Kaiser et al., 2011).

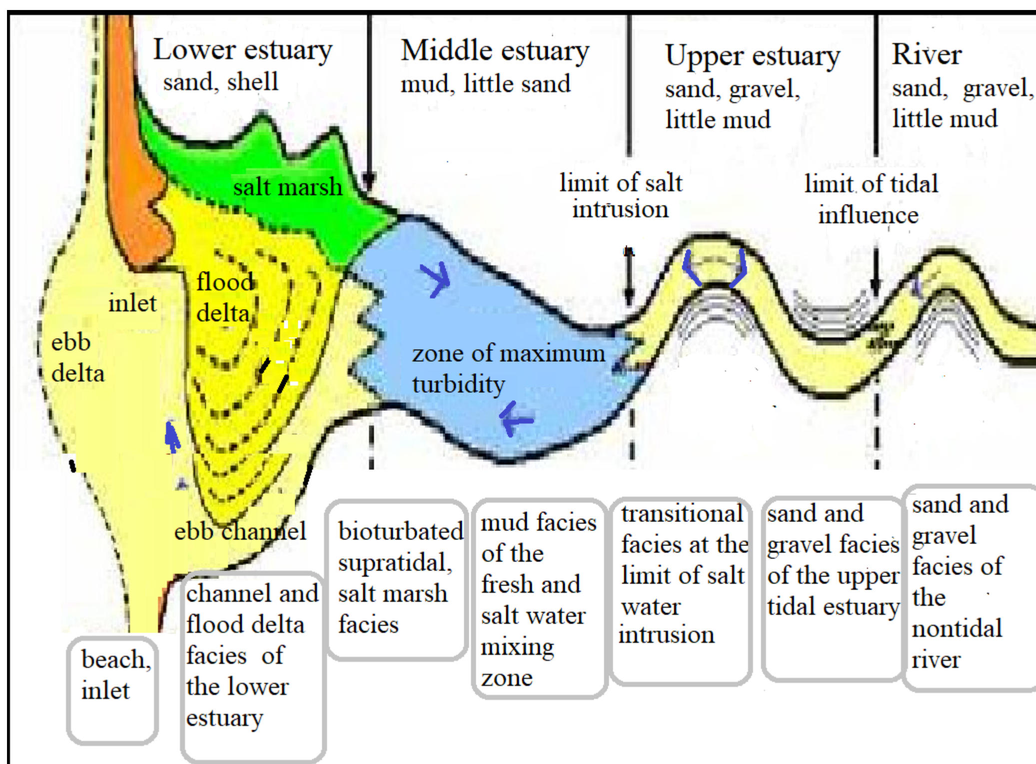


Fig. 1.1. Sedimentary facies zonation of estuaries (modified after Flemming, 2011).Map of the Schelde estuary, showing its lower section (Westerschelde) and connection to the open sea (North Sea), as well as some of the prominent habitats in the estuary. Image modified from (Post et al., 2017).

1.2 Intertidal flat habitat

Intertidal flats are defined as "areas between the average lowest and highest sea water level at low tide and high tide" (https://land.copernicus.eu/user-corner/technical-library/corine-land-cover-nomenclature-guidelines/html/index-clc-423.html), and they are also referred to as shores (Kaiser et

al., 2011). They are well known as both sources and sinks of nutrients and organic matter (Bella et al., 1972), and are therefore among the most productive ecosystems, despite an often strong heterogeneity and variability in environmental factors.

1.2.1 Environmental characteristics

Intertidal flats are characterized by strong physical variability introduced by water movements such as tidal currents and waves, and by variable environmental parameters such as temperature, salinity, acidity and oxygen (Platt and Warwick, 1980; Giere, 2009; Compton et al., 2013), with some of the major gradients being (inter)tidal position, sediment type and salinity (Elliott and McLusky, 2002; Kaiser et al., 2011; Flemming, 2011). Tidal and wave action act together to form characteristic tidal zonation patterns, which drive the distribution of many intertidal organisms, depending on their ability to adapt to life in such dynamic and variable environments (McLachlan et al., 1996; vanTamelen, 1996; Widdows and Brinsley, 2002; Rodil et al., 2007; Gomes and Rosa, 2009; Bird et al., 2013).

Variation in sediment type is mainly related to the deposition of sediments (Voulgaris, 1999) (Fig. 1.3), and can show clear gradients both along the estuary (Elliott and McLusky, 2002; Kaiser et al., 2011; Flemming, 2011) and across the intertidal (Evans, 1965). Whilst the head or upper part of many estuaries is characterized by pronounced currents/river flow, allowing sedimentation of only coarse particles, the middle reaches usually have less pronounced water flow and a strong mixing of fresh and marine waters, which in organically loaded estuaries can typically lead to the formation of 'estuarine snow' (estuarine turbidity maximum) and an enhanced deposition of finer sediment particles (Day Jr et al.,1989). This leads to the formation of often extensive, muddy intertidal flats in the oligo- to mesohaline reaches of estuaries, whereas the lower reaches are again characterized by stronger currents and hence coarser-grained sediment deposits (Jordan, 2012). Across the intertidal, low-dynamic muddy sediments, characterized by a high macrobenthos biomass, can be found at the lower-intertidal – upper-subtidal interface (verify with graph?) (Herman et al., 2001). Depending on the morphology of the intertidal flat, this ecologically highly valuable low-dynamic zone is bordered by slightly to substantially coarser sediments that experience more hydrodynamic influences, followed in turn by a gradual transition to siltier sediments and eventually vegetated salt marsh sediments at or above the mean high-water spring-tide level (Elliott and McLusky, 2002; Kaiser et al., 2011; Flemming, 2011). Many organisms have a preference to live in sediments with a specific granulometry – nematodes and many other invertebrates, for instance, obtain their highest densities in silty sediments, but a higher species diversity in sandier sediments (Giere, 2009; Moens et al., 2013).

Salinity varies over different spatial and temporal scales in estuaries. The Schelde estuary has a complete salinity gradient, i.e. stretching from freshwater to marine. Tidal action directly and

indirectly causes substantial salinity variations on a (twice a) daily basis; this tidal variation can vary from less than 1 psu at the head of the estuary, a few psu near the mouth of the estuary, to large variations (up to 20 psu or more) in the middle part of some estuaries. This extremely high salinity variation may be one reason why animal species diversity in the meso- to oligohaline reaches of estuaries is often substantially lower than in the freshwater and polyhaline reaches. An alternative explanation is that there are few, if any, true estuarine species; the diversity of estuarine organisms is then composed of marine species tolerant of lowered salinity and freshwater species that are – to an extent – halotolerant. This would make sense particularly in estuaries in boreal and temperate climatic regions, since these are almost without exception geologically very young: they only exist in their current state and bed since after the last glaciation, leaving little time for extensive speciation to have taken place.

In addition to this estuarine-wide salinity gradient, several other factors can cause much smaller-scale salinity gradients or fluctuations (Kaiser et al., 2011). Among these factors are exposure time and evaporation, temperature, and the input of fresh water, e.g. through precipitation (De Jonge and Van Beusekom et al., 1995). Many intertidal organisms can actively move up or down in sediments to avoid desiccation and extreme salinity fluctuations at the sediment surface (Giere, 2009).

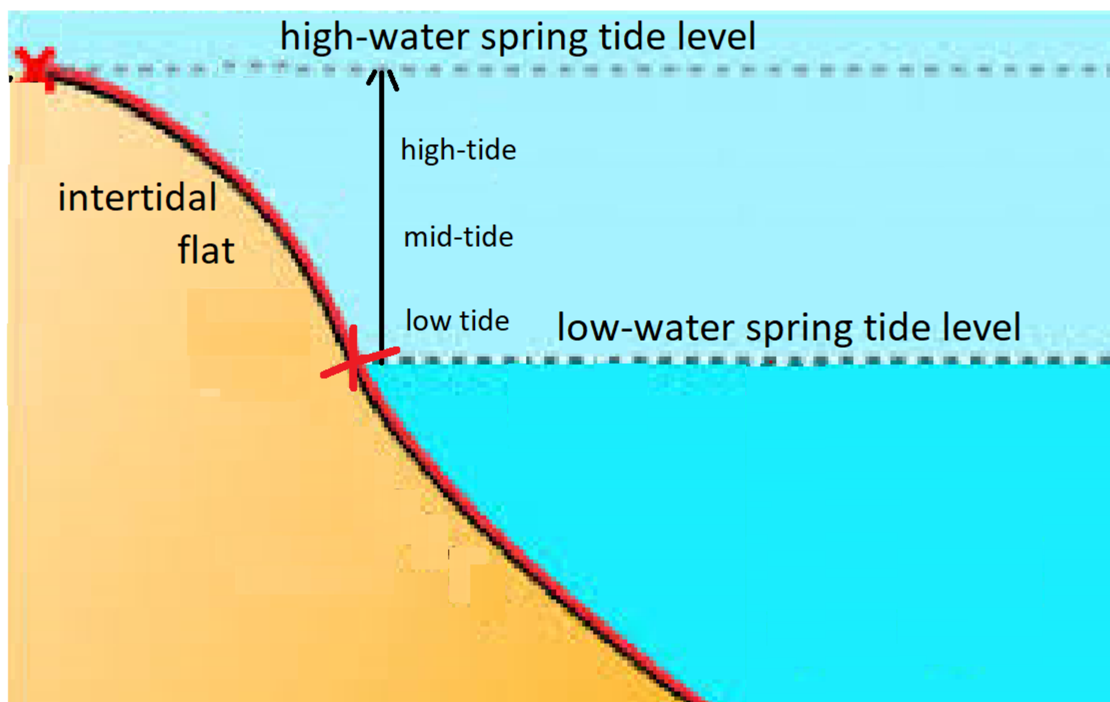


Fig. 1.2. Intertidal flat zone, image modified from online resource: https://vfa.vic.gov.au/recreational-fishing/restricted-fishing-locations/restricted-areas-intertidal-zone

Intertidal flat heterogeneity is not only related to the major environmental gradients. Several physical and chemical factors are also involved, even including atmospheric process factors (Fig. 1.3): wind,

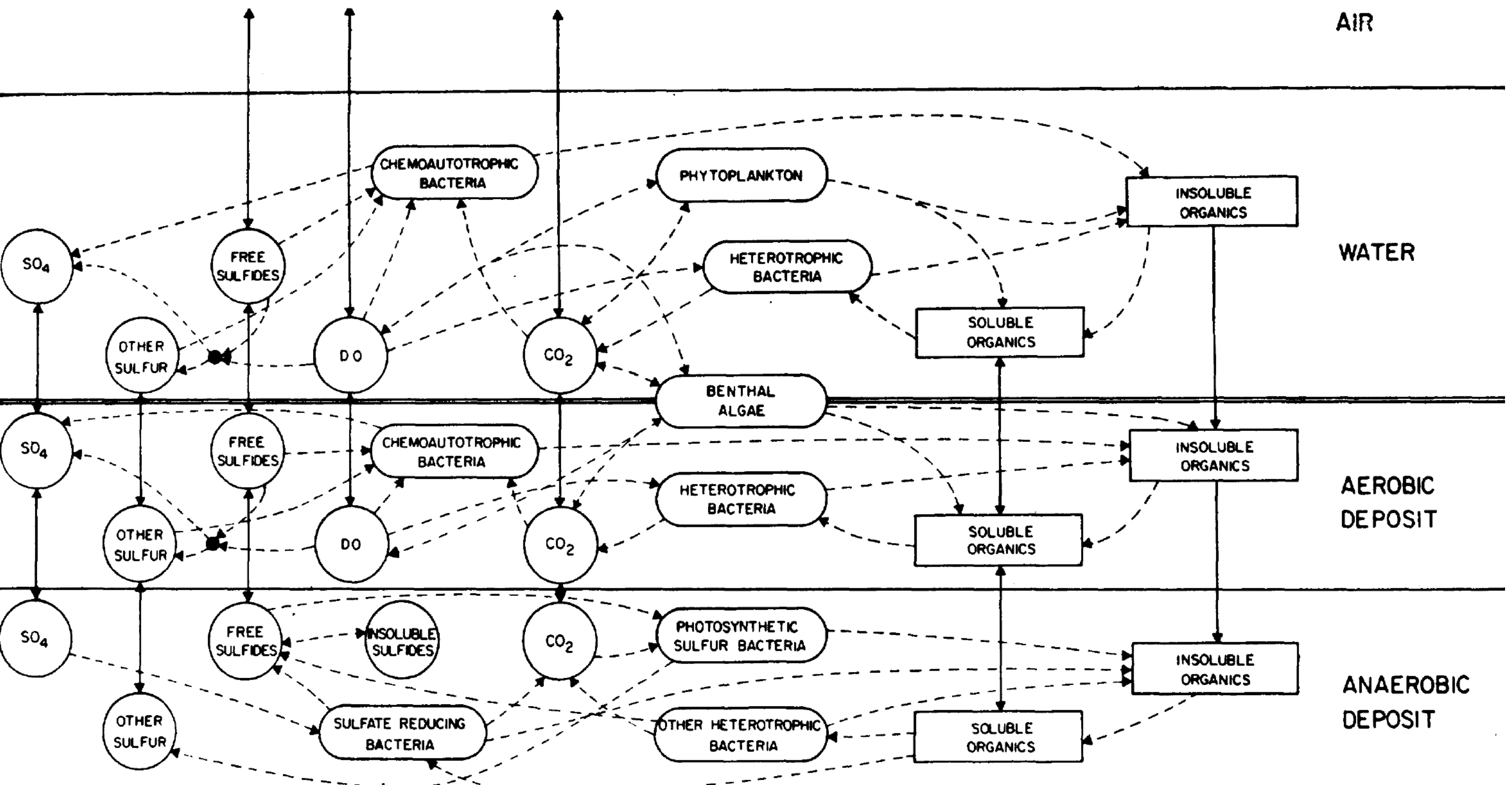


Fig. 1.3 A conceptual model of structuring factors in the tidal flat ecosystems, image from Bella et al. (1972).

rain, temperature and light, and impacts of human activities (e.g. pollutions, dredging) (De Jonge and Van Beusekom, 1995); Black and Paterson, 1998), together with other factors (Fig. 1.3 and Fig. 1.5) shape the biodiversity of intertidal flat organisms.

1.2.2 Intertidal flat MPB and benthos

Intertidal flats harbour several organisms, including primary producer such as microphytobenthos and several consumers, e.g. benthic fauna and migratory birds.

Intertidal flats are among the most biologically productive ecosystems. They are fascinating areas for ecologists mainly due to two reasons (Raffaelli and Hawkins, 1999): they are generally easy to access, especially during the low-tide period, and they provide model sites for an array of ecological questions/challenges related to, among others, their high biological productivity and rates of organic matter decomposition, their often high biodiversity, their variety of sediment types and the presence of prominent environmental gradients. Moreover, tidal flats can provide farming grounds for commercial fisheries (such as, fish, mollusks (e.g. cockles) and shellfish: mainly crabs and shrimps). The food provisioning by tidal flats is estimated to be up to 10-20 times larger than in deeper coastal waters (Miththapala, 2013).

1.2.2.1 Microphytobenthos (MPB)

The secondary productivity of intertidal flats ranks among the highest across ecosystems, which is driven by diverse inputs of organic matter, including settling phytoplankton, allochtonous organic matter from riverine and marine inputs, vascular plant detritus from salt marshes, and – last but not least – by a highly prominent presence of *in situ* primary producers (mainly microphytobenthos and cyanobacteria) and heterotrophic microorganisms (mainly bacteria). Microphytobenthos (MPB) refers to 'the microscopic, photosynthetic eukaryotic algae and cyanobacteria that live at or near the sediment surface' (MacIntyre et al., 1996). These often contribute a lot to the total primary production in estuaries (MacIntyre et al., 1996; Underwood and Kromkamp, 1999), with contributions of local microphytobenthic production typically in the order of 100 gC m^{-2} yr^{-1} (Heip et al., 1995; MacIntyre et al., 1996; Underwood and Kromkamp, 1999). Intricate interactions exist among MPB microalgae and heterotrophic bacteria (Gerbersdorf et al., 2009; Van Colen et al., 2014) making MPB biofilms very complex interaction webs of eu- and prokaryotic microorganisms, which have, or affect, multiple ecosystem functions (Hubas et al., 2010; Stock et al., 2014; Van Colen et al., 2014). One of these ecosystem functions is the stabilization of surface sediments through the production of copious extracellular polymeric substances (EPS) as part of the formation of biofilm (Goto et al., 1999; Paterson and Black, 1999). In addition, MPB plays a central role in moderating carbon flow in intertidal sediments (Middelburg et al., 2000). Moreover, they provide a quantitatively and especially qualitatively important carbon source for benthos (Herman et al., 1999; Moens et al., 2005a), as they provide high nutritional values for various organisms, for instance in the form of essential fatty acids

which cannot be synthetized de novo by their heterotrophic grazers (De Troch et al., 2012; Mensens et al., 2018). Among the main grazers of MPB are heterotrophic protists as well as benthic meio- and macrofauna (Montagna et al., 1995; Epstein, 1997; Herman et al., 1999), As such, MPB forms the basis of a grazer food web, in which carbon and energy from MPB is transferred to herbivores, which in turn are prey to secondary consumers (Gee, 1989; Coull, 1990). Moreover, dead MPB, EPS and faeces of MPB grazers provide highly palatable food sources for heterotrophic bacteria and thus fuel a high microbial activity (Evrard et al., 2008). However, much of this bacterial secondary production does not transfer up the food chain, but largely enters decomposition pathways, as the fate of much bacterial production is mortality, for instance through viral lysis (Herman et al. 2001; Van Oevelen et al. 2006), resulting in a weak trophic link between benthic invertebrates and microbial production.

1.2.2.2 Benthos

Benthos is a term that refers to all living organisms that live inside or on aquatic sediments. They can be classified based on their size into macro-, meio- and microfauna. Meiofauna is operationally defined as those organisms retained on a sieve with a mesh size of 38/32 µm, but passing through a sieve with pores of 500 or 1000 µm (there is discrimination on these size limits) (Giere, 2009), with the dominant taxa being Nematoda (see 1.3). Meiofauna provides an important (trophic) link between macro- and microbenthos, as they consume microorganisms and also serve as food sources for macrofaunal (Gee, 1989; Coull, 1990, 1999).

Compared to macrofauna and microbenthos, much fewer studies have been conducted on meiofauna (Moens and Beninger, 2018). This is due to several reasons, such as the time-consuming nature of sorting and identifying meiofauna due to their small size and large numbers and scarcity of taxonomic expertise. As a consequence, our knowledge of meiofauna ecology and functioning in tidal flat sediments is still limited.

1.2.3 The interplay among biota and among biota and abiotic factors

Intertidal flats are very heterogeneous environments. The interplay of physical factors such as hydrodynamics and tidal activity, of physico-chemical conditions and gradients and of a variety of trophic resources shapes the biodiversity and community structure of benthic invertebrates.

1.2.3.1 Relationships between organisms (food web)

Food web research is pivotal to a broad range of ecological questions, including the relationships between animal community structure and diversity and their roles in ecosystem functioning, or the study of the factors that drive the dynamics of populations and communities (Loreau et al., 2002). Trophic interactions can be quite complex and may require multiple approaches to elucidate them. For instance, figure 1.4 depicts the carbon flows through the benthic food web of an intertidal flat in

the Schelde estuary, as modelled by inverse modelling using constraints from a pulse-chase labelling experiment, from sediment community oxygen consumption measurements, and from various literature sources (Van Oevelen et al., 2006). In this food web, primary production by MPB, deposition of phytoplankton and suspended particulate organic matter, and production of pseudofaeces (what is this?) by macrobenthic suspension feeders are the principal carbon inputs. Dissolved organic matter (DOC) enters the system mostly through the secretion of EPS by MPB and bacteria, and is consumed by bacteria and microbenthos but not by benthic invertebrates. Detritus is produced (death and faeces) and consumed by all heterotrophic compartments in this food web model. MPB and bacteria are grazed upon by heterotrophic microbenthos, nematodes, other meiofauna and macrobenthos. Microbenthos in turn is potential prey for nematodes, other meiobenthos and macrobenthos. Nematodes are preyed upon by predatory nematodes and macrobenthos, and other meiobenthos is eaten by macrobenthos. The main carbon outflows are respiration (diamond head arrows in Fig. 1.4), export of macrobenthos biomass through consumption by fish or birds, and burial. It is clear that in this food web, nematodes have multiple carbon sources, including MPB, bacteria, microbenthos and other nematodes, MPB being somewhat more important than the other sources. In this thesis, we will, for instance, use natural stable isotopic signatures, fatty-acid biomarkers, pigment analyses and genetic 'diet analysis' to better elucidate what are the most important food sources for tidal flat nematodes. Note that the low quantitative importance of nematodes, as indicated by the thin flux lines, cannot be generalized, so this figure is mainly used here to illustrate the flows, not their quantitative importance.

1.2.3.2 Influence of environmental heterogeneity on intertidal flat organisms

The broad range of environmental factors which vary on tidal flats results in a very pronounced spatiotemporal heterogeneity in tidal flat sediments, and this at different spatial and temporal scales.

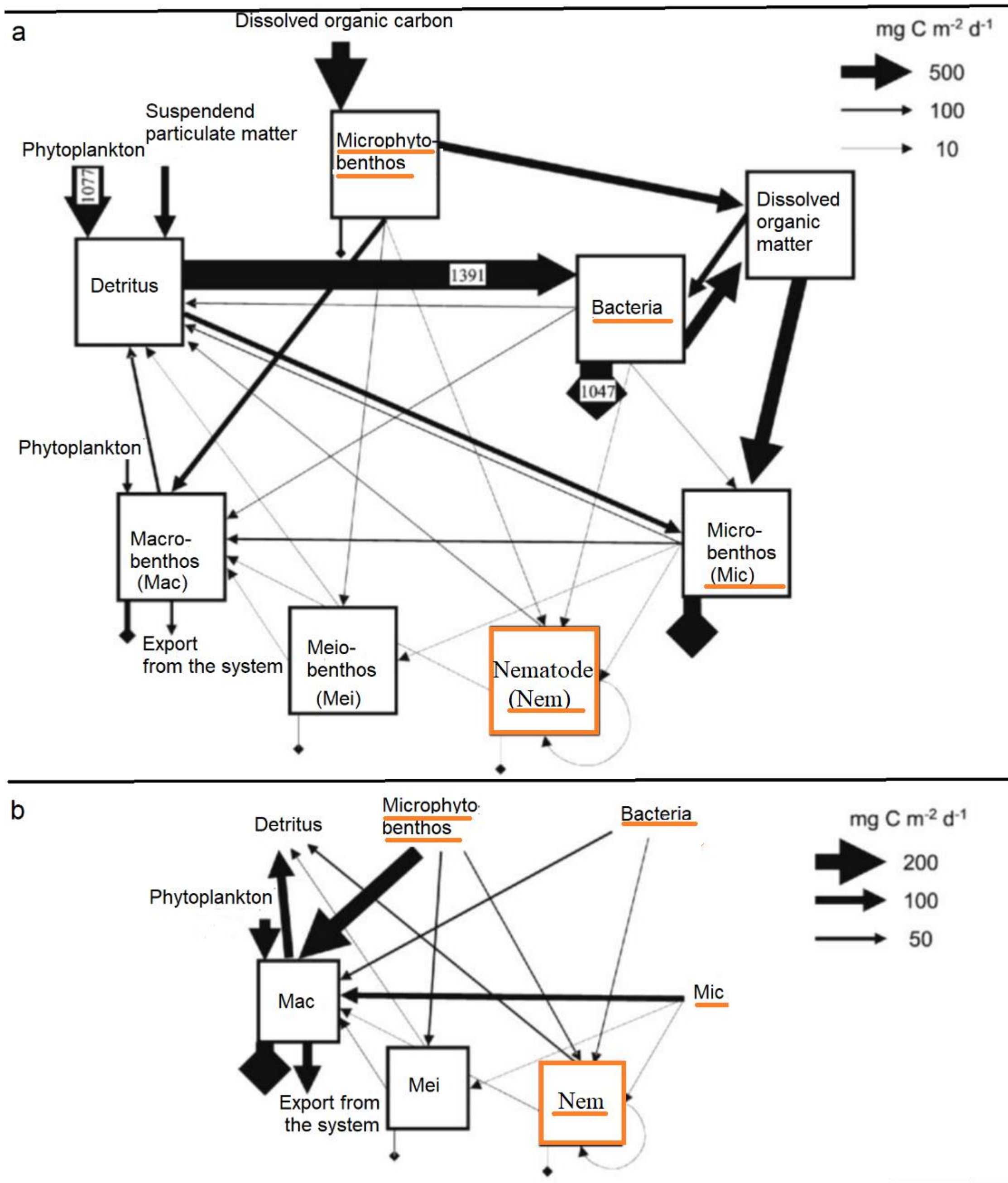


Fig. 1.4. Benthic food web flows at two scales (a, b) on an intertidal flat in the Schelde estuary (Van Oevelen et al., 2006), with carbon input and outflow indicated by arrow and diamond, respectively. Only non-zero flows are pictured. In *a*, the arrows with indicated values are not scaled, because their dominance would otherwise mask the thickness differences among other arrows. The lower panel- *b* shows nematodes, meiobenthos and macrobenthos on a different scale to better indicate the flow structure. Note that the nematode compartment has been highlighted with an orange frame, while the sources yielding inputs into the nematode compartment have been underlined in orange.

The result is that intertidal flats present a large degree of patchiness in environmental conditions, which in turn creates many potentially different microhabitats for the organisms inhabiting tidal flat sediments. Moreover, the bioturbation activity of certain macrofauna, but also biotic interactions –

both top-down and bottom-up – further contribute to this microspatial patchiness and may be important in structuring the biodiversity of these intertidal habitats (Giere, 2009; Gingold et al., 2010). An overview of the main factors that affect the structure and diversity of meiofaunal communities in tidal flats is presented in Fig. 1.5.

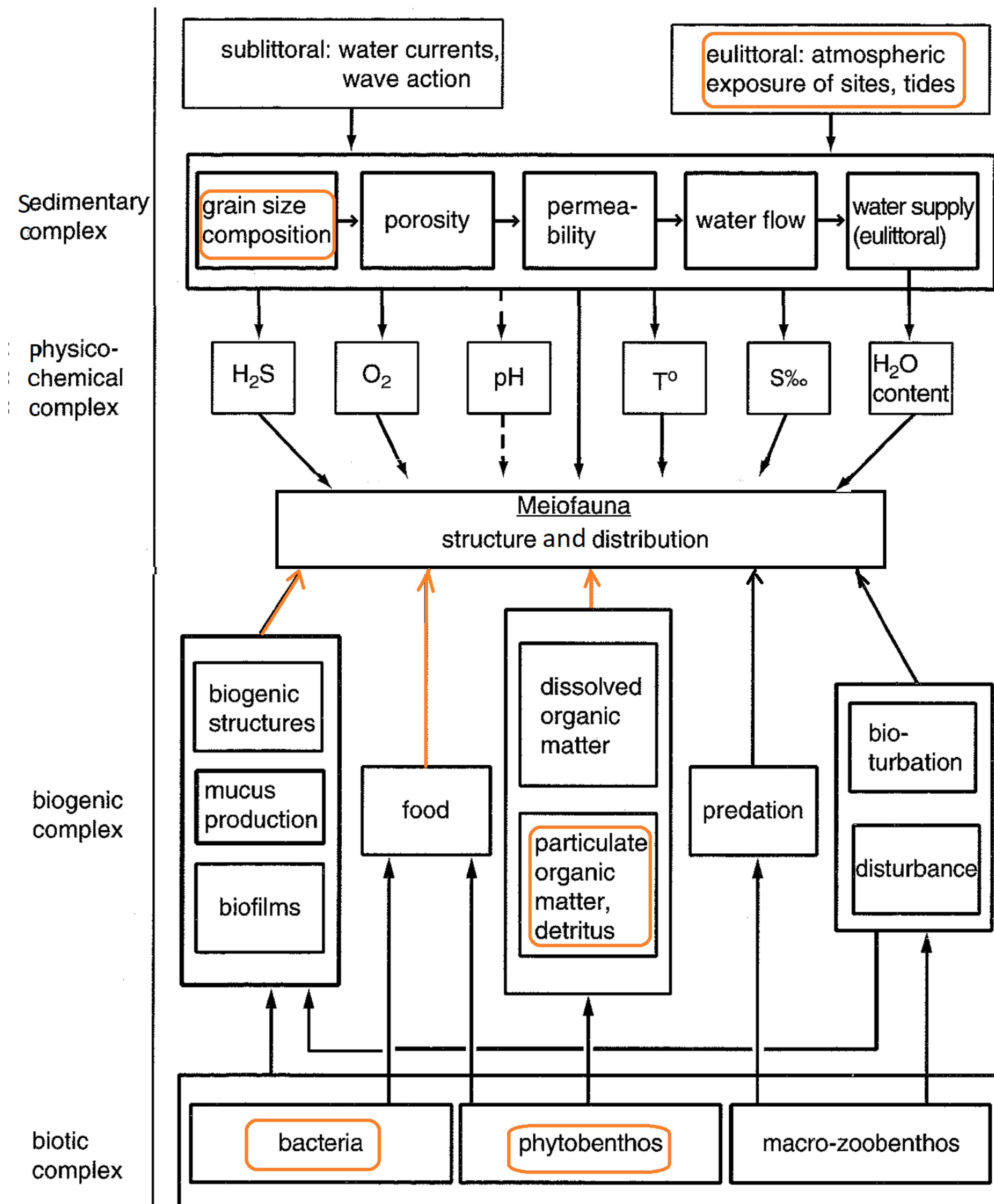


Fig. 1.5 A schematic factorial web of the different abiotic and biotic factors acting on, and structuring meiobenthos communities (Giere, 2009). Boxes and arrows which have been studied in this PhD have been highlighted.

1.3 Nematodes

Nematodes are by far the most abundant benthic invertebrates and cover different ecological functions (Giere, 2009; see 1.3.1). The global species richness of marine nematodes is unknown, with estimates ranging from 10,000 to 1,000,000 (including deep-sea nematodes), depending on the extrapolation methods used (Mokievsky and Azovsky, 2002; Lambshead and Boucher, 2003; Appeltans et al., 2012; Moens et al., 2013). Biodiversity of marine nematode communities is often described at genus level because only ca 15 % of the extant marine nematode species have been properly described (Appeltans et al., 2012). Moreover, these descriptions are based on morphological attributes, and hence overlook the substantial cryptic diversity that exists (e.g. Derycke et al., 2005, 2008a,b, 2010; Bhadury et al., 2006). For example, multiple (> 10) cryptic species of *Litoditis marina* have been observed (Sudhaus, 2011), but although plausible, it remains to be established whether such cryptic diversity is very widespread among marine nematodes. Cryptic species may occupy distinct ecological niches (De Meester et al., 2012; De Meester et al., 2015; Derycke et al., 2016) and have subtle differences in their ecological roles (De Meester et al., 2016).

1.3.1 Nematodes' roles in ecosystems

Nematodes are highly abundant, with densities in intertidal flats ranging from a multiple of 10^5 to more than 10^7 ind m^{-2}; at the same time, their local diversity is generally high, tens of species co-occurring within a single m^2 being a typical value of species richness (Heip et al., 1985; Moens et al., 2013). Moreover, nematodes can also be prey for secondary consumers (such as other nematodes or other meiofauna (e.g. Kennedy, 1994) and macrobenthos). Although nematode biomass standing stock is typically low (0.2-0.5 g carbon m^{-2}) compared to the organic carbon inputs in coastal marine sediments (50-150 g C m^{-2} $year^{-1}$) (Vranken and Heip, 1986; Li et al., 1997; Moens and Vincx, 1997), they may nevertheless represent significant carbon and energy flows because their biomass turnover is considerable larger than that of macrofauna (Kuipers and Dapper, 1984; Li et al., 1997; Coull, 1999). As such, when nematodes are significant grazers of bacteria and diatoms or significant prey to macrofauna, they can act as an important trophic intermediate, linking primary producers and detritus to higher trophic levels" (Giere, 2009). In addition, nematodes may also contribute to a range of other ecosystem processes (see section 1.3.1.2) (Moens et al., 2013). They have therefore been suggested to be important players in tidal flat ecology, leading Platt and Warwick (1980) to the contention that "any general assessment of intertidal habitats is incomplete if the nematode fauna is not taken into consideration" (Platt and Warwick, 1980).

1.3.1.1 Trophic position of tidal flat nematodes

Nematodes represent different trophic strategies and trophic levels (Vranken and Heip, 1986), which serve as prey for predacious nematodes (Moens and Vincx, 1997; Moens et al., 2000). Diatoms and bacteria have long been suspected to be the principal carbon sources for nematodes (Giere, 2009; Moens et al., 2013; Moens et al., 2014), In reality, however, nematodes are not merely 'primary consumers'; they can span multiple trophic levels, and many, if not most, species are omnivorous, meaning they obtain food from more than one (lower) trophic level (see chapter 3 for more information). Nevertheless, information from natural stable isotope abundances of carbon and nitrogen underlines the importance of MPB as a basal carbon source fuelling a majority of nematode species from intertidal flats (Carman and Fry, 2001; Moens et al., 2005a, 2014; Rzeznik-Orignac et al., 2008; Van der Heijden, 2018). Whether the majority of species that obtain this MPB carbon through direct grazing on MPB, or whether most species feed on a trophic intermediate between MPB and nematodes, remains a matter of debate.

In any case, whereas a reasonably good picture exists about the possible food sources for nematodes as a higher taxon, the trophic ecology of individual species, and the role of the environmental context therein, remains largely undiscovered. For lack of other information, the feeding modes and principal food sources of marine nematodes are often 'deduced' from morphological characteristics of their feeding apparatus, especially the size and structure of their mouth (Wieser, 1953) (Fig. 1.6). These 'deductions' have led to a classification of marine nematodes into four feeding types. A primary distinction is made between nematodes with and without a mouth armature such as a tooth, onchia (?) etc. In both groups, two subgroups are recognized. As such, nematodes which lack a mouth armature are classified as deposit feeders and further subdivided based on the size of their mouth opening into: 1A = selective deposit feeders (tiny buccal cavity, allowing ingestion of particles no bigger than bacterial cells) and 1B = non-selective deposit feeders (more spacious buccal cavity, allowing ingestion of considerably larger cells). Nematodes with a buccal armature are further subdivided based on the prominence of this armature and of the musculature of the pharynx in 2A = epistratum feeders (with a relatively small tooth, or denticles or other sclerotized structures which can serve to puncture cells before sucking out the contents, and/or to scrape off cells from a substrate), and 2B = predators/omnivores (with strong tooth/teeth, sometimes supplemented with jaw-like structures, and usually very muscular pharynx). However, this feeding-type classification has several limitations. First of all, it is based on assumptions about the relationship between mouth morphology and feeding mode; these assumptions can be misleading. For instance, the genera *Metachromadora* and *Hypodontolaimus* were considered predators due to the presence of a big tooth and a very muscular pharynx (Wieser, 1953), but evidence from natural stable isotope ratios and some

observations on the feeding behaviour of these genera (Moens and Vincx, 1997; Moens et al., 2014) demonstrate that they are 'epistratum feeders' which use a tooth to pierce or crack (mostly diatom) cells and empty their contents. While *Praeacanthonchus* was previously classified as an epistratum feeder (Wieser, 1953), observations show it ingests diatom cells whole, suggesting it is a non-selective deposit feeder (Moens and Vincx, 1997). Secondly, food sources of different feeding types may show considerable overlap, because Wieser's (1953) feeding-type classification is based on mode of feeding rather than

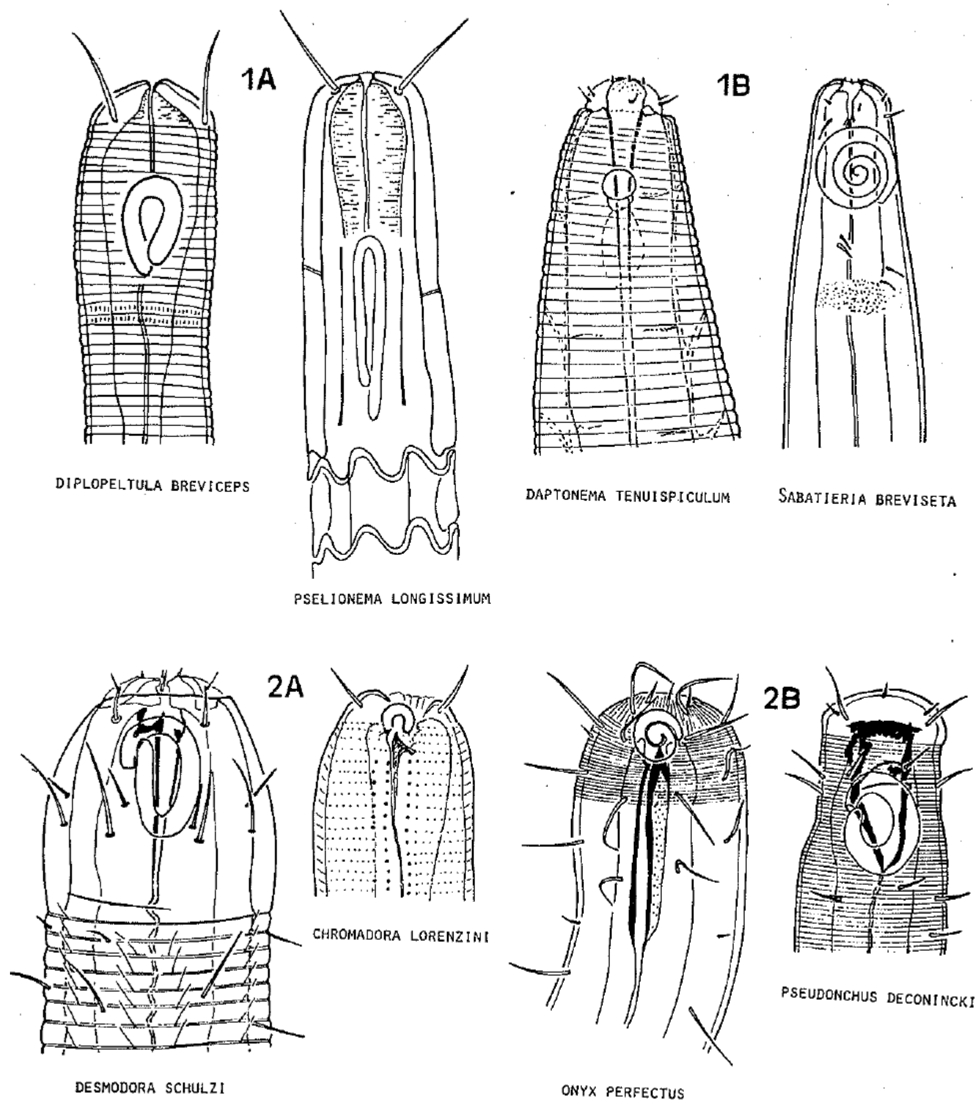


Fig. 1.6 The feeding type classification of Wieser (1953), depicting examples of the four main feeding types: 1A = selective deposit feeders, 1B = non-selective deposit feeders, 2A = epistrate feeders, 2B = omnivores/predators. Image from Heip et al. (1985).

on actual resources. Hence, non-selective deposit feeders and epistratum feeders from tidal flats may both feed predominantly on microphytobenthos as 'unicellular eukaryote feeders' (Moens et al.,

2004). Thirdly, feeding types have been treated too conservatively and restrictively, ignoring potential flexibility in feeding strategies in relation to fluctuations in food availability (Moens et al., 2004). As such, a predatory nematode may, for instance, supplement its diet with – or even largely shift to feeding on – microalgae (Moens et al., 2014). More generally, nematodes may in some cases belong to more than one feeding type, a flexibility the original scheme by Wieser (1953) does not account for.

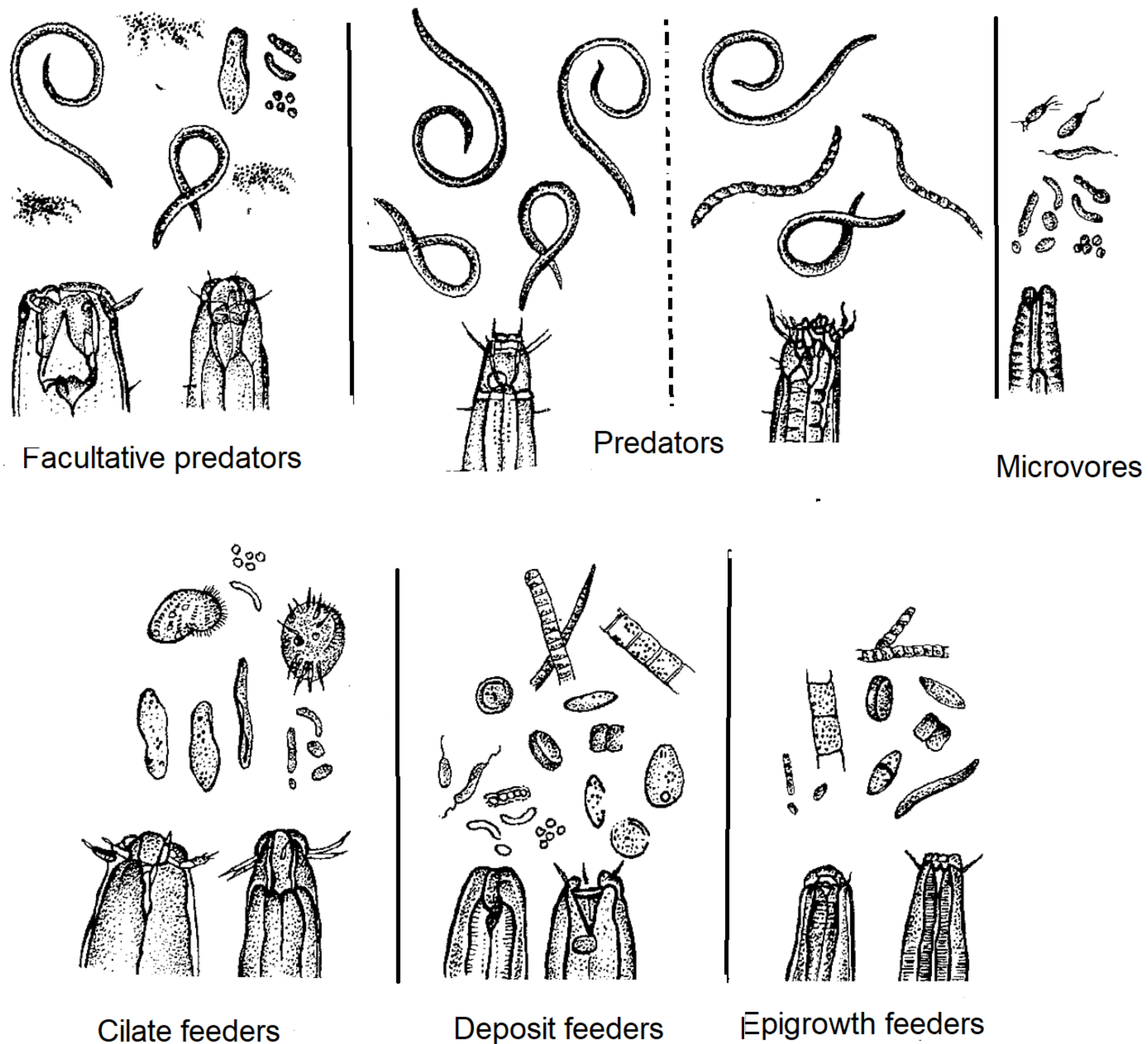


Fig. 1.7 Feeding types and observed food sources for nematodes represented by each feeding type according to the feeding-type classification of Moens and Vincx (1997).

Alternative feeding-type classifications have been proposed, such as the one by Moens and Vincx (1997), which is based on observations on the feeding behaviour of living nematodes (Fig. 1.7). Nevertheless, in the absence of such observations for a majority of marine nematodes, most applications of this scheme also use similarity in mouth morphology to a species for which observations have been published to infer feeding type.

1.3.1.2 Functional roles played by nematodes

Nematodes have the ability to affect the growth and/or activity of bacteria (De Mesel et al., 2003; Blanc et al., 2006; Bonaglia et al., 2014) as well as of diatoms (D'Hondt et al., 2018). This may result from several mechanisms. First, the grazing activity of nematodes may maintain bacterial and/or diatom populations in an exponential growth phase for a longer time (Blanchard, 1991; Epstein, 1997). Second, when feeding on bacteria or microalgae, nematodes may enhance the recycling of nutrients, particularly of N, because the C:N ratio of nematodes is higher than that of bacteria and microalgae (Montagna et al., 1995; Pascal et al., 2013). As a consequence, if food is sufficiently abundant to satisfy the carbon requirements of nematodes, nematodes will take up more N than they need, and they will excrete this excess N, mostly in the form of NH_4^+, which can be taken up by bacteria and microalgae. This mechanism is likely to be important only in soils/sediments with low nutrient concentrations. Third, nematode grazing can be quite selective (Moens et al., 2005b; Estifanos et al., 2013; Weber and Traunspurger, 2013), potentially inducing shifts in microbial and/or microalgal species composition (De Mesel, 2004; D'Hondt et al., 2018), which in turn can affect the activity and functioning of these micro-organisms. Fourth, nematodes can microbioturbate the surficial layers of sediment (Cullen, 1973; Alkemade et al., 1992), thereby stimulating the fluxes of oxygen and nutrients into the sediment (Aller and Aller, 1992; Bonaglia et al., 2014). This can substantially stimulate the activity of bacteria. Finally, nematodes can produce substantial amounts of mucus when they move. Such mucus may transport bacteria, but may also facilitate attachment to, and early colonization of detrital substrates by bacteria (Riemann and Schrage, 1978; Moens et al., 2005b), in some cases potentially involving a remarkable mutualistic relationship (Riemann and Helmke, 2002). Indeed, it has been suggested that some nematode species secrete exo-enzymes which could initiate the decomposition of organic matter, which subsequently facilitates bacterial colonization. Bacteria then take over the decomposition, while the nematodes can feed on the copious ‘soup’ of dissolved organic matter released from the detritus and on the associated bacteria (Riemann and Helmke, 2002). As a consequence of these intricate nematode-bacteria relationships, nematodes can stimulate the decomposition and mineralisation of organic matter (Riemann and Schrage, 1978; Abrams and Mitchell, 1980; Danovaro, 1996), but the opposite, i.e. a general slowing down of OM decomposition, has also been observed (De Mesel et al., 2003; De Mesel et al., 2006; De Meester et al., 2016). Nematodes can also stimulate biomass production (D’Hondt et al., 2018) and EPS production (Hubas et al., 2010), and affect species composition in a diatom biofilm; here too, selective grazing may be an essential mechanism in inducing shifts in biofilm composition (D'Hondt et al., 2018). Given the multiple functions of biofilms in sediment, such as sediment stabilization, nematodes may indirectly play an important role in these ecosystem processes.

1.3.2 Nematodes' distribution

1.3.2.1 Patterns

Nematodes' distributions are strongly dependent on spatio-temporal scales and habitats (see review Moens et al., 2013). According to these authors, studies conducted at one intertidal flat belong to meso- and microscales, whereas research of at least two intertidal flats fall to macroscales. Most studies are performed at one tidal flat and therefore belong to meso- and microscales, with horizontal zonation and aggregated patterns observed, respectively (Blome et al., 1999; Rodriguez et al., 2001; Steyaert et al., 2001, 2003; Gheskiere et al., 2004, 2005; Somerfield et al., 2007; Gingold et al., 2010). There have been rather few studies at macroscales (but see Lee and Riveros, 2012, for sandy beaches), rendering the distributional patterns of nematodes at this scale rather poorly documented. A recent one (Hua et al., 2016) found a low density of nematodes at subtropical tidal beach compared to those at tropical and temperate areas. In addition to the patterns at a horizontal dimension, nematodes are vertically distributed, with most nematodes present in the top sediment layer (mm to 2 cm). Moreover, the horizontal and vertical dimensions both have structuring effects on nematode communities which do not only act in isolation, but may also interact (Somerfield et al., 2007; Vieira and Fonseca, 2013). For instance, the horizontal patchiness in MPB biofilms will likely affect the vertical distribution of oxygen in the sediment. These distribution patterns are linked to various factors, including abiotic (e.g. water currents) and biotic (e.g. trophic interactions) factors, physical and chemical factors (e.g., oxygen concentration, organic matter content, tidal exposure) in sediment matrix. These factors create heterogeneous environments where nematodes reside, leading to different nematode distribution patterns. Here, we describe some of the well-recognised main factors and classified them in three groups: sediment granulometry, food availability/quality and other factors such as hydrodynamics and water movements at tidal flats.

1.3.2.2 Drivers

1.3.2.2.1 Sediment granulometry

Grain size has been considered particularly important. Firstly, it has been considered as a key structuring factor (Heip et al., 1985; Giere, 2009; Moens et al., 2013), due to its direct determination of spatial and structural conditions and indirect determination of the physical and chemical features in sediments (Giere, 2009). Relatively low nematode diversity is usually found in sediment with a high content of clay and detritus, and high values appear in coarser sediments (Heip et al., 1985). Secondly, it may affect nematode density by influencing the nematode feeding by scraping material off sediment particles (Hodda, 1990). For instance, at sandy beaches, nematode density tends to increase in finer sediments, with the lowest values being observed in exposed, coarse beaches (Heip et al., 1985). This is mostly because finer sediments accumulate more OM and therefore have higher food availability.

Additionally, a small shift in sediment composition can already result in a strong effect on nematodes, such as the predacious genus *Enoploides*, which loses much of its ability to capture nematode prey when sediments become siltier (Moens et al., 2000; Gallucci et al., 2005). Given the major impact of predatory nematodes on their prey assemblages, even subtle shifts in sediment granulometry can potentially translate in significant changes in nematode community abundance and structure.

1.3.2.2.2 Food availability/quality

Food sources for nematodes range from diatoms and other microalgae to heterotrophic protists, bacteria and various forms of carnivory (both scavenging on dead animals and predation on living prey) (Jensen, 1987; Moens and Vincx, 1997).

Qualitatively, fresh sources, such as diatoms and other microalgae, indicated by the pigments diatoxanthin and β-carotene (Wright and Jeffrey, 1997; Peters et al., 2005), separately, are better than their degraded forms (indicated by pheophytin a), and both are better than faeces (indicated by pyropheophytin *a*).

Quantitatively, decreased concentrations of these pigments were observed with increased sediment depth (Evrard et al., 2008). However, these differences were not revealed in seized variations in nematode composition among different sediment depths. This may partly be because of a broad range of food sources for nematodes and the ability to selectively feed of nematodes, which enable nematodes to use their preferred sources efficiently. Some nematodes have the ability to migrate towards their preferred food sources. A migration of *Sabatieria* to freshly deposited phytoplankton has been observed in fine sediments (Franco et al., 2008a). Availability of phytodetritus was observed to increase density of deep-sea meiofauna and species number of nematodes (Fonseca and Soltwedel, 2007). Appearance of 'stout' nematode assemblages characterised by low length/width (<15) was observed in response to a large amount of phytodetritus input at one coast station with a depth of 20m (Vanaverbeke et al., 2004).

Diatoms/algae, as the dominant microphytobenthos, have been often linked to small scale variation in nematode distribution, due to the overlapping microscale distribution patterns of them, and largely relate to nematode feeding preferences (Montagna et al., 1983; Blanchard, 1990; Blanchard, 1991). Microphytobenthos, which often contribute a lot to the total primary production (MacIntyre et al., 1996; Underwood and Kromkamp, 1999), have been suggested to play a central role in moderating the carbon flow in intertidal sediments (Middelburg et al., 2000) and are an important carbon source for the benthos (Herman et al., 1999). Additionally, they have been well-known for stabilizing surface sediments through the production of extracellular polymeric substances (Paterson and Black, 1999). Effects of diatoms on nematodes can go through direct grazing, or more indirectly through predation

on MPB grazers (Moens et al., 2014; Wu et al., chapter 3 of this PhD). In any case, MPB is a major carbon source for tidal flat nematodes as inferred from natural stable isotopes of carbon and nitrogen (Carman and Fry, 2001; Moens et al., 2005; Rzeznik-Orignac et al., 2008; Van der Heijden, 2018). What is less well known is which nematodes can feed on MPB and how. For instance, one could assume that for nematodes which ingest diatoms whole (deposit feeders), size of the diatom cells would be a more limiting factor than for nematodes which use a tooth to puncture diatoms and empty them. One could also assume that larger nematodes can automatically consume larger cells. This is not entirely true, because the feeding mechanism also plays a role (Moens et al., 2014). For instance, small Chromadoridae still fed proportionally more from large diatom cells than did some larger nematodes (Moens et al., 2014).

Zooplankton faeces may largely release from both deposit and suspension feeders (Turner, 2002). Faeces have been considered as a poor direct food source for benthos. However, it can contain high proportions of undigested, protein-rich matter, like undigested diatom cells. They may be biochemically or trophically upgraded by bacteria, which can mobilize nitrogen from the surrounding water and therefore use energy from nitrogen-poor organic compounds from the faecal pellets to biosynthesize new proteins; in addition, the bacteria associated with faeces may be grazed upon by nematodes (Valiela, 1984).

However, food sources can alter sediment composition, through the density of extracellular polysaccharides produced by diatoms and their associated bacteria to bind and trap sediment particles and even form cohesive surface biofilms that ultimately protect the sediment surface against erosion (Kromkramp et al., 2016). Hence, microphytobenthos and the trophic interactions among nematode species can modify the effect of MGS (median grain size) on nematode communities. A previous study has observed species richness and diversity of nematode assemblages did not differ in muddy and sandy sediments and concluded that sediment granulometry or trophic differences alone could not be the only determining factors after a comprehensive evaluation of literature data (Boucher, 1990).

1.4 Microbiomes

It is well-known that intertidal flat sediments are partly shaped by the benthos, MPB and their interactions through several processes (Raffaelli and Hawkins, 1999; Van De Koppel et al., 2001; Passarelli et al., 2012). However, increasing evidence shows that microorganisms are also key drivers in many of the relevant processes (Hicks et al., 2018). Microbes are everywhere, with an estimated global abundance of up to 10^{30} cells (Turnbaugh and Gordon, 2008). The term microbiome is used to refer to the collective genomic content of the microbiota associated with a habitat and/or a living organism (Tremaroli and Backhed, 2012). In the present study, we use the term microbiomes to refer

to all the microbial taxa/Operational Taxonomic Units (OTUs) detected by sequencing the V4 region of the 16s rRNA gene of an individual organism/host or of an environmental DNA sample using high throughput sequencing (HTS). The former is called a host-associated microbiome, while the latter is an environmental microbiome.

1.4.1 Host associated microbiomes and environmental microbiomes

Host-associated microbiomes include the gut microflora, endo- and ectosymbionts and (remains of) ingested bacteria (Derycke et al., 2016). Microbiomes in general have been receiving increasing attention, at least partly thanks to methodological progress in the development of high-throughput sequencing technologies (e.g., IIlumina HiSeq and MiSeq platforms). These technologies now allow the generation of very extensive sequence data at affordable costs and in a very short time (Caporaso et al., 2012). In addition, the increased interest for host-associated microbiomes relates to the fact that microbiomes appear to be involved in many important aspects of the 'functioning' and 'fitness' of their hosts, such as the health situation and immune system of the host (Costello et al., 2012). For example, specific aspects of human health are associated with specific bacterial groups (Cho and Blaser, 2012; Greenblum et al., 2012), which could only be revealed through studies of the human-associated microbiomes.

Environmental microbiomes are generally quite dynamic, and can rapidly change between stations and seasons (Rusch et al., 2001; Schulenburg and Félix, 2017). To what extent this also applies to host-associated microbiomes is less clear. Some prokaryotic taxa have the ability to physiologically adapt to changing conditions, which enables them to survive in unfavorable environments, be they in the environment or in a temporary or permanent host (Schulenburg and Félix, 2017). Recent studies stress the importance of environmental microbiomes, as these tend to differ from the host-associated microbiomes (Samuel et al., 2016; Schulenburg and Félix, 2017; Zhang et al., 2017).

1.4.2 Nematode associated microbiomes (NAM)

Studies on host-associated microbiomes have only recently included nematodes as hosts. Most studies have hitherto focused on terrestrial free-living and/or parasitic nematodes, with no more than two studies so far on marine free-living species (Derycke et al., 2016; Schuelke et al., 2018). Nematode microbiomes (NAM) vary among host nematode species and their feeding strategies, and – in case of parasitic nematodes – may also depend on the host of the nematodes (Baquiran et al., 2013; Alves et al., 2016; Derycke et al., 2016; Samuel et al., 2016; Elhadyl et al., 2017; Schuelke et al., 2018). In animals, the microbiome comprises both a microbiome *sensu stricto*, i.e. all bacteria living in or on an animal host, from pathogens to mutualists, and a microbiome *sensu lato*, i.e. the bacteria taken up as food or attached to food organisms (Derycke et al., 2016; Schulenburg and Félix, 2017). And therefore,

some nematode-associated bacteria form part of a beneficial gut microflora, whilst others may negatively impact nematodes through the production of toxic metabolites, the expression of virulence factors etc., potentially affecting host physiology, metabolism, immune responses and behaviour in multiple ways (Samuel et al., 2016; Schulenburg and Félix, 2017; Zhang et al., 2017).
Most studies to date have focused on only one or a few species with a known or potentially important ecological function, such as the free-living model species *Caenorhabditis elegans* and the animal and plant-parasitic species *Haemonchus contortus* (Sinnathamby et al., 2018) and *Pratylenchus penetrans* (Elhady et al., 2018), respectively. *Haemonchus contortus* associated microbiomes varied with nematode life stages and encompassed the prominent presence of endosymbionts (*Weissella* and *Leuconostoc*) (Elhadyl et al., 2017; Sinnathamby et al., 2018). In *C. elegans*, the nematode-associated microbiomes differed from those of their immediate environment (i.e. their native soil) (Schulenburg and Félix, 2017; Zhang et al., 2017).

As mentioned above, in marine nematodes, studies of nematode-associated microbiomes have hitherto been restricted to two publications (Derycke et al., 2016; Schuelke et al., 2018). The former study investigated the microbiomes of three congeneric species of the morphospecies complex *Litoditis marina*, a marine representative of the same nematode family as *C. elegans,* and demonstrated highly species-specific differences. Bacteria are the main food source of *L. marina* and part of the species-specific differences in the microbiomes of these cryptic species undoubtedly reflect differences in resource selectivity. Nevertheless, a large part (ca. 50 %) of the microbiomes of these species could not be directly linked to nematode diet and belonged to the microbiome *sensu stricto* (Derycke et al., 2016). Schuelke et al. (2018) conducted their study at multiple habitats, analysing the microbiomes of 281 nematodes covering 33 genera as well as all nematode trophic groups. Surprisingly, these authors did not find clear differences in nematode-associated microbiomes between different geographical areas, marine habitats nor between nematode feeding types; moreover, host phylogenetic relationships did not explain the extent of the observed differences in microbiomes between nematode genera. However, the dataset did reveal a variety of new ecological interactions, including putative symbionts and parasites as well as associations with prokaryotic taxa involved in methane and nitrogen cycling. Furthermore, environmental microbiomes may affect host microbiomes, causing variability at much smaller scales than the habitat and geographic scales in the study by Schuelke et al. (2018). This is supported by observations on the variation in host microbiomes in two species of soil-inhabiting nematodes, which differ substantially among locations and even microhabitats (Elhadyl et al., 2017; Schulenburg and Félix, 2017). Ectosymbiotic bacteria have been observed in marine nematodes belonging to the Stilbonematidae (Blome and Riemann, 1987; Polz et al., 1992) and endosymbionts in the mouthless *Astomonema* (Musat et al., 2007) (Siphonolaimidae,

Desmodorida). Stilbonematidae move up and down across the redox potential discontinuity layer in certain marine sediments, thus exposing their ectosymbionts sequentially to oxygenated and sulphidic environments (Polz et al., 1992), allowing them to oxidize chemolithotrophic sulfide (Schiemer et al., 1990). In turn, members of the genus *Astomonema* contain endosymbiotic sulphur-oxidizing bacteria in a 'blind' gut pouch (Musat et al., 2007). In both, Stilbonematidae and *Astomonema*, the symbiotic bacteria probably provide nutrition for their hosts (Ott et al., 1991), although the exact way they do this still remains under debate.

1.5 Study objectives and outline of this PhD thesis

1.5.1 Study objectives and outline of this thesis

The *in situ* productivity of microbial biofilms fuels a major part of the secondary production on many estuarine intertidal flats. These biofilms are complex consortia of benthic microalgae and heterotrophs embedded in a biogenic polymer matrix. They play key roles in a range of important ecosystem functions, such as sediment stabilization and water quality improvement. Nevertheless, several unknowns still exist about the complex interplay between microphytobenthos (MPB), prokaryotes and benthic invertebrates in microbial biofilms on tidal flats.

Their interactions with biofilm-forming organisms may affect the activity and community structure of both MPB and of sediment bacteria, thereby potentially affecting some of the ecosystem processes mediated by these micro-organisms. The high local-scale species diversity of nematodes has puzzled ecologists for decades. Differential resource use is often invoked as a basis for niche differentiation among species, yet the vast majority of studies demonstrating that this would be prominent in marine nematodes are based on laboratory experiments on single species or on highly simplified assemblages, leaving the issue of resource differentiation under natural conditions rather understudied until present.

In order to improve our understanding of the functional roles of nematodes in tidal flat sediments, it is imperative that both trophic and non-trophic interactions among nematodes, and between nematodes and biofilm-forming organisms, are documented and understood. The overarching goal of this PhD was therefore to **elucidate trophic relationships between nematodes and microphytobenthos and bacteria** on an intertidal flat.

Because one needs to know patterns before one can understand the underlying processes, we first set out to **describe the horizontal and vertical distribution of nematode communities on an intertidal flat in relation to a number of potential drivers**, including sediment granulometry and intertidal position, but with a major focus on food/biofilm-related drivers (**chapter 2**). Sampling was performed

on two different spatial scales: on a mesoscale, with ten stations covering a replicated gradient in tidal regime (high, mid and low) and spanning different sediment grain sizes, while five additional stations were sampled on a 'microscale', with interdistances < 50 m, including stations with visibly different biofilm growth at the sediment surface at the time of sampling. We quantified the microphytobenthos and its different components by analyzing the phytopigment concentrations of the sediments. In accordance with the general ideas described above, we hypothesized that specific food sources, as represented by different pigments, would be important drivers of nematode assemblage composition at the microscale, while grain size and tidal level would be more important drivers at the mesoscale. We further addressed the following specific questions: (1) Which environmental variables relate best to the horizontal patterns in nematode community composition at meso- and microscale, and (2) are similar horizontal patterns observed at different depth layers?

We subsequently selected nine common nematode species, representing different feeding guilds, from this intertidal flat and used natural stable isotope ratios of carbon and nitrogen, as well as fatty acid profiles, to **elucidate resource use, resource partitioning and trophic structure** of these nine species (**chapter 3**). Firstly, we assessed the trophic level of several nematode species which are presumed to be mainly consumers of MPB and of others which are known as predators of other nematodes, and evaluated the hypothesis that these represent clearly separate trophic levels, i.e. primary and secondary consumers, respectively. Inherent to this hypothesis is the alternative hypothesis where omnivory is common in tidal-flat nematodes and nematodes do not occupy discrete and nicely distinct trophic levels. Secondly, our sampling comprised multiple species each that under the previous hypothesis would classify as primary and secondary consumers, allowing us to test the degree of resource partitioning among species that presumably belong to the same trophic level. We used isotopic niche spaces as well as multivariate analysis of fatty acid profiles to assess this concept. Thirdly, we used fatty acid biomarkers to investigate the contribution, if any, of hitherto poorly documented resources such as dinoflagellates and zooplankton (dead and/or faecal pellets) in the diet of intertidal nematodes.

Because the 'biomarker' approaches of chapter 3 do not adequately address trophic **relationships between nematodes and bacteria**, in **chapter 4** we used next-generation sequencing on individual nematode specimens to analyse the microbiomes (i.e. the bacteria on and inside nematodes, including all kinds of symbioses as well as remnants of bacteria ingested as food) of the three nematode species which in chapter 3 proved most dependent on microphytobenthos. We investigated whether nematode microbiomes were a random subset of the microbiomes of the environment they inhabit, or rather indicated specific nematode-bacteria relationships. We assessed whether the nematode microbiomes were species-specific, and whether and to what extent they varied spatially (two stations

with contrasting sediment properties) and temporally (three subsequent seasons). We also investigated whether any species-specific differences could be related to differences in the feeding ecology of these species. Although host microbiomes cannot be unambiguously interpreted as reflecting diet (and diet alone), we hoped to find indication of the feeding ecology of these three nematode species as well as to their non-trophic relationships with bacteria. Microbiomes are in many ways relevant to the fitness of organisms, and as such may have diverse consequences for their ecology.

Finally, in **chapter 5**, we integrate some of the main findings of the different chapters, and provide an outlook to future research perspectives to further enhance our understanding of the roles of nematodes in tidal-flat ecosystem functioning.

1.5.2 Study area and model species

Samplings were conducted at the Paullina polder intertidal flat in the polyhaline reach of the Schelde estuary (Fig. 1.7), a temperate macrotidal estuary situated across the Dutch-Belgian border. The

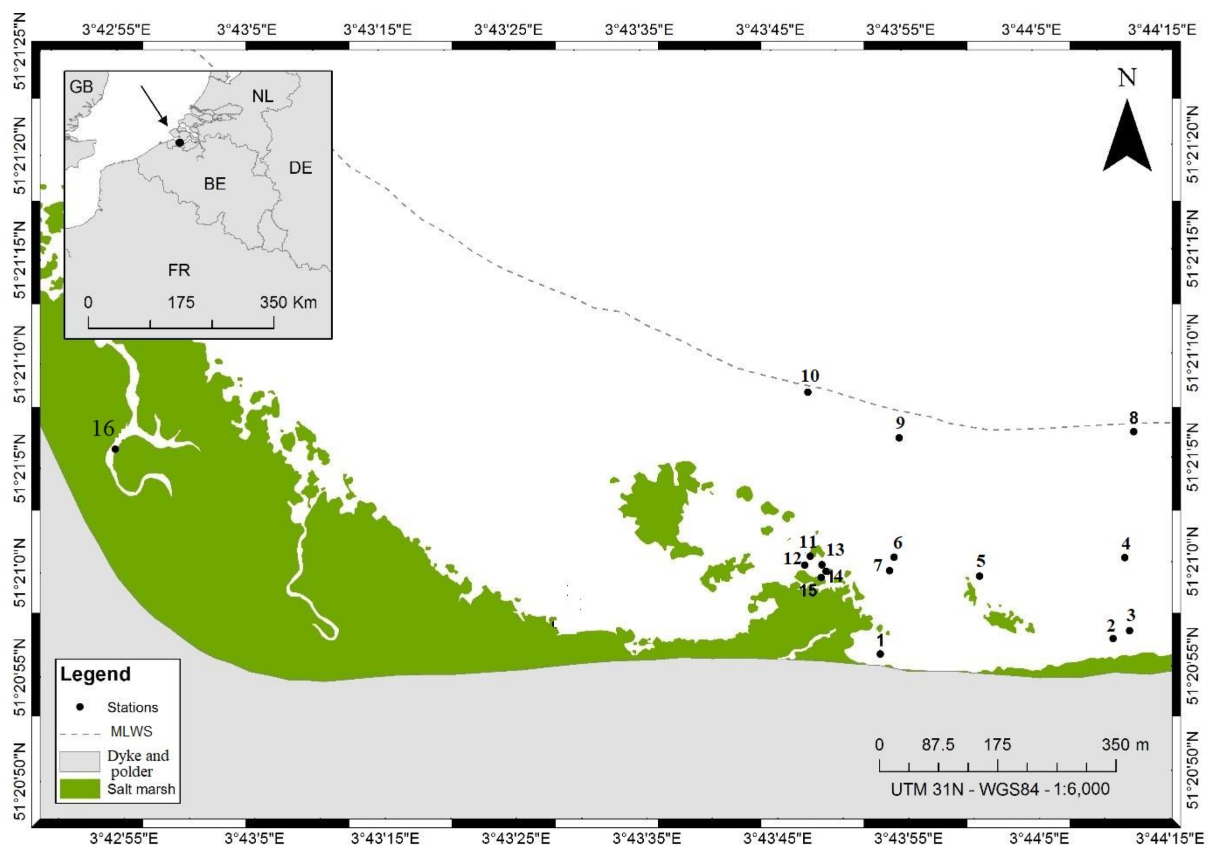


Fig. 1.8 Location of sampling stations (indicated by numbers) covering meso- and microsclae variation at the Paulina intertidal flat, Schelde estuary, the Netherlands. Mean low water spring tide level was indicated by MLWS, high water spring tide level coincided with the position of the dyke.

estuary has a fully oxygenated water column and is bordered by extensive intertidal flats and several (mostly small) salt marshes (Meire et al., 2005), the Paulina intertidal flat being one such tidal flat system with a salt marsh. The Paulina intertidal flat covers ca. 2 km^2 (including the salt marsh), with an average tidal range of ca. 4.1 m (Gallucci et al., 2005). Its sediments range from silty to medium sandy and from bare to vegetated (salt marsh); microphytobenthos biofilms can be very prominent on the bare tidal flats, particularly where sediments are silty.

Our sampling stations are within a 1-km^2 area of the tidal flat (Fig. 1.8). Most stations are located in the bare tidal flat just upstream of the salt marsh and amidst the most upstream (scattered) vegetation patches. In this area, sediment granulometry tends to comprise mostly fine sands with little or no silt in the more upstream, upper intertidal stations, and very fine sands with a substantial silt fraction in the low-tide stations and in close proximity of the vegetation patches. One station (st16) was located in a major drainage gully of the marsh and has the finest sediment of all stations. Stations 1 to 15 were studied in the framework of chapter 2. In chapter 3, most samples were obtained from stations 1 and 6, whereas one species was collected from st16. Finally, nematodes for chapter 4 were collected from stations 1 and 16 (note, the name of stations may change in other chapters). Chapter 2 performed community analyses at genus level resolution. Eight common and easily identifiable species from this tidal flat, and a ninth 'enigmatic' species where information on feeding ecology and trophic level has been completely missing, collected in the marsh gully, were studied in chapter 3. Three of those, all with a trophic level equal or very close to 2 (primary consumers), were the model species for chapter 4.

Chapter 2 Environmental drivers of spatial patterns of nematode abundance and genus composition at two spatial scales on an estuarine intertidal flat

Submitted to Hydrobiologia by Xiuqin Wu, Ann Vanreusel, Freija Hauquier and Tom Moens

Chapter 2 Environmental drivers of spatial patterns of nematode abundance and genus composition at two spatial scales on an estuarine intertidal flat

Abstract

Estuarine intertidal flats are biologically rich ecosystems. We studied spatial patterns and drivers of nematode density and genus composition at two different spatial scales (i.e. meso- and microscale), with drivers including sediment granulometry (median grain size, % silt), inundation period and food quality/quantity as indicated by various phytopigments. The mesoscale included 10 stations covering three different intertidal positions, while the microscale included 5 stations at one intertidal level with interdistances of < 50 m. Our results revealed mesoscale zonation and patchiness patterns at microscale. These patterns are more pronounced in the surface layer than the deeper layers. In terms of drivers, these patterns are both driven by food quality/quantity indicated by several pigments; grain size and tidal level are important too at least in the surface layers of the sediment, while their assumed larger importance at the mesoscale is not outspoken. Our study indicates that the microphytobenthos is an important driver for the nematode community.

Keywords: nematodes, abundance, genus composition, spatial patterns, tidal flat, microphytobenthos, sediment granulometry

2.1 Introduction

Estuarine intertidal flats are important ecosystems, as they are sites of intense primary production, organic matter decomposition and nutrient cycling (Herman et al., 2001). Moreover, surface sediments in intertidal areas are highly variable in terms of *in situ* primary production and decomposition, associated with strong environmental gradients and resulting in a high patchiness at different spatial scales (Joint et al., 1982; Hodda, 1990). Although the importance of microphytobenthos (MPB) for the functioning of shallow estuarine ecosystems has been well established (Heip et al., 1995), its relationships with benthic fauna are less well understood. Microalgae living in the top few millimeters of the sediment are consumed by benthic invertebrate fauna (Middelburg et al., 2000), but to what extent the microalgal biodiversity and biomass are linked to the community composition and biodiversity of the benthic fauna remains largely unclear until today.

Nematodes are numerically by far the most abundant invertebrate taxon in almost all marine and estuarine benthic habitats (Heip et al., 1985). In addition to being highly abundant, their communities

tend to be species-rich (Heip et al., 1985; Moens, et al., 2013) and trophically diverse (Wieser, 1953). Although the ecological significance of tidal flat nematodes has repeatedly been stressed (Platt and Warwick, 1980; Coull, 1999; Moens et al., 2013), considerable uncertainty remains about the precise mechanisms through which nematodes affect tidal flat ecosystem processes as well as about their quantitative importance. Progress in our knowledge of ecological processes requires a thorough understanding of the underlying ecological patterns (Underwood et al., 2000). Hence, important ecosystem properties such as primary production, bioturbation and organic matter mineralization may be strongly related to the (distribution) patterns of benthic fauna (Hooper et al., 2005). As an example, it was recently demonstrated that an estuarine intertidal nematode assemblage stimulated the biomass production of a mixed-species diatom biofilm, while having the opposite effect on a single-species diatom biofilm (D'Hondt et al., 2018).

Generally, patterns in nematode community abundance and composition are strongly dependent on multiple drivers that act at different spatial and temporal scales and dimensions (Moens et al., 2013). At spatial scales of meters to a few kilometers (mesoscale), physical-chemical properties of sediments, such as salinity, sediment grain size and tidal exposure, are often invoked as the principal drivers of nematode community patterns (Heip et al., 1985; Steyaert et al., 2003; Gheskiere et al., 2004). Among these sediment characteristics, grain size has been considered particularly important (Heip et al., 1985; Giere, 2009; Moens et al., 2013), due to its direct determination of spatial and structural conditions, but also its indirect effects on physical and chemical features in the sediment (Giere, 2009). Its reflection in nematode distribution patterns has been observed in several studies, for instance through a tendency for lower nematode diversity in fine sediments with a high content of clay and organic matter, while higher diversity values often appear in coarser sediments (Heip et al., 1985); or at sandy beaches, where nematode density tends to increase in finer sediments, with lowest values being observed in very exposed coarse sandy beaches (Heip et al., 1985).

Tides additionally cause substantial variation in hydrodynamics, sediment temperature, water content and oxygen concentration, which in turn can generate specific horizontal distribution patterns (Evans, 1965; McLachlan and Jaramillo, 1996; Armonies and Reise, 2000; Le Hir et al., 2000; Herman et al., 2001; Gheskiere et al., 2004). However, whether these horizontal patterns are similar at different vertical sediment layers has rarely been studied (Somerfield et al., 2007; Vieira and Fonseca, 2013). One study concluded that there is less variability in abundance, diversity and composition of nematode communities from the top oxygenated layers compared to communities from the reduced subsurface layers (Vieira and Fonseca, 2013). According to these authors, the deeper reduced sediments are inhabited by a set of tolerant species that are more restricted in their mobility, while the surface fauna has a higher chance of being resuspended, resulting in a wider distribution and less

spatial variability. At smaller spatial scales (microscale: cm to a few m), nematodes on intertidal flats tend to exhibit aggregated distribution patterns (Findlay, 1981; Gingold et al., 2011; Boldina et al., 2014), caused mainly by biotic interactions such as the distribution of food (Steyaert et al., 2001; Vanaverbeke et al., 2008), the activity of macrofauna and/or anthropogenic or other disturbances (Braeckman et al., 2011a, b). The spatial correlation between patches of microphytobenthos and of nematodes suggests they are causally related or constrained by a common (set of) driver(s)(Steyaert et al., 2003; Moens et al., 2013). Stable-isotope studies have demonstrated that at intertidal flats, most nematode species appear to be fuelled by microphytobenthos carbon (Moens et al., 2002; Moens et al., 2005a; Rzeznik-Orignac et al., 2008) although it is not always clear whether they obtain this carbon directly, by grazing on microalgae, or indirectly, via trophic intermediates such as bacteria, heterotrophic protists and/or herbivorous invertebrates (Moens et al., 2014; Vafeiadou et al., 2014).

Despite the clear influences of granulometry, tidal action and food availability on nematode communities, it is often unclear which fraction of the total variability in nematode distribution on estuarine intertidal flats is captured at different spatial scales by each of the different environmental factors. Estuarine tidal flats often combine a high *in situ* primary productivity by microphytobenthos with the deposition of substantial amounts of particulate organic matter from the water column, resulting in a potentially high food availability. While food availability as a driver of nematode community abundance and composition at the microscale has received considerable attention (Hodda, 1990), its importance at the mesoscale remains less well understood in intertidal flats. Moreover, many studies have used chl*a* concentration and TOM as bulk measures of the availability of primary producer biomass and organic matter (Steyaert et al., 2003; Gheskiere et al., 2004), respectively, for benthic invertebrates. However, MPB biofilms and settled phytoplankton, while often dominated by diatoms, may comprise a variety of microalgal taxa, the importance of each of which as a food source for nematodes remains to be established.

This study aims to investigate the importance of sediment granulometry, inundation period and food quality and quantity on nematode distribution at an intertidal flat in the Schelde Estuary, SW Netherlands. Sampling was performed at two different spatial scales: meso- and microscales (see 2.2.1). In accordance with the general ideas described above, we hypothesized that specific food sources, as represented by different pigments, would be important drivers of nematode assemblage composition at the microscale, while grain size and tidal level would be more important drivers at the mesoscale. We further addressed the following specific questions: (1) Which environmental variables relate best to the horizontal patterns in nematode community composition at meso- and microscale, and (2) are similar horizontal patterns observed at different depth layers?

2.2 Material and Methods

2.2.1 Study area and sampling

Sampling was conducted at the Paulina polder intertidal flat, which is located in the polyhaline reach of the Schelde estuary (see Meire et al., 2005), a temperate macrotidal estuary situated along the Dutch-Belgian border. The Paulina intertidal flat covers ca. 1 km^2 (excluding salt marsh) and is subject to semidiurnal tides with an average tidal range of ca. 4.1 m (Gallucci et al., 2005). It is characterized by a substantial variability in sediment properties, with granulometry ranging from very fine sands with high proportions of silt on the western side (where the tidal flat borders a salt marsh) to medium sands devoid of silt on the eastern, most upstream side (Gallucci et al., 2005). This east-west gradient is, however, complicated by additional variability depending on the intertidal position of stations and

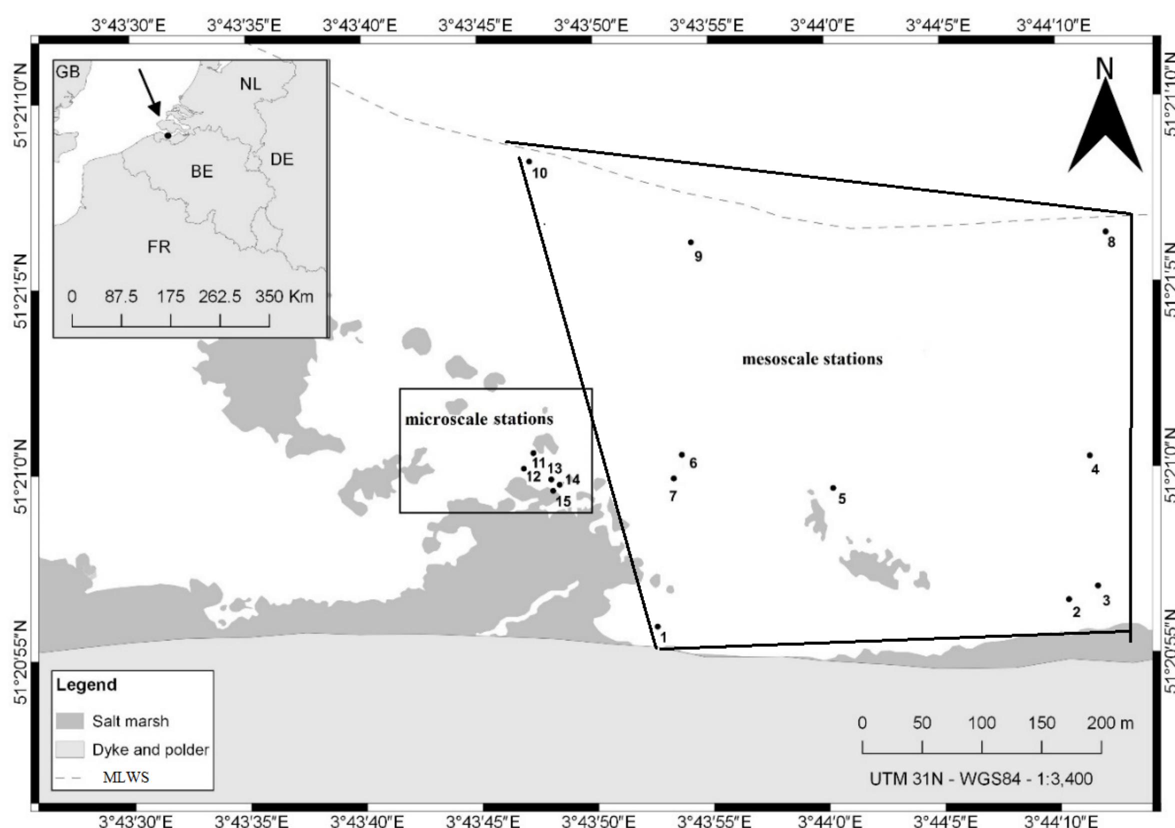


Fig. 2.1 Location of sampling stations (indicated by numbers) covering meso- and microscale variation at the Paulina intertidal flat, Schelde estuary, the Netherlands. Mean low water spring tide level was indicated by MLWS, high water spring tide level coincided with the position of the dyke.

by the presence of vegetation (a salt marsh on the western side, with scattered vegetation patches extending more eastward). As a consequence, some parts of this tidal flat exhibit a mosaic of patches which vary substantially in sediment characteristics at scales of tens of meters (Gallucci et al., 2005).

Sampling was primarily conducted to cover mesoscale spatial variability, from east to west (but not entering the silt-rich western part of the tidal flat) and from high to low-tide level. Three (stations 1,

2 and 3), four (stations 4, 5, 6 and 7) and three (stations 8, 9 and 10) stations were located at the high, middle and low-tide level, respectively. Stations at a particular tidal level were not equally spaced (Fig. 2.1), mostly to avoid local anthropogenic impacts (mostly from digging for *Arenicola marina*, which is used as life bait in recreational fishing, at the mid-tide level, and from tourist activities at the high tide level). In addition, five more stations with interdistances smaller than 50 m were chosen near the mid-tide level at the easternmost vegetation (*Spartina*) extensions of the salt marsh. This area displays a high variability in sediment characteristics, particularly in biofilm cover. These stations (11, 12, 13, 14 and 15) are henceforth referred to as the 'microscale stations'. At each station, we also investigated vertical variability, by sectioning cores in three depth layers: 0-2, 2-4 and 4-6 cm. Sampling was performed on June 12th 2012.

At each station, four replicate samples for nematode community analysis were taken using a cylindrical plexiglas corer of 10 cm^2 surface area to a depth of ≥ 8 cm. Replicates were taken within 5 m of the station 'midpoint' identified by its GPS location. Two additional sets of 4 replicates each were taken within the same 5-m radius at each station for sediment granulometry and phytopigment analysis, respectively. Every sediment core was sliced per 2 cm down to 6 cm, yielding 3 sediment layers (0-2 cm, 2-4 cm, and 4-6 cm). Nematode samples were preserved in 4% formaldehyde upon arrival in the lab. Samples used for environmental variables were stored in a dark cooling box immediately after slicing on site, and they were stored frozen at -20 °C or -80 °C upon arrival in the lab for organic matter and grain size samples and for phytopigment samples, respectively.

2.2.2 Sample processing and analytical procedures

2.2.2.1 Environmental variables

Sediment granulometry was analysed using a Malvern Mastersizer 2000, based on the principle of laser diffraction. Median grain size (MGS) and percentage of silt (%) were used to characterize sediments and to classify them into different types based on the Wentworth classification (Wentworth, 1922). Total organic matter (TOM) content was calculated from the difference in sediment dry weight before and after combustion at 500°C for 2 h.

Sediment samples for phytopigment analyses were first lyophilised, then extracted in 90% acetone at 4°C in the dark. They were separated by reversed phase high-performance liquid chromatography (HPLC) and measured with a fluorescence detector according to methods described by Wright and Jeffrey (1997). Table 2.1 provides an overview of the pigments we analysed and of their most common (primary producer) organisms of origin (Wright and Jeffrey, 1997; Peters et al., 2005). In addition, we calculated the ratio of phaeopigments to the sum of chlorophyll a plus phaeopigments (PAP ratio) as an indicator of the freshness of the primary producer biomass in the sediment (Boon and Duineveld, 1997).

Table 2.1. Phytopigments and their most likely origins, based on Wright and Jeffrey (1997).

Variables	Abbreviation	Major organism	Additional organism
Chlorophyll a	chl a	photosynthetic algae, higher plants	
Pheophorbide a	phor	marine detritus, terrestrial detritus	
Pheophytin a	pheo a	living higher plants, plant and algal detritus	
Pyropheophytin a	pyro	zooplankton faecal pellets	
Peridinin	perdinin	photosynthetic dinoflagellates	
Diadinoxanthin	diadino	diatoms, prymnesiophytes	chrysophytes, dinoflagellates
Diatoxanthin	diato	diatoms, prymnesiophytes	chrysophytes,dinoflagellates
Chlorophyll c2	chl c2	chromophyte algae, brown seaweeds	
β-Carotene	b-car	all algae, except for cryptophytes, chlorophytes, rhodophytes	
α-Carotene	a-car	cryptophytes, prochlorophytes, rhodophytes	
Fucoxanthin	fuco	diatoms, prymnesiophytes, brown seaweeds, raphidophytes	dinoflagellates with endosymbionts

Inundation was taken into account as a categorical factor in mesoscale analyses, with three levels: high (H), middle (M) and low (L), with a given number 1, 2 and 3, respectively. Inundation was not taken as a factor in microscale analyses, as all these five stations were very close to each other at the mid-tidal level; they were labelled MP in a PCA of all stations (meso- and microscale together; Fig. 2.2).

2.2.2.2 Nematode community analysis

Nematode samples were rinsed with tap water over a stack of two sieves with respective mesh sizes of 500 µm and 32 µm in order to separate the macrofauna and larger detritus fragments from the nematodes; nematodes retained by the 500-µm sieve were also counted, sorted and added to the nematode samples for this study. Nematodes were extracted from the rinsed samples by density centrifugation (3000 rpm for 12 minutes) using the colloidal silica gel Ludox® HS 40 at a specific density of 1.18 g/cm^3 (Higgins and Thiel, 1988; Somerfield et al., 2005); this step was repeated three times. After each centrifugation, the supernatant was washed over a 32-µm sieve, and the fractions retained on that sieve were pooled, preserved in 4 % formaldehyde and stained with a drop of 1 % Rose Bengal to facilitate visual detection of nematodes under a binocular microscope.

The stained nematode samples were rinsed over a 32-µm sieve to remove formaldehyde prior to counting and collected into a lined counting tray, where they were counted under a Leica binocular microscope (Wild M10; 20-40X) and 100 nematodes were randomly sorted from each sample using a fine Tungsten wire and transferred stepwise to dehydrated glycerol (Seinhorst, 1959; De Grisse, 1965) before being mounted on slides and identified to genus level according to pictorial keys (Platt and Warwick, 1983; Warwick et al., 1998) and the NeMys database (Bezerra et al., 2018).

2.2.3 Data processing and statistical analysis

All the following analyses were performed in PRIMER v6 (Clarke and Gorley, 2006). We used a principal component analysis (PCA) based on Euclidean distance on the environmental data of all stations to visualize whether and to what extent the microscale samples would differ 'environmentally' from the mesoscale samples (L, M, H). The dataset was first checked for skewness and collinearity by using Draftsman plots, after which variables were individually transformed when necessary and standardized (see legend of Figs. 2.2, 2.6, 2.7 and Tables S2.4 and S2.5). Strongly correlated ($\rho > 0.90$) variables were excluded from the analysis (see legend of Fig. 2.2 for an overview of such collinear variables).

The nematode community composition was standardized to total density of nematodes per sample and analysed using a Bray-Curtis similarity matrix. Nematode total density (univariate) was not transformed and further analysis in PRIMER was based on a Euclidean similarity matrix. Visualization of nematode total density (univariate) was done using boxplot function in R (R Core Team 2013). Non-metric multidimensional scaling (nMDS) was performed to visualize patterns of nematode community variation across all stations at two scales and three depth layers. The boxplot function in R (R Core Team, 2013) was used to visualize patterns of distribution of nematode density.

To test whether horizontal spatial variation of nematode communities would be more pronounced at the surface than in deeper layers, we performed a two-way crossed ANOSIM (analysis of similarity; 9999 permutations) with factors 'inundation' (values assigned to three levels: H, M and L) and 'depth' of the sediment (three levels: 0-2, 2-4 and 4-6 cm) at mesoscale, and an ANOSIM with factors 'station' and 'depth' at meso- and microscales. ANOSIM tests were also conducted on the nematode total density dataset to compare patterns of total nematode abundances with patterns of community similarity. A SIMPER (similarity of percentages) analysis was performed to check the percentage of similarity among, and the nematode genera characteristic for, the different depth layers and the different tide levels in the mesoscale samples.

To reveal the relative importance of the environmental variables for the nematode distribution at meso- and microscales, distance-based linear models (DistLM; stepwise selection procedure based on adjusted R^2 and 9999 permutations) were constructed to find the predictor variables that best explained the variation in total nematode density and nematode community structure. In DistLM, marginal tests and sequential tests were implemented, the former treating each variable separately and indicating whether they individually contribute significantly to the variation in nematode communities, while the latter considers all variables simultaneously and chooses the combination(s) of environmental variables that best explain variation in the nematode dataset (Anderson and

Robinson, 2003). Results of DistLM were visualized with distance-based redundancy analysis (dbRDA) plots.

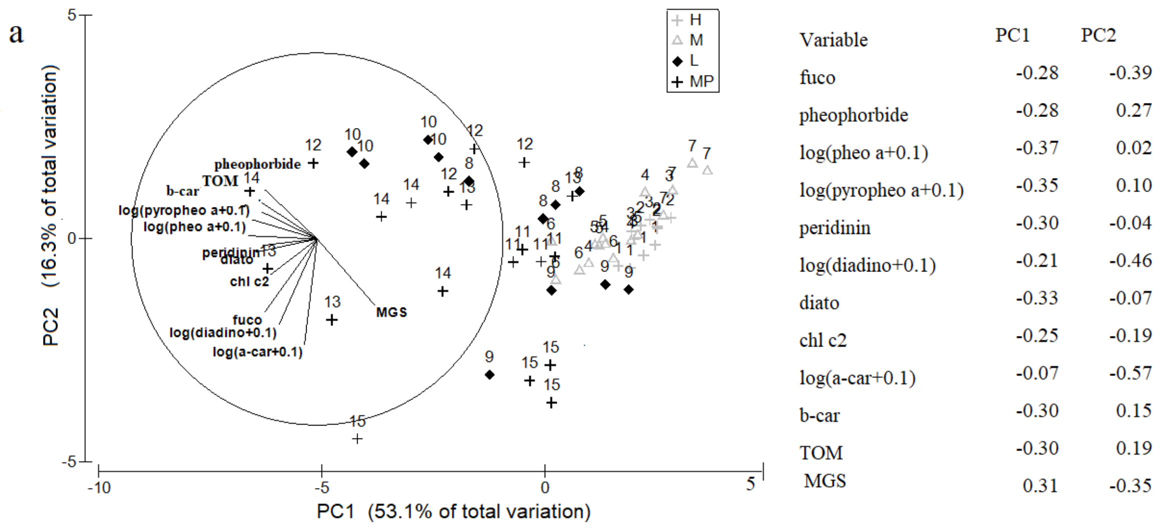
2.3 Results

2.3.1 Sediment characteristics

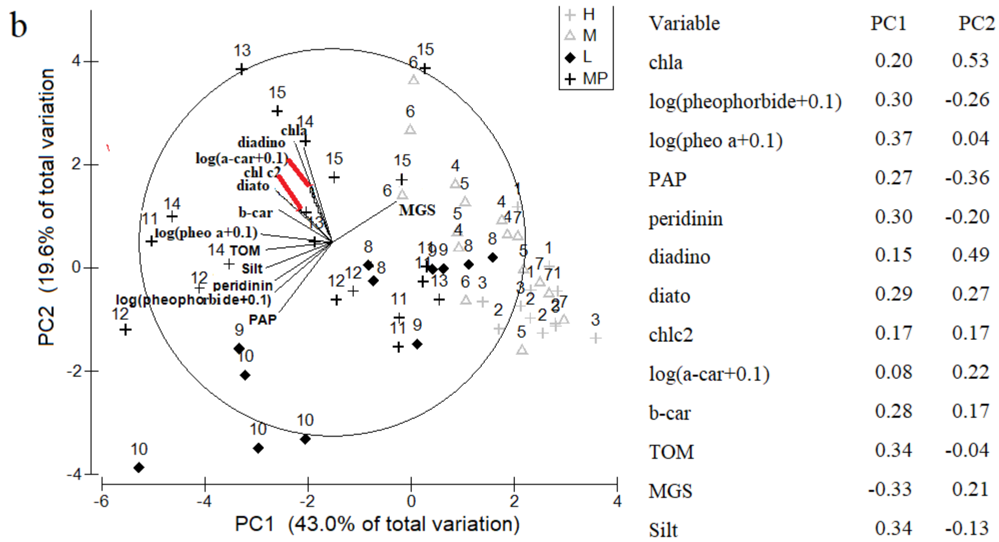
Sediment in the sampling stations consisted mainly of very fine to medium sand (MGS: 66-256 µm, silt: 0-47.8 %) with TOM concentrations varying from 0.6 % to 3.4 %. Chl*a* was the most abundant pigment in the majority of stations and depth layers varying from 0.38 to 21.08 µg/g sediment dry weight, followed by fucoxanthin with concentrations varying between 0.32 and 11.07 µg/g. All other pigments showed concentrations lower than 2 µg/g sediment dry weight. The first two axes of a PCA based on these sediment characteristics (Table S2.1) of all stations (meso- and microscale) explained more than 60 % of the environmental variation for each of the three depth layers analyzed separately (Fig. 2.2). The variability among the microscale stations (MP) was visually the largest, followed by that among the low-tide stations. The separation of low-tide stations from high- and mid-tide stations was rather inconsistent, with different low-tide stations becoming separated from H and M stations depending on sediment depth.

In all three depth layers, sediment granulometry (MGS and silt (%)), TOM and different pigments all contributed to both axes. In general, pigment concentrations were higher in the finer sediments. Especially some of the microscale stations separated based on higher pigment and TOM contents. H and M stations were characterized by higher values of MGS and lower values of TOM.

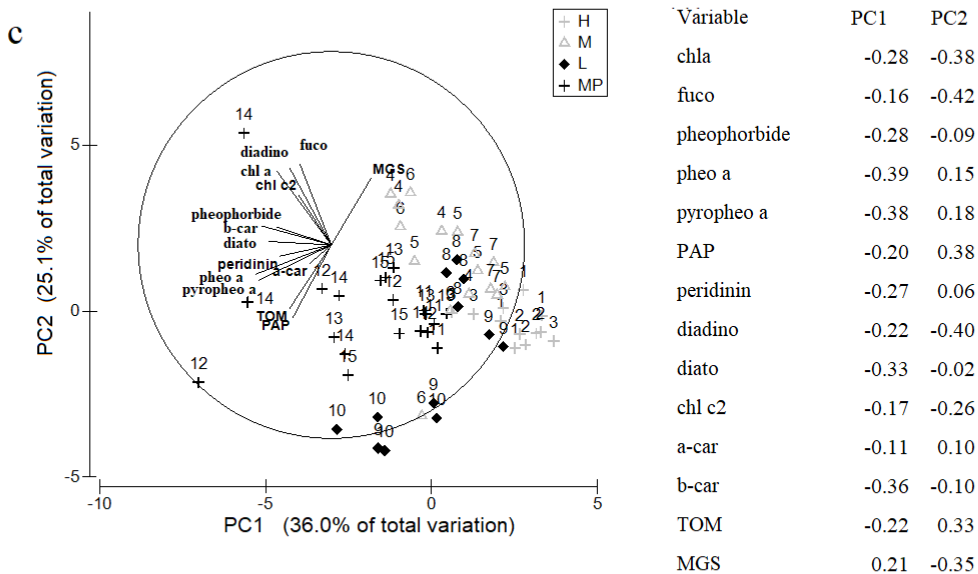
Pigment concentrations decreased with increasing sediment depth at most sites. Moreover, a higher pigment concentration was observed at the microscale stations, and at the low-tide stations as well. PAP, fucoxanthin, diadinoxanthin and phaeophorbides were among the most important food-related drivers of the environmental variation among stations, as indicated by their higher correlations to PCA axes and their longer vectors (Fig. 2.2). The contributions of other pigments varied substantially between the different depth layers. For example, α-carotene and chl*a* contributed more to the variation in the top two layers, while chlc2 more to the variation in the 4-6 cm layer.



Variable	PC1	PC2
fuco	-0.28	-0.39
pheophorbide	-0.28	0.27
log(pheo a+0.1)	-0.37	0.02
log(pyropheo a+0.1)	-0.35	0.10
peridinin	-0.30	-0.04
log(diadino+0.1)	-0.21	-0.46
diato	-0.33	-0.07
chl c2	-0.25	-0.19
log(a-car+0.1)	-0.07	-0.57
b-car	-0.30	0.15
TOM	-0.30	0.19
MGS	0.31	-0.35



Variable	PC1	PC2
chla	0.20	0.53
log(pheophorbide+0.1)	0.30	-0.26
log(pheo a+0.1)	0.37	0.04
PAP	0.27	-0.36
peridinin	0.30	-0.20
diadino	0.15	0.49
diato	0.29	0.27
chlc2	0.17	0.17
log(a-car+0.1)	0.08	0.22
b-car	0.28	0.17
TOM	0.34	-0.04
MGS	-0.33	0.21
Silt	0.34	-0.13



Variable	PC1	PC2
chla	-0.28	-0.38
fuco	-0.16	-0.42
pheophorbide	-0.28	-0.09
pheo a	-0.39	0.15
pyropheo a	-0.38	0.18
PAP	-0.20	0.38
peridinin	-0.27	0.06
diadino	-0.22	-0.40
diato	-0.33	-0.02
chl c2	-0.17	-0.26
a-car	-0.11	0.10
b-car	-0.36	-0.10
TOM	-0.22	0.33
MGS	0.21	-0.35

Fig. 2.2 Principal component analysis (PCA) depicting the environmental variation of 15 intertidal stations across the Paulina tidal flat, Schelde estuary, as based on measurements of 15 variables in each of three sediment depth layers: figure panels a, b and c show the patterns for the 0-2 cm, 2-4 cm and 4-6 cm layer, respectively. Strongly collinear (ρ >0.90 in draftsman plot analysis) variables were removed from the analysis: silt (vs MGS), chl*a* (vs fucoxanthin) and ratio of phaeophorbides to the sum of chl*a* and phaeophorbides (vs pheophorbide) at 0-2 cm, fucoxanthin (vs chl*a* and diadinoxanthin) and pyrophaeophytin a (vs phaeophytin a) at 2-4 cm, silt (vs MGS) at 4-6 cm. H, M and L refer to the intertidal position of the stations at mesoscale, i.e. high, mid- and low intertidal, respectively; MP refers to the microscale stations. Coefficients in the linear combinations of variables making up PC's were listed in the right side of each figure.

2.3.2 Nematode densities

2.3.2.1 Mesoscale

ANOSIM tests (Table S2.2) revealed that nematode density at the mesoscale differed among all inundation levels and most stations, with highest densities observed at the low-tide level, particularly at station 10 (Fig. 2.3). Average densities at high, mid and low tide were 597 (± 515) (standard deviations), 354 (± 330) and 1154 (± 920) individuals.10cm^{-2}, respectively. Nematode density in the top 0-2 cm layer was higher than in the lower two layers (pairwise R =0.371 (vs 2-4 cm), 0.459 (vs 4-6 cm); pairwise p = 0.0001 in both cases) (Fig. 2.3), the latter two being relatively similar as indicated by the pairwise R value close to 0 (0.057 or 0.016). Average densities at 0-2 cm, 2-4 cm and 4-6 cm were 1227 (± 852), 481 (± 463) and 302 (± 190) individuals.10cm^{-2}, respectively.

2.3.2.2 Microscale

Nematode density differed among stations and sediment depth layers (Table S2.2). Differences among stations were mainly caused by a higher density at st14 than in the other four stations; st12 also had a higher density than st11 (Fig. 2.3). Much like for the mesoscale stations, nematode density in the top 0-2 cm layer was higher than in the lower two layers (pairwise R= 0.633 (vs 2-4 cm), 0.8 (vs 4-6 cm), pairwise p = 0.0001 in both cases), the latter two being relatively similar as indicated by a low pairwise R value (0.144) (Table S2.2, Fig. 2.3). Average densities at 0-2 cm, 2-4 cm and 4-6 cm were 3557 (± 2068), 1065 (± 599) and 921 (± 617) individuals.10cm^{-2}, respectively.

2.3.3 Nematode community

2.3.3.1 Mesoscale

nMDS revealed a clear separation of the low-tide stations from the high- and mid-tide stations in the 0-2 and 2-4 cm layers, while H and M stations partly overlapped. At a depth of 4-6 cm, there was overlap between all three tidal levels (Fig. 2.4). Two-way ANOSIM (Table S2.2) largely supported these patterns: it revealed a significant difference in nematode community composition between tidal levels (global R=0.461, p = 0.0001) as well as between depth layers (global R=0.511, p = 0.0001). The top 0-2 cm layer was well-separated from the lower two layers (pairwise R=0.688 (vs 2-4 cm), 0.771 (vs 4-6 cm), p = 0.01 in both cases), the latter two being relatively similar as indicated by a pairwise R value

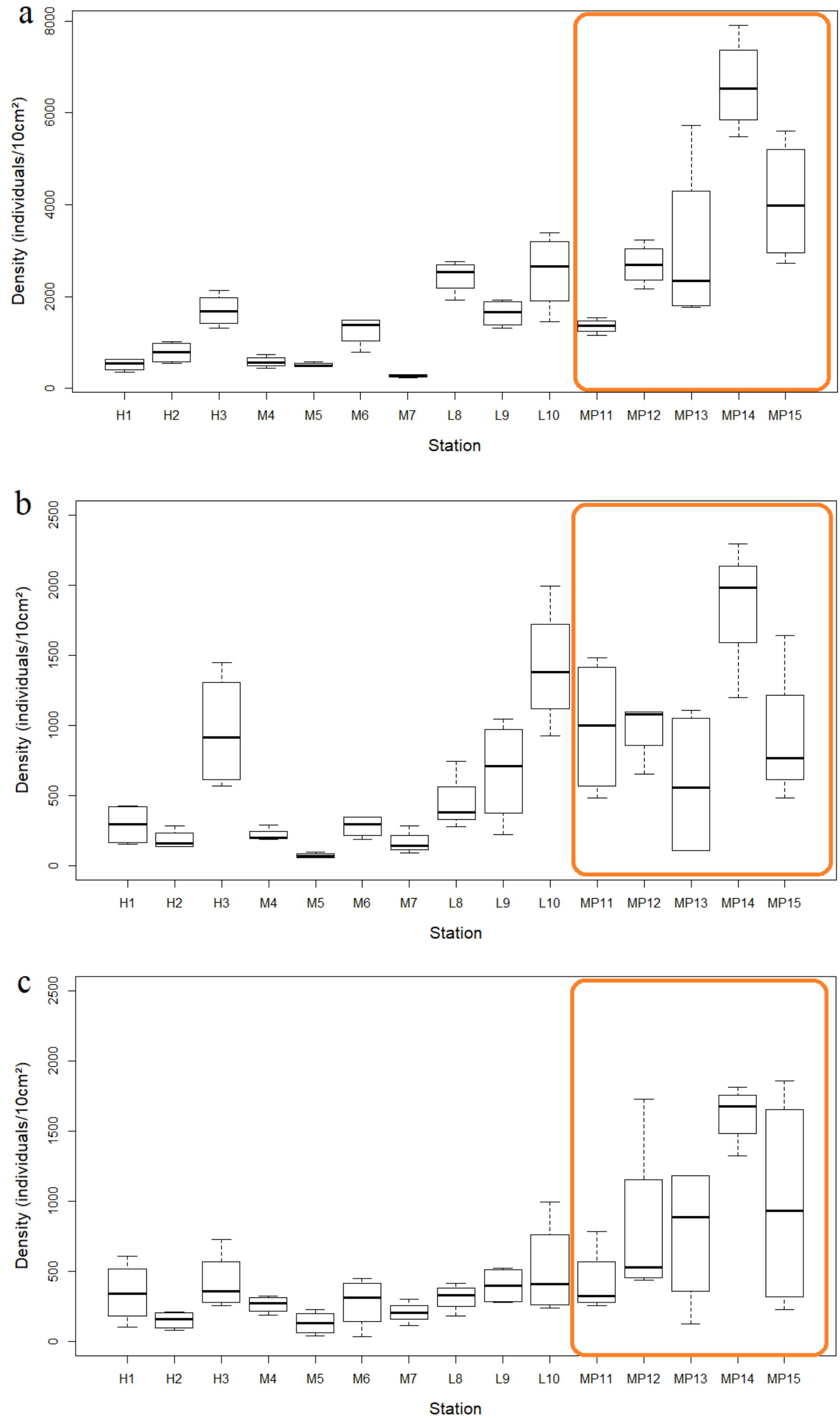


Fig. 2.3. Nematode density (individuals/10cm^2) at 15 intertidal sampling stations displayed by box-whisker plots, showing the following five measures of variation and data distribution: minimum, first quartile, median, third quartile, and maximum. Panels a, b and c show the results for the depth layers of 0-2cm, 2-4cm and 4-6cm, respectively. H, M and L refer to the intertidal position of the stations at mesoscale, i.e. high, mid- and low intertidal, respectively; MP (mid-protected intertidal) refers to the microscale stations (in orange frame).

close to 0 (0.018). SIMPER analysis (Table S2.2) added further support for the dissimilarity of the low-tide stations from the H and M stations.

Genera that contributed most to the dissimilarities among tidal regimes and depths are listed in table S2.3a. Among tidal regimes, *Paramonohystera*, *Odontophora, Oncholaimellus*, *Metalinhomoeus, Daptonema* and *Trefusia* together contributed ca. 50 % dissimilarity between low-tide level and both other tidal regimes, with a higher density of these genera present at low-tide level. *Microlaimus, Theristus*, *Enoploides*, *Chromadora*, *Hypodontolaimus, Daptonema* and *Odontophora* mainly contributed to the (small) difference between mid- and high-tide levels, with higher densities of the first 6 genera and lower densities of the latter two present at the high-tide level. Among sediment depth layers, higher densities of *Enoploides*, *Daptonema*, *Metachromadora*, *Chromadora* and *Oncholaimellus* in the top layer largely differentiated this layer from deeper layers.

Moreover, a larger R value for the factor depth (global R = 0.482, p = 0.0001) than for station (global R = 0.328, p = 0.0001) indicated that at the mesoscale, nematode communities were more dissimilar with depth than horizontally. Genera that contributed most to the dissimilarities among stations and depths are listed in table S2.3b.

2.3.3.2 Microscale

nMDS revealed a large variation in nematode genus composition among and within the five microscale stations (Fig. 2.5). In the upper 0-2 cm, replicates from the stations 11 and 12, as well as three replicates of station 14, each formed separate groups. The same group of three replicates of station 14 persisted in the two deeper sediment layers, whereas stations 11 and 12 formed one cluster at 4-6 cm and largely merged with the other stations at 2-4 cm. ANOSIM tests (Table S2.2) confirmed the difference between station14 and the rest, while they also highlighted a difference between stations 11 and 12. With respect to sediment depth, the top 0-2 cm layer differed significantly from the other two layers. These differences were not always reflected by proportional dissimilarities in a SIMPER analysis due to the large variation among replicates of mainly stations 12 and 14. However, the considerably larger global R-value for the factor depth (0.493) than for the factor station (0.305) indicates that also at the microscale, nematode communities were more dissimilar with sediment depth than horizontally.

Genera that contributed most to the dissimilarities among stations and depths are listed in table S2.3c, with a high density of *Odontophora*, *Metalinhomoeus*, *Anoplostoma*, *Terschellingia* and *Sabateria* at station 12 largely differentiating this station from station 11, with 40 % dissimilarity between the two stations. The distinction between station 14 and other stations was mainly due to a high abundance of *Metalinhomoeus* and *Metachromadora* at station 14, with these two genera capturing ca. 40 % of

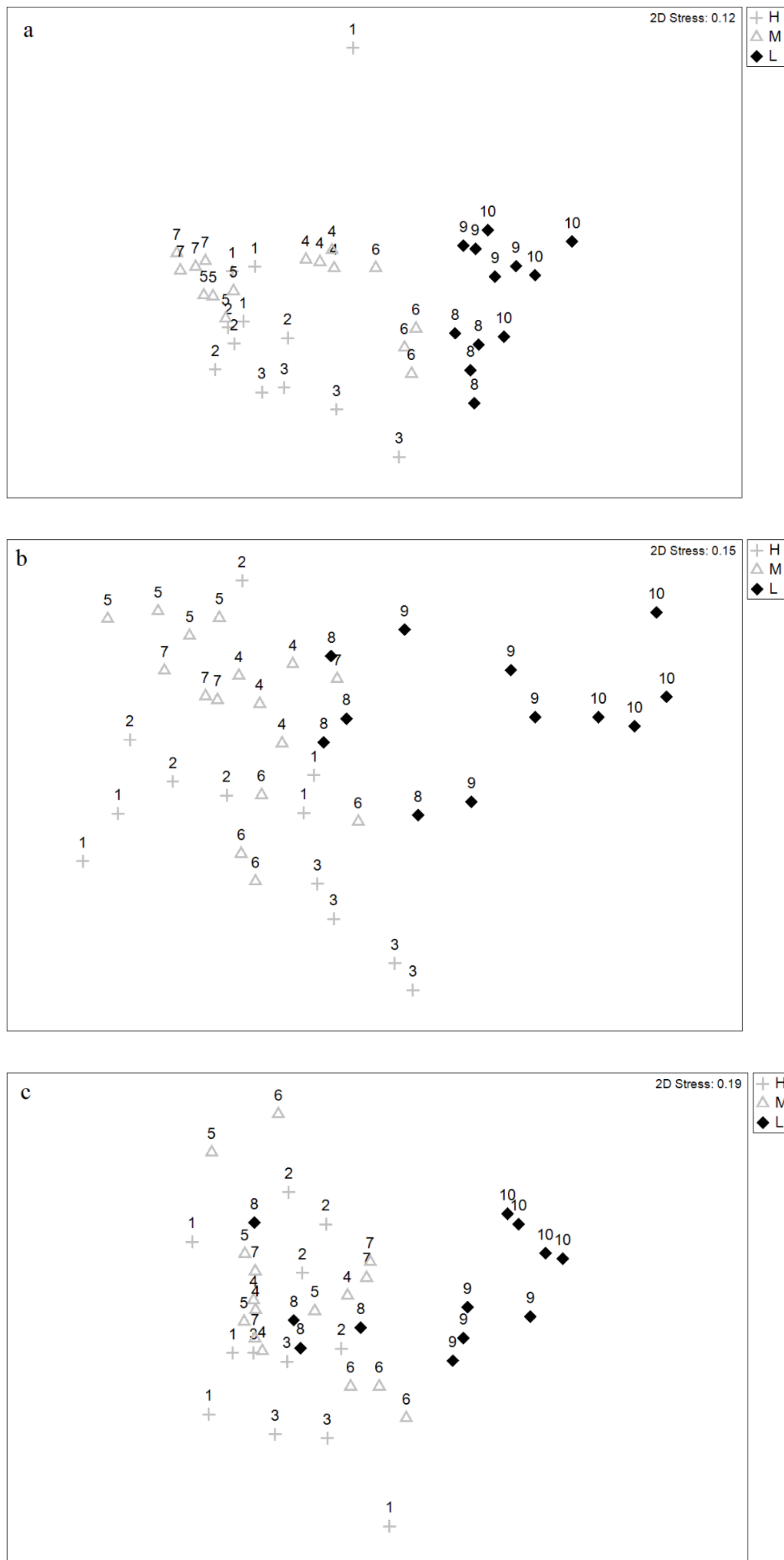


Fig. 2.4. Non-metric multidimensional scaling (nMDS) ordination based on Bray-Curtis similarity of nematode genus composition (absolute densities) at 10 field stations across the Paulina tidal flat in the Schelde estuary. Panels a, b and c show the patterns for the depth layers of 0-2cm, 2-4cm and 4-6cm, respectively. H, M and L refer to the intertidal position of the stations, i.e. high, middle and low intertidal, respectively.

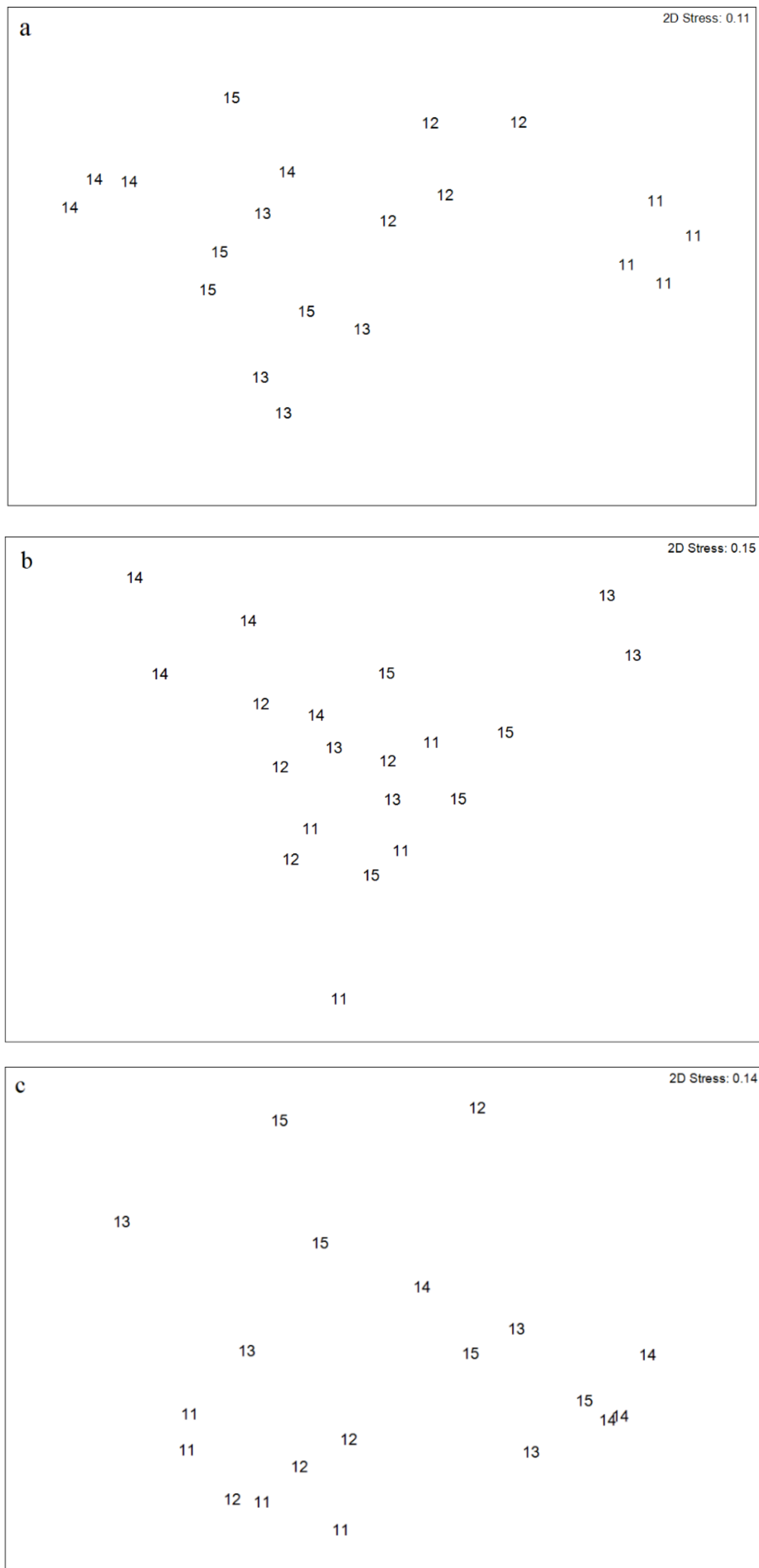


Fig. 2.5. Non-metric multidimensional scaling (nMDS) ordination based on Bray-Curtis similarity of nematode genus composition (absolute densities) at 5 microscale stations (indicated by numbers). Panels a, b and c show the patterns for the depth layers of 0-2 cm, 2-4 cm and 4-6 cm, respectively.

the dissimilarities between station 14 and the rest of the stations. The differentiation of the top layer from the deeper layers was mostly caused by the high abundances of *Metachromadora*, *Odontophora*,

Daptonema, *Ptycholaimellus*, *Metalinhomoeus*, *Chromadora* and *Oncholaimellus* in the top layer, summing up to a dissimilarity of ca 50 %.

2.3.4 Environmental drivers of nematode density

2.3.4.1 Mesoscale

DistLM marginal test (Table S2.4a) revealed that up to 4 pigments (pyropheophytin a, β-carotene, pheophytin and diatoxanthin) had a significant individual effect on nematode density at the mesoscale, with the first three pigments having an individual effect in all three layers and diatoxanthin only in the two deeper layers. Sequential tests (Table S2.4b) revealed the explanatory variable(s): pyropheophytin a at 0-2 cm, a combination of diatoxanthin and β-carotene at 2-4 cm, and diatoxanthin at 4-6 cm, explaining respectively 13 %, 63 % and 13 % of the total variation in nematode abundances.

2.3.4.2 Microscale

DistLM tests (Table S2.4c, S2.4d) revealed that the measured variables captured 24 - 46 % of the variation in nematode density at the top 0-2 cm layer, and almost none in the two deeper layers. Marginal tests (Table S2.4c) revealed that five food indicators (β-carotene, log(pheophytin a + 0.1), log(pyropheophytin a + 0.1), diatoxanthin and log(α-carotene + 0.1)) and sediment granulometry (MGS and silt) had an individual effect, and β-carotene was the best explanatory variable detected by sequential tests (Table S2.4c) at 0-2 cm, with 46 % in total being explained by this pigment.

2.3.5 Environmental drivers of nematode genus composition

2.3.5.1 Mesoscale

Marginal DistLM tests and dbRDA (S5a; Fig. 2.6) on the nematode genus composition of the H, L and M stations showed that most environmental variables (14 of 16, i.e. all except diadinoxanthin and chlorophyll c2) had a significant individual effect on nematode distribution patterns in the 0-2 cm layer. Considerably fewer variables contributed significantly in the deeper sediment layers (Table S2.5a). Generally, sediment granulometry significantly affected nematode community composition in all three depth layers. TOM was significant in the top and deepest layer but not in the middle layer, while a significant effect of inundation was only detected in the upper two layers, not in the deepest one. Indicators of food availability and/or quality such as β-carotene, diatoxanthin, PAP, pheophytin a and pyropheophytin a were marginally significant in all three depth layers, while the effects of other pigments, including the dominant pigments chl*a* and fucoxanthin, depended on the different layers; for example, an individual effect of chl*a* and fucoxanthin was only present in the top two layers.

Sequential tests (Table S2.5b) revealed that the best combinations of environmental variables explained up to 40 % of the variation in nematode community composition in the upper 2 cm of the sediment, whilst only ≤ 23 % was explained in the deeper sediment strata. A combination of 5 pigments (pheophytin a, α-carotene, chlorophyll c2, chl*a* and diatoxanthin) and silt concentration

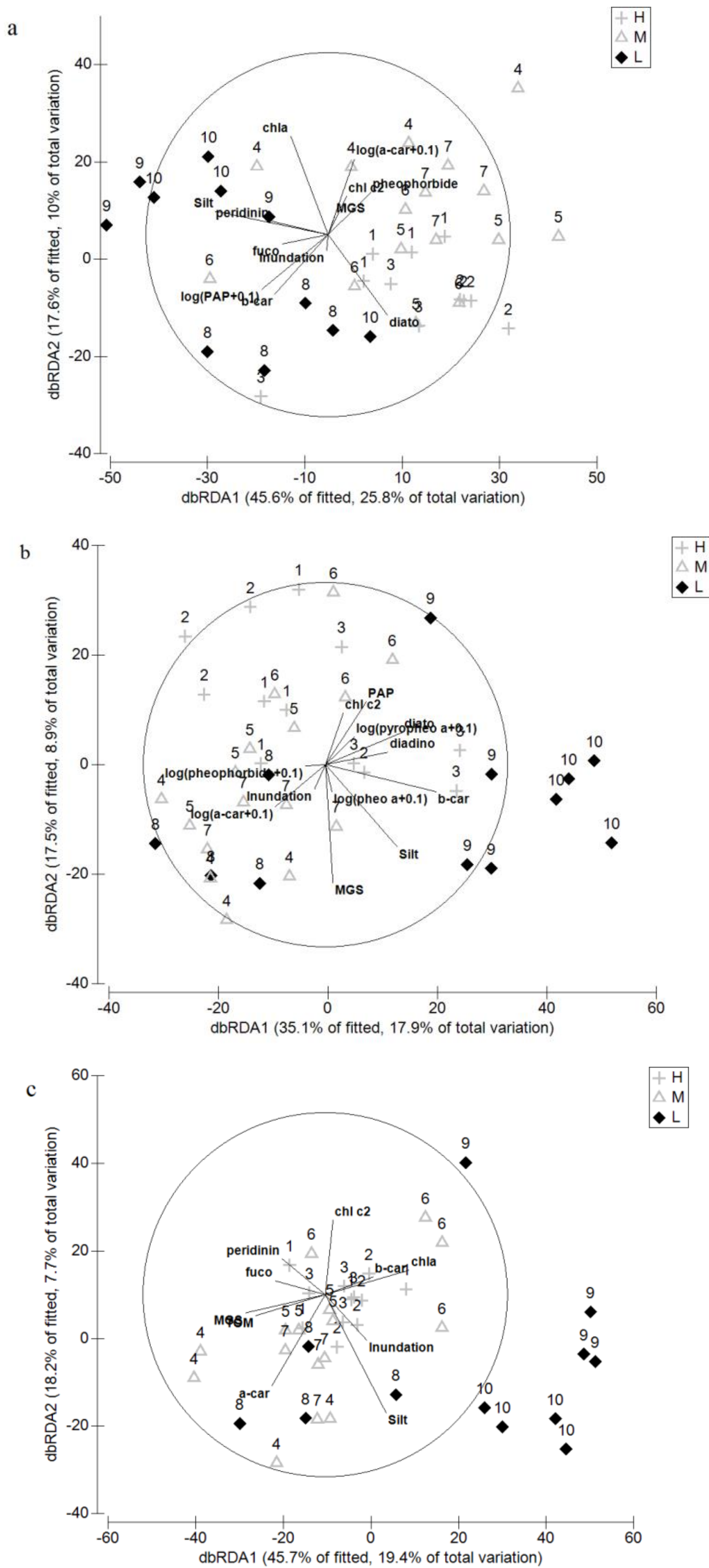


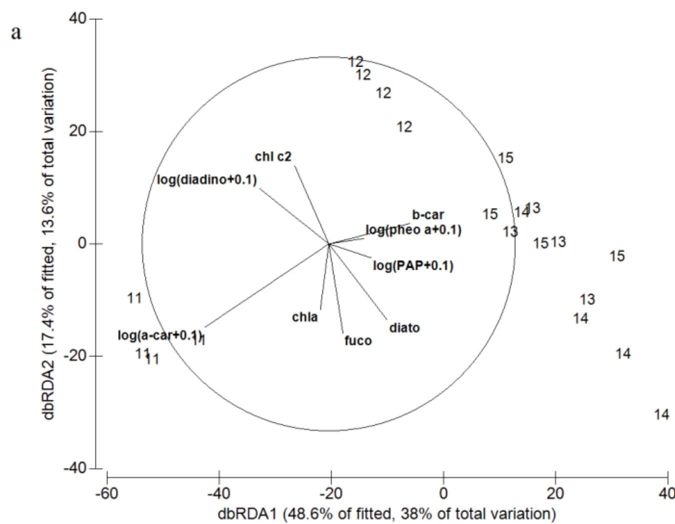
Fig. 2.6. Distance-based redundancy analysis (dbRDA) plots illustrating the DistLM models based on nematode genus composition (absolute densities) and fitted environmental variables with their vectors (i.e. strength and direction of effect of the variable on the ordination plot) at mesoscale, with axis legends showing the percentage of variation explained by the fitted model and by each axis. Panels a, b and c show the results for the depth layers of 0-2 cm, 2-4 cm and 4-6 cm, respectively. H, M and L refer to the intertidal position of the stations, i.e. high, middle and low intertidal, respectively. Abbreviation of variables followed Table 1.

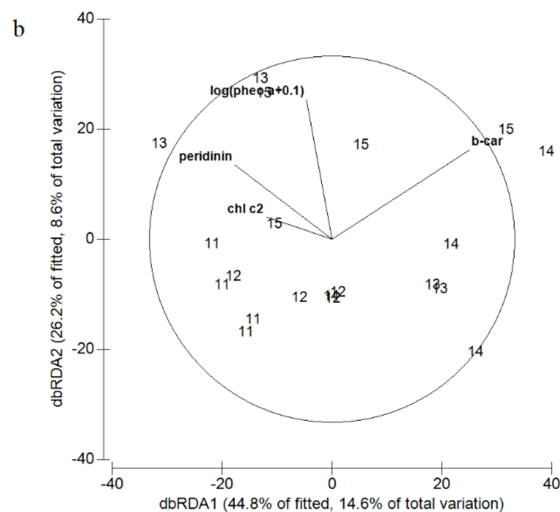
explained 40 % of the variation in nematode community composition in the top sediment layer. β-carotene, diatoxanthin and percentage of silt together explained 23 % of the variation at 2-4 cm depth, while pheophytin a, α-carotene and inundation explained 20 % of the total variation in horizontal patterns of nematode communities at 4-6 cm depth.

2.3.5.2 Microscale

DistLM marginal tests and dbRDA (S5c; Fig. 2.7) for the microscale stations revealed that MGS, silt, TOM and five food indicators (pheophytin a, pyropheophytin a, PAP, α- and β-carotene) had a significant individual effect on nematode distribution patterns in the 0-2 cm layer at the microscale. In contrast, only one pigment (β-carotene) explained a significant portion of the variation in nematode community composition at 2-4 cm and two pigments (α- and β-carotene) did so at 4-6 cm.

Sequential tests (Table S2.5d) revealed that α-carotene captured most of the variation (30 %) in nematode genus composition in the top layer. In deeper layers, β-carotene was the only explanatory variable, with 9 % at 2-4 cm and 18 % variation at 4-6 cm being explained.





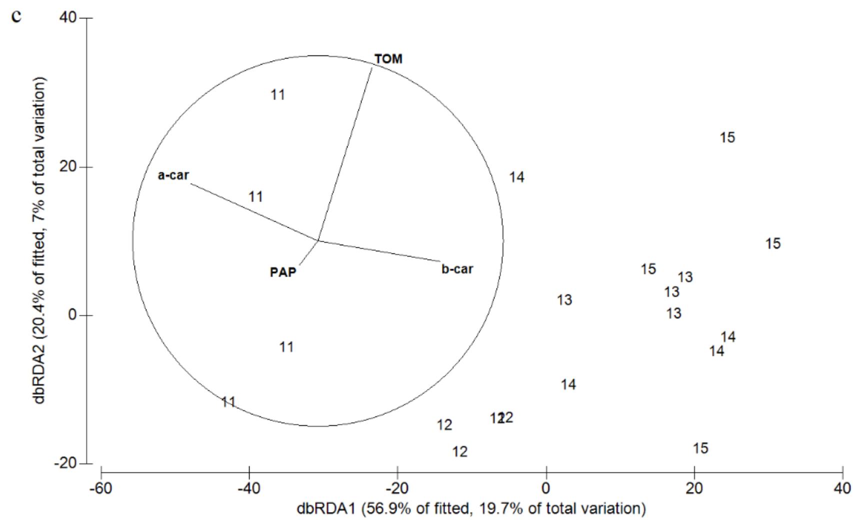


Fig. 2.7. Distance-based redundancy analysis (dbRDA) plots illustrating the DistLM model based on nematode genus composition (absolute densities) and fitted environmental variables with their vectors (strength and direction of effect of the variable on the ordination plot) at microscale, with axis legends showed percentage of variation explained by the fitted model and percentage of total variation explained by the axis. Panels a, b, c show the results at the depth layers of 0-2 cm, 2-4 cm and 4-6 cm, respectively. Abbreviation of variables followed Table 1.

2.4 Discussion

2.4.1 Environmental drivers at mesoscale

The environment of the Paulina tidal flat as characterized mainly based on pigment concentrations and granulometric features, was only partly structured by tidal zonation. PCA analysis showed no outspoken separation of the three tidal zones for any of the depth layers, while the microscale stations, which were all within the same tidal zone, exhibited the highest variability of the entire dataset. The lack of a clear tide-related environmental separation may be due to the fact that the tidal exposure gradient at the Paulina tidal flat is relatively small: the difference in exposure time between high- and mid-tide and between high- and low-tide stations during a typical low tide is in the order of 1-1.5 and 2-3 h, respectively. Despite the lack of such clear tide-related environmental separation of stations, nematode communities from the low-tide level were clearly different from those of mid- and high-tide level, with only minor differences between the latter two. However, the differences between tidal levels disappeared gradually towards the deepest sediment layer. The importance of tidal level for the structure of the communities of the surface layers at mesoscale was confirmed by the marginal tests implemented in DistLM.

The effect of tidal exposure on nematode community abundance, diversity and composition has been well-studied on dissipative sandy beaches (Gheskiere et al., 2004; Maria et al., 2013). However, unlike at those sandy beaches, where a mid-tide zone is often separated from both high- and low-tide zones (Gheskiere et al., 2004; McLachlan and Jaramillo, 1996), the distinction at the Paulina tidal flat was

mostly evident between the high- and mid-tide zones on the one hand and the low-tide zone on the other, with higher total abundances and higher densities of deposit-feeding nematodes such as *Daptonema*, *Paramonohystera*, *Metalinhomoeus* and *Trefusia* and of a few predatory/omnivorous genera such as *Odonthophora* and *Oncholaimellus* in the latter. Other predators/omnivores (mainly *Enoploides*) and so-called epistratum feeders, which tend to puncture or scrape off microalgae with a partly evertible tooth (here mainly *Microlaimus, Chromadora* and *Hypodontolaimus*), were proportionally more abundant towards the high-tide stations. These predators/omnivores and epistratum feeders were mostly restricted to the upper 2 cm of sediment, which for the latter feeding type likely relates to their intimate trophic relationship with microphytobenthos (Moens and Vincx, 1997; Commito and Tita, 2002; Sahraean et al., 2017). The abundant predator/omnivore *Enoploides* is commonly restricted to the surface layers of the sediment (Steyaert et al., 2001), where it can exert a significant top-down control on both the total abundances and the species composition of its prey communities (Moens and Vincx, 2000; Gallucci et al., 2005). The ability of this species to efficiently forage on prey nematodes is strongly hampered by the presence of silt (Gallucci et al., 2005), but probably also by sediment compaction (Steyaert and Moens, unpubl.), explaining its absence, and hence also the absence of its structuring effect on nematode assemblages, from the muddier low-tide stations and from the deeper layers at high- and mid-tide stations. This may be one important reason for the lack of a significant tidal effect on nematode community structure in the deepest sediment layer (4-6 cm). Alternatively, nematode community structure at this depth may be mainly determined by lack of oxygen (Vieira and Fonseca, 2013), and while at or near the sediment surface, tides may affect oxygen penetration (with considerably deeper penetration at sandier sediments), this is no longer the case at greater depth in these very fine to fine/medium sandy sediments.

On dissipative beaches, the tidal zonation is often explained in relation to different stressors: high-tide communities are less abundant and diverse because they are more exposed to abiotic fluctuations such as heat and desiccation. Low-tide communities are more exposed to wave action, but also tend to accumulate more organic matter and hence are often characterized by steep oxygen and biogeochemical gradients, leading to relatively species-poor yet very abundant nematode communities. The mid-tide level represents an intermediate zone where hydrodynamics and low-tide exposure are generally more at equilibrium, allowing more species to co-exist (Nicholas and Hodda, 1999; Armonies and Reise, 2000; Gheskiere et al., 2004; Gingold et al., 2010). Essentially the same explanation as for these dissipative beaches can explain the higher total abundance of nematodes at the low-tide level of the Paulina tidal flat, which was characterized by a higher concentration of silt, OM and several phytopigments, demonstrating that stations at the low-tide level accumulated substantial amounts of labile OM. These conditions tend to favour deposit-feeding genera like

Daptonema and *Paramonohystera* (Sahraean et al., 2017; Saidi et al., 2017), *Metalinhomoeus* (Blome et al., 1999; Ferreira et al., 2015) and *Trefusia* (Urkmez et al., 2015), probably through a combination of a higher food availability and these genera's good tolerance to hypoxic conditions. The overall small differences observed in this study between high- and mid-tide nematode communities likely reflect the small differences in tidal exposure and wave action between both.

The intertidal gradient at the Paulina tidal flat also encompasses clear differences in granulometry between low-tide stations on the one hand and mid- and high-tide stations on the other, the latter being characterized by a considerably larger median grain size and a much lower contribution of silt. The structuring role of sediment granulometry for nematode communities, in terms of abundance, diversity and taxonomic composition is well known and is intimately tied with the above-mentioned differences in biogeochemistry and food availability (Steyaert et al., 2003; Gheskiere et al., 2004), as well as with the presence/abundance of predators such as the above-mentioned *Enoploides*.

Indeed, in addition to sediment, specific food sources such as diatoms, detritus and zooplankton fecal pellets, as indicated by the respective concentrations of diatoxanthin, pheophytin a and pyropheophytin a (Wright and Jeffrey, 1997; Peters et al., 2005), also contributed significantly to the variation in nematode communities in all three sediment layers, along with more general biomarkers of algal abundance (β-carotene) and freshness (PAP). The contribution of some of these food indicators in explaining the variation in nematode community composition was even larger than that of the silt fraction. Food availability and/or quality have been suggested as important factors by multiple studies (for an overview see Giere, 2009; Moens et al., 2013), but mostly so at microscales, where good correlations between abundances of major meiofauna taxa and chl*a* or other proxies of microalgal biomass have often been demonstrated (Montagna et al., 1983; Blanchard, 1990; Pinckney and Sandulli, 1990; Moens et al., 1999; Netto and Meneghel, 2014); at the mesoscale, this has been much less the case.

Microphytobenthos (MPB) often contribute significantly to the total primary production of intertidal areas (MacIntyre et al., 1996; Underwood and Kromkamp, 1999) and have been suggested to play a central role in moderating carbon flow in intertidal sediments (Middelburg et al., 2000) by being an important carbon source for benthos (Herman et al., 1999). MPB was identified as a major carbon source for a variety of tidal-flat nematodes (e.g. the genera *Metachromadora*, *Daptonema*, *Enoploides*, *Praeacanthonchus* and *Theristus*) as inferred from $\delta^{13}C$ analysis (Moens et al., 2005a; Rzeznik Orignac et al., 2008; Moens et al., 2014) and from microscopical observations of the intestinal contents of nematodes (Moens and Vincx, 1997). Often, however, the precise pathway from MPB to nematodes remains to be elucidated; either it results from direct grazing on MPB, or indirectly through predation

on MPB grazers (Moens et al., 2014) or through grazing on bacteria that metabolize the extracellular polymeric substances produced by MPB (Carpentier et al., 2014). Furthermore, not only fresh material but also labile phytodetritus, here quantified from the amount of pheopigments, can provide a food source for intertidal nematodes (Moens et al., 2002; Urban-Malinga and Moens, 2006). In the present study, phytodetritus affected both total nematode densities and community composition at the mesoscale: abundances were considerably higher at the low-tide stations, where mainly deposit feeders benefited from the larger supply of organic matter from the water column.

Whereas a broad variety of environmental factors, including tidal level, sediment granulometry and multiple pigment concentrations as indicators of food availability/quality, significantly impacted nematode genus composition, nematode density was only impacted by few variables. These were mostly the phytodetritus algal biomarker pigments β-carotene and pheophytin, the zooplankton faecal pellet indicator pyropheophytin a, and the diatom indicator diatoxanthin (the latter surprisingly only in the two deeper layers). It makes sense that total nematode abundances correlate with general markers of algal biomass and quality, with higher nematode abundances where inputs of fresh and detrital algal material are higher, i.e. at the low-tide stations. An important contribution of zooplankton faecal pellets to the nutrition of Antarctic deep-sea nematodes has been suggested based on natural stable-isotope data (Moens et al., 2007), but the present data are, to our knowledge, the first to indicate such a link for intertidal sediments.

While comparatively fewer environmental variables contributed to the variation in nematode abundances than in nematode genus composition, the total amounts of variation in nematode data explained by the best combination of environmental data were roughly similar for density (up to 40 %) and genus composition (up to 60 %). A striking difference, however, was that genus composition variation was best explained in the surficial sediment layer, whereas total nematode abundance variation was much better explained by environmental variables at 2-4 cm than at the surface or in the deeper layer. Given that (biomarkers of) MPB and deposited phytoplankton likely show the largest patchiness at the sediment surface, and that tidal and sedimentary effects on abundant predacious and epistrate-feeding nematodes are also likely most pronounced in the depth stratum where these nematodes are most abundant (i.e. in the upper 2 cm, see above), the results of the genus composition data are according to expectation. That food-related biomarkers better explained total nematode density in the subsurface suggests that the burial of fresh organic matter, either by hydrodynamic or biological activity, may be an important determinant of nematode abundances in this depth stratum.

2.4.2 Environmental drivers at microscale

Similar to the mesoscale, a significant variation in nematode community composition was observed at microscales, separating most stations in a multivariate analysis, especially for the surface layer. Multivariate comparison separated station 14 from all other stations and station 12 from station 11, with these differences being more pronounced at the top layer than in the deeper layers. Our results agree with those of previous studies in which patchily distributed food sources correlated well with the variation in nematode abundances at a microscale (Montagna et al., 1983; Blanchard, 1990; Hodda, 1990), since multiple phytopigments had significant individual effects on the nematode communities. These pigments were pheophytin a, pyropheophytin a, α- and β-carotene at 0-2 cm, β-carotene at 2-4 cm, and α- and β-carotene at the 4-6 cm layer. In other words, the best explanatory variables reflected the general abundance and 'quality' of algal food sources and of zooplankton faecal pellets, rather than the abundance of specific algal groups like diatoms.

Some of the dominant nematode genera are known to be favored by patchily distributed food sources. For example, *Metalinhomoeus*, *Terschellingia* and *Sabatieria* have been observed to respond with increased densities to decaying OM (Fonseca et al., 2011), although it is unclear whether this reflects a resource effect or a higher tolerance of these genera to hypoxia. *Sabatieria* is known to migrate to the sediment surface in response to the deposition of fresh phytoplankton in fine sandy sediments (Franco et al., 2008a). In our study, the high numbers of *Metalinhomoeus* and *Terschellingia* at station 12 could have benefitted from higher amounts of phytodetritus and zooplankton faeces deposition, while *Sabatieria* could have been attracted to higher inputs of fresh algae as indicated by higher β-carotene concentrations. Also at station 14, *Metalinhomoeus* and *Metachromadora* were linked to higher algae concentrations as indicated by β-carotene. Based on natural stable isotope data, the latter genus is almost certainly an MPB feeder at the Paulina tidal flat (Moens et al., 2005a).

The magnitude of the effect of specific food sources on the nematode genus composition did not always reflect their concentrations. For instance, although decreased pigment concentrations were observed with increased sediment depth, these changes were not always reflected in the amount of variation in nematode composition explained by these variables: for example, the general algal marker β-carotene captured 9 % and 13 % of the variation in nematode genus composition in the top two layers, while its concentration was much higher at the top layer than in the subsurface 2-4 cm layer. This observation indicated that the effect of specific food sources on nematode composition was not proportional to their concentration in the environment. This should point to the additional effect of biotic factors and interactions in explaining differences in the present nematode communities.

Moreover, our study also reflected that sediment granulometry (MGS, silt) still had an important effect on nematode density and genus composition at the microscale, especially at the top layer. However, MPB can alter the local sediment composition, for example through the presence of extracellular polysaccharides produced by diatoms and their associated bacteria, which can trap fine sediment particles and even forming cohesive surface biofilms that ultimately act against erosion and contribute to a further fining of the sediment (Kromkamp et al., 2016). It is also known that even a small shift in sediment composition can result in a strong effect on specific nematodes, such as shown for the predacious genus-*Enoploides* (Moens et al., 2000; Gallucci et al., 2005), which can further cascade down since the nematode community composition may be significantly influenced by predator –prey dynamics (Steyaert, 2003). Therefore, a proper evaluation of the effect of sediment granulometry on nematode communities may be biased without considering the effects of trophic interactions and the presence of food sources. A study by Boucher (1990) observed no differences in species richness and H' index of nematode assemblages from muddy and sandy sediments and concluded that sediment granulometry or trophic differences alone could not be the only determining factors. Unlike at mesoscale, nematodes density and composition are driven by similar factors in the top sediment layer at microscale.

2.5 Conclusion

Our study has shown that specific food sources, as represented by different pigments and measures for freshness, are important drivers of nematode genus composition and densities both at the micro- and mesoscale, especially for the surface layers of the sediments. Grain size and tidal level are important too, at least in the surface layers of the sediment, while their assumed larger importance at the mesoscale is not outspoken. Both mesoscale zonation and microscale patchiness are more pronounced in the surface layers compared to the deeper sediment layers, supporting the importance of MPB as an important environmental driver.

Acknowledgements

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Supplementary information of Chapter 2

Table S2.1. Environmental variables at 15 intertidal stations at the Paulina tidal flat, Schelde estuary, SW Netherlands. Ten stations covered a mesoscale, including different inundation times (H, M, L for high-, mid- and low-tide level, respectively), whilst the remaining five covered a microscale (indicated by MP) and were all located at the mid-tide level. Sediment granulometry is indicated by median grain size (MGS) and % silt (<63 µm). indicators of food availability are total organic matter content (TOM) and several phytopigment concentrations as well as the ratio of phaeopigments to the sum of chlorophyll a plus phaeopigments (PAP) as an indicator of quality/freshness of primary producer biomass. For abbreviations of pigments, see Table 2.1. All pigments are recorded in µg/g sediment dry weight. Records at microscale were labelled as MP in factor of inundation.

a

Station	Depth (cm)	MGS (µm)	silt%	TOM (%)	Inundation	Chl*a*	fuco	PAP
st1	0-2	256	0.0	0.7	H	9.93	4.8	0.01
st2	0-2	238	0.0	0.7	H	5.94	3.03	0.01
st3	0-2	246	1.2	0.9	H	4.7	2.63	0.02
st1	2-4	245	1.1	0.8	H	4.65	2	0.01
st2	2-4	217	0.8	0.7	H	1.95	0.85	0.04
st3	2-4	244	1.4	0.9	H	2	1.16	0.03
st1	4-6	239	0.0	1	H	0.85	0.37	0.03
st2	4-6	208	0.0	0.7	H	0.38	0.2	0.03
st3	4-6	247	1.8	0.8	H	1.27	0.56	0.04
st4	0-2	230	0.0	0.7	M	7.8	3.84	0.02
st5	0-2	235	0.0	0.7	M	8.46	3.77	0.02
st6	0-2	215	8.5	1.3	M	14	7.03	0.01
st7	0-2	234	0.0	0.9	M	3.12	1.35	0.01
st4	2-4	227	0.0	0.7	M	6.58	2.83	0.02
st5	2-4	232	0.0	0.6	M	4.92	2.21	0.04
st6	2-4	209	10.5	1.2	M	9.59	4.31	0.03
st7	2-4	230	0.0	0.9	M	3.93	1.7	0.01
st4	4-6	226	0.0	0.7	M	5.51	2.37	0.03
st5	4-6	231	0.0	0.7	M	3.32	1.29	0.03
st6	4-6	195	17.3	1.6	M	5.24	2.29	0.08
st7	4-6	228	0.0	0.9	M	3.15	1.28	0.01
st8	0-2	178	15.9	1.7	L	8.54	4.81	0.04
st9	0-2	204	10.3	1.7	L	8.03	5.11	0.03
st10	0-2	78	39.0	2.6	L	9.57	5.21	0.14
st8	2-4	199	7.9	1.2	L	6.63	2.92	0.03
st9	2-4	137	25.5	2.4	L	4.79	2.64	0.03
st10	2-4	77	39.3	2.2	L	3.88	1.69	0.15
st8	4-6	209	6.7	1.1	L	3.61	1.28	0.02
st9	4-6	133	26.8	3	L	1.43	0.49	0.05
st10	4-6	66	47.8	2.7	L	1.06	0.32	0.2
st11	0-2	207	4.2	0.9	MP	12.02	6.14	0.04
st12	0-2	110	33.4	3.4	MP	7.82	4.72	0.1
st13	0-2	144	27.4	2.5	MP	21.08	11.07	0.04
st14	0-2	135	30.7	2.7	MP	17.7	9.09	0.06

Station	Depth (cm)	MGS (µm)	silt%	TOM (%)	Inundation	Chl*a*	fuco	PAP
st15	0-2	243	0.0	1.6	MP	20.11	10.55	0.02
st11	2-4	186	8.5	1	MP	4.77	1.82	0.06
st12	2-4	122	29.1	2.2	MP	6.05	2.66	0.07
st13	2-4	155	24.9	1.9	MP	10.17	3.89	0.03
st14	2-4	161	25.7	2.4	MP	9.83	4.27	0.08
st15	2-4	176	19.0	2.2	MP	11.82	5.22	0.02
st11	4-6	164	12.0	1.1	MP	2.68	0.69	0.06
st12	4-6	124	27.5	1.9	MP	4.29	1.14	0.07
st13	4-6	180	20.4	1.6	MP	4.25	0.9	0.03
st14	4-6	188	18.2	2	MP	7.23	2.42	0.06
st15	4-6	225	7.1	1.5	MP	5.5	1.09	0.05

b

Station	Depth (cm)	diadino	diato	pheophorbide	pheo a	pyropheo a	peridinin	chl c2	a-car	b-car
st1	0-2	0.91	0.09	0	0.05	0.01	0	0.22	0.01	0.37
st2	0-2	0.52	0.14	0	0.06	0.01	0	0.1	0	0.26
st3	0-2	0.52	0.08	0	0.09	0.03	0.11	0.07	0.01	0.23
st1	2-4	0.41	0.09	0	0.04	0.01	0	0.11	0	0.18
st2	2-4	0.15	0.16	0	0.09	0.03	0	0.05	0	0.15
st3	2-4	0.2	0.06	0	0.08	0.04	0.04	0.08	0	0.12
st1	4-6	0.08	0.04	0	0.02	0.02	0	0.07	0	0.05
st2	4-6	0.04	0.04	0	0.01	0	0	0.02	0	0.03
st3	4-6	0.09	0.08	0	0.07	0.04	0.07	0.06	0	0.09
st4	0-2	0.72	0.15	0	0.12	0.07	0.02	0.23	0.01	0.34
st5	0-2	0.66	0.07	0.05	0.14	0.04	0	0.4	0.01	0.44
st6	0-2	1.35	0.23	0	0.13	0.09	0.01	0.2	0.02	0.54
st7	0-2	0.23	0.06	0	0.02	0.01	0	0.06	0	0.16
st4	2-4	0.62	0.18	0.02	0.12	0.07	0	0.26	0.01	0.31
st5	2-4	0.38	0.12	0.03	0.09	0.04	0	0.37	0.01	0.23
st6	2-4	0.82	0.45	0	0.11	0.05	0.01	0.17	0.03	0.51
st7	2-4	0.3	0.06	0	0.03	0.01	0	0.09	0	0.19
st4	4-6	0.51	0.18	0	0.12	0.09	0.02	0.3	0.01	0.27
st5	4-6	0.21	0.16	0.01	0.07	0.06	0	0.26	0	0.18
st6	4-6	0.43	0.28	0	0.14	0.09	0	0.1	0.01	0.32
st7	4-6	0.21	0.1	0	0.04	0.03	0	0.09	0	0.17
st8	0-2	0.53	0.74	0.14	0.2	0.61	0.1	0.17	0.01	0.44
st9	0-2	3.04	0.24	0.1	0.14	0.05	0.25	0.12	0.34	0.08
st10	0-2	0.69	0.49	0.9	0.65	0.35	0.52	0.4	0.01	0.52
st8	2-4	0.34	0.56	0.09	0.12	0.08	0.05	0.16	0.01	0.33
st9	2-4	0.4	0.24	0.04	0.09	0.05	0.21	0.07	0.23	0.06
st10	2-4	0.28	0.27	0.38	0.29	0.21	0.22	0.21	0	0.29
st8	4-6	0.2	0.37	0.01	0.05	0.05	0	0.11	0	0.23
st9	4-6	0.02	0.22	0	0.08	0.05	0.04	0.04	0.18	0.05
st10	4-6	0.09	0.21	0	0.25	0.22	0.04	0.13	0	0.22
st11	0-2	0.91	0.17	0.19	0.29	0.1	0.13	0.57	0.02	0.53
st12	0-2	0.76	0.63	0.48	0.58	0.38	0.23	0.31	0.02	0.67
st13	0-2	1.01	1.28	0.14	0.6	0.47	0.13	0.44	0.03	0.87
st14	0-2	1.73	1.16	0.35	0.78	0.52	0.29	0.28	0.03	1.29

Station	Depth (cm)	diadino	diato	pheophorbide	pheo a	pyropheo a	peridinin	chl c2	a-car	b-car
st15	0-2	1.8	0.76	0.07	0.28	0.17	0.26	0.4	0.75	0.14
st11	2-4	0.25	0.29	0.09	0.2	0.13	0.07	0.28	0.01	0.35
st12	2-4	0.44	0.68	0.16	0.37	0.25	0.21	0.2	0.02	0.68
st13	2-4	0.43	0.97	0.01	0.3	0.27	0.05	0.2	0.01	0.58
st14	2-4	0.76	0.89	0.13	0.65	0.43	0.15	0.32	0.02	0.78
st15	2-4	0.86	0.74	0	0.21	0.16	0.03	0.17	0.53	0.11
st11	4-6	0.03	0.31	0	0.16	0.13	0.02	0.2	0.01	0.29
st12	4-6	0.28	0.52	0.07	0.25	0.16	0.09	0.11	0.01	0.58
st13	4-6	0.16	0.6	0	0.15	0.15	0.01	0.12	0.02	0.51
st14	4-6	0.46	0.78	0.05	0.31	0.21	0.06	0.23	0.02	0.7
st15	4-6	0.2	0.92	0	0.26	0.17	0.02	0.09	0.55	0.11

Table S2.2. Results of similarity analyses: two-way ANOSIM and SIMPER showing the dissimilarity of nematode density/communities between different stations, inundation levels and sediment depth layers at meso- and microscales. Results of the main effects and of pairwise comparisons are given. Significant P values are in bolded and italic or indicated by * when p<0.0001.

			Density		Composition		
			ANOSIM		ANOSIM		SIMPER
	Test	Groups	R	P	R	P	Dissimilarity (%)
mesoscale	Global	between inundation	0.239	***0.0001***	0.461	***0.0001***	
		between depth	0.281	***0.0001***	0.511	***0.0001***	
	Pairwise	H, M	0.078	***0.019***	0.241	***0.01***	70.8
		H, L	0.0169	***0.0008***	0.636	***0.01***	83.9
		M, L	0.447	***0.0001***	0.555	***0.01***	79.6
		0-2, 2-4	0.371	***0.0001***	0.688	***0.0001***	82.3
		0-2, 4-6	0.459	***0.0001***	0.771	***0.0001***	86.5
		2-4, 4-6	0.016	0.243	0.018	0.236	68.2
	Global	between station	0.328	***0.0001***	0.328	***0.0001***	
		between depth	0.482	***0.0001***	0.482	***0.0001***	
	Pairwise	st1, st2	0.109	0.159	0.109	0.159	66.5
		st1, st3	0.403	***0.006***	0.403	***0.006***	73.2
		st1, st4	-0.009	0.478	-0.009	0.478	70.4
		st1, st5	0.285	***0.017***	0.285	***0.017***	70.4
		st1, st6	0.16	0.12	0.16	0.12	75.8
		st1, st7	0.309	***0.011***	0.309	***0.011***	67
		st1, st8	0.302	***0.02***	0.302	***0.02***	79.4
		st1, st9	0.358	***0.018***	0.358	***0.018***	86.5
		st1, st10	0.507	***0.001***	0.507	***0.001***	93
		st2, st3	0.618	***0.0001***	0.618	*	69.9
		st2, st4	0.142	0.116	0.142	0.116	68.9
		st2, st5	0.243	***0.036***	0.243	***0.036***	60.1
		st2, st6	0.271	***0.037***	0.271	***0.037***	73.6
		st2, st7	0.141	0.109	0.141	0.109	62.3
		st2, st8	0.583	***0.0007***	0.583	***0.001***	77.1
		st2, st9	0.559	***0.0003***	0.559	*	83.5

Test		Groups	ANOSIM		ANOSIM		SIMPER
			R	P	R	P	Dissimilarity(%)
		st2, st10	0.649	***0.0001***	0.649	*	90.5
		st3, st4	0.526	***0.0007***	0.526	***0.001***	72.1
		st3, st5	0.701	***0.0001***	0.701	*	77.1
		st3, st6	0.182	***0.03***	0.182	***0.03***	74.9
		st3, st7	0.63	***0.0003***	0.63	*	76.6
		st3, st8	0.179	0.066	0.179	0.066	74.3
		st3, st9	-0.16	0.955	-0.16	0.955	82
		st3, st10	-0.003	0.465	-0.003	0.465	88.9
		st4, st5	0.408	***0.004***	0.408	***0.004***	60.4
		st4, st6	0.271	***0.028***	0.271	***0.028***	70.2
		st4, st7	0.347	***0.012***	0.347	***0.012***	56.2
		st4, st8	0.41	***0.006***	0.41	***0.006***	63.6
		st4, st9	0.507	***0.001***	0.507	***0.001***	79
		st4, st10	0.569	***0.0004***	0.569	*	85.3
		st5, st6	0.625	***0.0003***	0.625	*	79.7
		st5, st7	0.415	***0.005***	0.415	***0.005***	51
		st5, st8	0.691	***0.0002***	0.691	*	76.8
		st5, st9	0.729	***0.0001***	0.729	*	87.2
		st5, st10	0.688	***0.0002***	0.688	*	92
		st6, st7	0.385	***0.009***	0.385	***0.009***	78.5
		st6, st8	0.219	***0.043***	0.507	***0.002***	75
		st6, st9	0.087	0.188	0.552	***0.001***	83.5
		st6, st10	0.403	***0.005***	0.403	***0.005***	85.2
		st7, st8	0.507	***0.002***	0.507	***0.002***	75
		st7, st9	0.552	***0.0006***	0.552	***0.001***	83.5
		st7, st10	0.635	***0.0005***	0.635	***0.001***	88.5
		st8, st9	0.17	0.102	0.17	0.102	70.1
		st8, st10	0.236	***0.026***	0.236	***0.026***	80.7
		st9, st10	0.163	0.075	0.163	0.075	58.9
		0-2, 2-4	0.671	***0.0001***	0.671	***0.0001***	78.6
		0-2, 4-6	0.763	***0.0001***	0.763	***0.0001***	85.1
		2-4, 4-6	0.057	0.168	0.057	0.168	57.6
microscale	Global	between station	0.305	***0.0001***	0.305	***0.0001***	
		between depth	0.493	***0.0001***	0.493	***0.0001***	
	pairwise	st11, st12	0.365	0.007	0.365	***0.007***	62.1
		st11, st13	0.174	0.089	0.174	0.089	78
		st11, st14	0.771	0.0001	0.771	*	87
		st11, st15	0.205	0.058	0.205	0.058	79.4
		st12, st13	0.042	0.328	0.042	0.328	73.5
		st12, st14	0.674	0.0002	0.674	*	76
		st12, st15	0.042	0.289	0.042	0.289	73.3
		st13, st14	0.573	0.0001	0.573	*	71.9
		st13, st15	-0.066	0.662	-0.066	0.662	67.2
		st14, st15	0.368	0.01	0.368	***0.01***	68.6
		0-2, 2-4	0.633	***0.0001***	0.633	*	75.7
		0-2, 4-6	0.8	***0.0001***	0.8	*	81.2
		2-4, 4-6	0.144	0.089	0.144	0.089	63.1

Table S2.3. Overview of nematode genera that contribute up to a cumulative % of ca. 50 % dissimilarity between communities from different tidal levels and depth layers for the mesoscale stations (a) and between stations and depth layers for the meso- (b) and microscale stations (c) in a SIMPER analysis. H, M, L indicate the different tidal regimes. 0-2, 2-4 and 4-6 cm are the different sediment depth layers.

a

Groups	Genus	Av.Abund	Av.Abund	Av.Diss %	Cum.%
H & M	*Microlaimus*	81.26	32.52	8.48	11.98
	Daptonema	32.56	65.93	5.37	19.57
	Theristus	53.31	12.8	4.99	26.63
	Enoploides	89.46	46.6	4.88	33.52
	Chromadora	67.89	8.14	4.34	39.65
	Odontophora	15.31	26.55	4.17	45.54
	Hypodontolaimus	40.43	0.63	3.78	50.88
H & L	*Paramonohystera*	3.45	188.3	13.04	15.54
	Microlaimus	81.26	40.81	6.25	22.99
	Odontophora	15.31	123.62	6.01	30.15
	Oncholaimellus	0.97	137.02	5.28	36.45
	Metalinhomoeus	1.96	48.57	4.32	41.6
	Daptonema	32.56	92.1	4.22	46.63
	Trefusia	5.91	30.93	3.53	50.83
M & L	*Paramonohystera*	7.38	188.3	14.7	18.46
	Odontophora	26.55	123.62	7.27	27.58
	Oncholaimellus	0.81	137.02	5.99	35.11
	Daptonema	65.93	92.1	5.12	41.54
	Metalinhomoeus	1.77	48.57	4.9	47.69
	Trefusia	10.51	30.93	4.25	53.03
0-2 & 2-4	*Enoploides*	124.22	7.22	14.62	17.78
	Daptonema	145.2	22.73	9.6	29.45
	Microlaimus	52.66	63.15	5.04	35.58
	Metachromadora	64.93	7.52	4.31	40.82
	Chromadora	77.91	6.43	3.95	45.62
	Oncholaimellus	116.06	7.75	3.78	50.22
0-2 & 4-6	*Enoploides*	124.22	5.52	14.79	17.1
	Daptonema	145.2	23.38	9.77	28.4
	Odontophora	77.76	33.6	5.02	34.2
	Oncholaimellus	116.06	1.35	4.47	39.37
	Metachromadora	64.93	2.27	4.42	44.47
	Chromadora	77.91	3.68	4.39	49.55
2-4 & 4-6	*Paramonohystera*	101.71	47.54	8.21	12.04
	Microlaimus	63.15	33.07	8.02	23.79
	Odontophora	45.53	33.6	6.71	33.63
	Daptonema	22.73	23.38	4.53	40.28
	Trefusia	17.39	21.42	3.85	45.92
	Theristus	22.41	17.29	3.46	50.99

b

Groups	Genera	Av.Abund	Av.Abund	Av.Diss	Cum.%
st1 & st2	*Enoploides*	53.4	98.34	6.73	10.12
	Microlaimus	32.02	15.5	5.27	18.05
	Theristus	28.18	35.3	4.36	24.6
	Chromadora	32.5	27.22	3.62	30.04
	Cyatholaimoides	34.42	0	3.57	35.41
	Cyatholaimidae	12.31	5.54	3.07	40.01
	Daptonema	16.07	21.22	2.68	44.04
	Eleutherolaimus	7.96	14.91	2.6	47.95
	Neochromadora	18.71	6.27	2.41	51.58
st1 & st3	*Microlaimus*	32.02	196.26	12.84	17.54
	Hypodontolaimus	5.14	108.44	6.96	27.05
	Chromadora	32.5	143.97	6.81	36.35
	Enoploides	53.4	116.64	6.55	45.3
	Theristus	28.18	96.47	5.78	53.2
st1 & st4	*Daptonema*	16.07	84.24	7.38	10.48
	Microlaimus	32.02	45.66	6.73	20.05
	Odontophora	16.94	52.03	6.66	29.5
	Chromadora	32.5	0.7	4.03	35.23
	Enoploides	53.4	32.74	3.92	40.8
	Cyatholaimoides	34.42	0	3.66	45.99
	Theristus	28.18	2.75	3.36	50.76
st1 & st5	*Enoploides*	53.4	83.96	7.1	10.09
	Microlaimus	32.02	17.89	6.1	18.76
	Odontophora	16.94	24.89	4.75	25.52
	Chromadora	32.5	8.45	4.71	32.21
	Cyatholaimoides	34.42	0	4.35	38.39
	Theristus	28.18	2.79	4.26	44.44
	Neochromadora	18.71	0.34	2.98	48.67
	Cyatholaimidae	12.31	1.21	2.64	52.43
st1 & st6	*Daptonema*	16.07	150.14	11.88	15.68
	Metachromadora	7.64	96.75	6.33	24.04
	Microlaimus	32.02	41.81	5.36	31.1
	Theristus	28.18	42.52	4.73	37.34
	Enoploides	53.4	9.37	3.9	42.49
	Trefusia	6.25	24.47	3.25	46.78
	Chromadora	32.5	19.51	3.2	51.01
st1 & st7	*Enoploides*	53.4	60.33	5.74	8.57
	Microlaimus	32.02	24.73	5.35	16.55
	Chromadora	32.5	3.91	4.75	23.64
	Cyatholaimoides	34.42	0.51	4.52	30.39
	Theristus	28.18	3.14	3.97	36.32
	Odontophora	16.94	25.52	3.8	41.99
	Paramonohystera	0.63	17.88	3.63	47.4
	Neochromadora	18.71	0.44	2.9	51.72
st1 & st8	*Bolbolaimus*	1.36	172.44	6.62	8.34
	Microlaimus	32.02	89.69	6.62	16.67

Groups	Genera	Av.Abund	Av.Abund	Av.Diss	Cum.%
	Trefusia	6.25	48.9	6.59	24.97
	Odontophora	16.94	66.59	5.34	31.69
	Enoploides	53.4	0.56	3.15	35.66
	Theristus	28.18	41.72	3.1	39.56
	Cyatholaimidae	12.31	12.51	3.02	43.37
	Ascolaimus	7.06	71.36	2.82	46.92
	Metachromadora	7.64	68.85	2.66	50.27
st1 & st9	*Paramonohystera*	0.63	119.71	12.88	14.9
	Oncholaimellus	0.98	199.24	10.19	26.69
	Daptonema	16.07	100.56	7.57	35.45
	Odontophora	16.94	98.56	5.99	42.38
	Microlaimus	32.02	28.08	3.89	46.87
	Trefusia	6.25	39.42	3.48	50.9
st1 & st10	*Paramonohystera*	0.63	428.92	26.92	28.94
	Metalinhomoeus	1.02	101.06	8.8	38.4
	Odontophora	16.94	205.7	7.74	46.73
	Oncholaimellus	0.98	147.85	5.52	52.67
st2 & st3	*Microlaimus*	15.5	196.26	16.56	23.69
	Hypodontolaimus	7.7	108.44	7.66	34.66
	Chromadora	27.22	143.97	5.58	42.65
	Theristus	35.3	96.47	5.24	50.14
st2 & st4	*Odontophora*	7.49	52.03	9.7	14.08
	Microlaimus	15.5	45.66	7.52	25.01
	Daptonema	21.22	84.24	6.38	34.28
	Enoploides	98.34	32.74	5.11	41.7
	Cyartonema	1.62	16.21	3.71	47.08
	Theristus	35.3	2.75	3.45	52.09
st2 & st5	*Odontophora*	7.49	24.89	6	9.99
	Theristus	35.3	2.79	4.82	18
	Microlaimus	15.5	17.89	3.99	24.64
	Eleutherolaimus	14.91	3.03	3.89	31.11
	Daptonema	21.22	17.09	3.54	37
	Enoploides	98.34	83.96	3.06	42.09
	Dichromadora	9.28	0	2.81	46.77
	Hypodontolaimus	7.7	0.8	2.56	51.03
st2 & st6	*Daptonema*	21.22	150.14	12.14	16.49
	Metachromadora	3.63	96.75	6.57	25.41
	Theristus	35.3	42.52	5.29	32.6
	Enoploides	98.34	9.37	4.72	39.01
	Microlaimus	15.5	41.81	4.15	44.66
	Trefusia	2.59	24.47	4.01	50.1
st2 & st7	*Paramonohystera*	7.75	17.88	5.21	8.35
	Odontophora	7.49	25.52	4.72	15.92
	Theristus	35.3	3.14	4.64	23.36
	Microlaimus	15.5	24.73	4.42	30.45
	Enoploides	98.34	60.33	4.24	37.25
	Eleutherolaimus	14.91	4.78	2.96	42

Groups	Genera	Av.Abund	Av.Abund	Av.Diss	Cum.%
	Praeacanthonchus	34.95	5.66	2.77	46.45
	Chromadora	27.22	3.91	2.71	50.81
st2 & st8	*Trefusia*	2.59	48.9	8.41	10.91
	Odontophora	7.49	66.59	7.66	20.86
	Bolbolaimus	1.33	172.44	6.15	28.83
	Microlaimus	15.5	89.69	5.53	36.01
	Enoploides	98.34	0.56	3.41	40.44
	Ascolaimus	3.87	71.36	3.09	44.46
	Cytolaimium	0.47	10.87	2.93	48.26
	Daptonema	21.22	56.01	2.57	51.59
st2 & st9	*Paramonohystera*	7.75	119.71	14.49	17.36
	Oncholaimellus	0.23	199.24	9.5	28.74
	Daptonema	21.22	100.56	7.86	38.15
	Odontophora	7.49	98.56	5.64	44.9
	Enoploides	98.34	1.17	4.23	49.97
	Trefusia	2.59	39.42	4.06	54.83
st2 & st10	*Paramonohystera*	7.75	428.92	29.61	32.72
	Metalinhomoeus	3.06	101.06	10.33	44.14
	Odontophora	7.49	205.7	7.67	52.61
st3 & st4	*Microlaimus*	196.26	45.66	12.3	17.08
	Hypodontolaimus	108.44	0	7.85	27.98
	Chromadora	143.97	0.7	7.05	37.76
	Theristus	96.47	2.75	6.19	46.35
	Enoploides	116.64	32.74	5.16	53.52
st3 & st5	*Microlaimus*	196.26	17.89	17.54	22.76
	Hypodontolaimus	108.44	0.8	8.89	34.29
	Chromadora	143.97	8.45	7.16	43.58
	Theristus	96.47	2.79	7.04	52.71
st3 & st6	*Microlaimus*	196.26	41.81	13.49	18.01
	Hypodontolaimus	108.44	1.47	7.45	27.94
	Daptonema	60.4	150.14	6.12	36.12
	Theristus	96.47	42.52	5.37	43.28
	Chromadora	143.97	19.51	5.11	50.1
st3 & st7	*Microlaimus*	196.26	24.73	14.76	19.26
	Hypodontolaimus	108.44	0.26	8.34	30.15
	Chromadora	143.97	3.91	7.78	40.3
	Theristus	96.47	3.14	6.83	49.21
	Enoploides	116.64	60.33	5.55	56.46
st3 & st8	*Microlaimus*	196.26	89.69	11.85	15.95
	Hypodontolaimus	108.44	2.5	6.61	24.85
	Trefusia	8.88	48.9	4.89	31.43
	Bolbolaimus	0.97	172.44	4.68	37.73
	Enoploides	116.64	0.56	3.7	42.7
	Theristus	96.47	41.72	3.65	47.61
	Chromadora	143.97	33.73	3.55	52.39
st3 & st9	*Microlaimus*	196.26	28.08	11.68	14.24
	Paramonohystera	1.98	119.71	9.28	25.56

Groups	Genera	Av.Abund	Av.Abund	Av.Diss	Cum.%
	Oncholaimellus	1.71	199.24	6.51	33.49
	Hypodontolaimus	108.44	3.21	5.97	40.77
	Odontophora	21.49	98.56	4.7	46.5
	Chromadora	143.97	8.99	4.68	52.21
st3 & st10	*Paramonohystera*	1.98	428.92	21.09	23.72
	Microlaimus	196.26	4.65	10.07	35.05
	Metalinhomoeus	1.81	101.06	7.27	43.23
	Odontophora	21.49	205.7	6.24	50.25
st4 & st5	*Microlaimus*	45.66	17.89	8.8	14.55
	Daptonema	84.24	17.09	7.65	27.21
	Odontophora	52.03	24.89	7.44	39.51
	Enoploides	32.74	83.96	5.63	48.83
	Cyartonema	16.21	0.72	4.75	56.69
st4 & st6	*Odontophora*	52.03	3.75	9.15	13.02
	Daptonema	84.24	150.14	8.81	25.57
	Metachromadora	8.73	96.75	6.44	34.75
	Microlaimus	45.66	41.81	5.83	43.06
	Theristus	2.75	42.52	4.31	49.19
	Ascolaimus	0.5	41.14	3.85	54.66
st4 & st7	*Daptonema*	84.24	12.24	8.93	15.9
	Odontophora	52.03	25.52	6.52	27.5
	Microlaimus	45.66	24.73	6.35	38.81
	Paramonohystera	7.76	17.88	3.88	45.73
	Cyartonema	16.21	0.86	3.72	52.35
st4 & st8	*Microlaimus*	45.66	89.69	6.77	10.64
	Trefusia	11.05	48.9	6.69	21.15
	Bolbolaimus	0.7	172.44	6.49	31.35
	Odontophora	52.03	66.59	3.67	37.11
	Ascolaimus	0.5	71.36	3.14	42.05
	Theristus	2.75	41.72	2.72	46.33
	Oncholaimellus	1.69	63.97	2.38	50.07
st4 & st9	*Paramonohystera*	7.76	119.71	12.69	16.06
	Oncholaimellus	1.69	199.24	10.04	28.77
	Odontophora	52.03	98.56	9.13	40.33
	Daptonema	84.24	100.56	6.72	48.84
	Microlaimus	45.66	28.08	5.22	55.45
st4 & st10	*Paramonohystera*	7.76	428.92	27.23	31.91
	Odontophora	52.03	205.7	9.97	43.59
	Metalinhomoeus	0.74	101.06	9.24	54.42
st5 & st6	*Daptonema*	17.09	150.14	15.06	18.88
	Metachromadora	13.36	96.75	7.27	27.99
	Odontophora	24.89	3.75	6.54	36.2
	Theristus	2.79	42.52	5.7	43.35
	Trefusia	0.18	24.47	5.2	49.86
	Microlaimus	17.89	41.81	4.88	55.98
st5 & st7	*Odontophora*	24.89	25.52	5.34	10.48
	Paramonohystera	2.17	17.88	5.34	20.94

Groups	Genera	Av.Abund	Av.Abund	Av.Diss	Cum.%
	Microlaimus	17.89	24.73	5.31	31.36
	Enoploides	83.96	60.33	5	41.17
	Metachromadora	13.36	9.09	2.12	45.32
	Trefusia	0.18	6.33	1.99	49.22
	Tubolaimoides	1.73	6.13	1.96	53.06
st5 & st8	*Trefusia*	0.18	48.9	9.56	12.44
	Bolbolaimus	0	172.44	7.08	21.65
	Microlaimus	17.89	89.69	6.41	29.99
	Odontophora	24.89	66.59	6.06	37.88
	Theristus	2.79	41.72	3.46	42.38
	Cytolaimium	0.28	10.87	3.43	46.84
	Ascolaimus	1.35	71.36	3.41	51.28
st5 & st9	*Paramonohystera*	2.17	119.71	16.6	19.05
	Oncholaimellus	0.35	199.24	10.7	31.33
	Daptonema	17.09	100.56	9.69	42.45
	Odontophora	24.89	98.56	8.24	51.9
st5 & st10	*Paramonohystera*	2.17	428.92	31.83	34.61
	Metalinhomoeus	0.98	101.06	11.15	46.73
	Odontophora	24.89	205.7	9.43	56.99
st6 & st7	*Daptonema*	150.14	12.24	14.35	18.28
	Metachromadora	96.75	9.09	7.29	27.56
	Odontophora	3.75	25.52	5.29	34.3
	Theristus	42.52	3.14	4.78	40.39
	Microlaimus	41.81	24.73	4.67	46.34
	Ascolaimus	41.14	0.86	4.42	51.98
st6 & st8	*Odontophora*	3.75	66.59	7.48	10.84
	Daptonema	150.14	56.01	7.11	21.14
	Trefusia	24.47	48.9	6.16	30.06
	Bolbolaimus	10.27	172.44	5.35	37.81
	Microlaimus	41.81	89.69	4.53	44.38
	Theristus	42.52	41.72	3.45	49.38
	Ascolaimus	41.14	71.36	2.65	53.22
st6 & st9	*Paramonohystera*	1.72	119.71	13.22	18.72
	Oncholaimellus	0.61	199.24	8.01	30.07
	Daptonema	150.14	100.56	6.04	38.62
	Odontophora	3.75	98.56	4.77	45.38
	Metachromadora	96.75	8.36	4.19	51.32
st6 & st10	*Paramonohystera*	1.72	428.92	27.36	32.14
	Metalinhomoeus	5.03	101.06	8.9	42.59
	Odontophora	3.75	205.7	7.14	50.97
st7 & st8	*Trefusia*	6.33	48.9	7.64	10.19
	Bolbolaimus	5.69	172.44	7.39	20.05
	Microlaimus	24.73	89.69	6.2	28.31
	Odontophora	25.52	66.59	4.98	34.95
	Paramonohystera	17.88	16.26	3.56	39.7
	Ascolaimus	0.86	71.36	3.45	44.31
	Theristus	3.14	41.72	2.92	48.21

Groups	Genera	Av.Abund	Av.Abund	Av.Diss	Cum.%
	Metachromadora	9.09	68.85	2.9	52.08
st7 & st9	*Paramonohystera*	17.88	119.71	12.89	15.44
	Oncholaimellus	0.61	199.24	11.58	29.31
	Daptonema	12.24	100.56	9.12	40.23
	Odontophora	25.52	98.56	7.56	49.28
	Ascolaimus	0.86	65.84	4.66	54.87
st7 & st10	*Paramonohystera*	17.88	428.92	27.35	30.9
	Metalinhomoeus	0.35	101.06	10.05	42.25
	Odontophora	25.52	205.7	8.8	52.19
s8 & st9	*Paramonohystera*	16.26	119.71	11.47	16.37
	Odontophora	66.59	98.56	6	24.93
	Daptonema	56.01	100.56	5.39	32.61
	Trefusia	48.9	39.42	4.89	39.58
	Bolbolaimus	172.44	6.12	4.71	46.31
	Oncholaimellus	63.97	199.24	4.22	52.33
st8 & st10	*Paramonohystera*	16.26	428.92	24.61	30.51
	Metalinhomoeus	21.31	101.06	7.8	40.17
	Odontophora	66.59	205.7	6.6	48.35
	Trefusia	48.9	4.46	4.74	54.22
st9 & st10	*Paramonohystera*	119.71	428.92	17.24	29.28
	Metalinhomoeus	23.35	101.06	5.55	38.71
	Daptonema	100.56	119.72	4.57	46.47
	Odontophora	98.56	205.7	4.47	54.07
0-2 & 2-4	*Enoploides*	124.22	7.22	14.6	18.57
	Daptonema	145.2	22.73	8.3	29.12
	Oncholaimellus	116.06	7.75	4.22	34.49
	Microlaimus	52.66	63.15	4.16	39.78
	Chromadora	77.91	6.43	4.1	44.99
	Metachromadora	64.93	7.52	3.72	49.72
0-2 & 4-6	*Enoploides*	124.22	5.52	14.56	17.11
	Daptonema	145.2	23.38	8.48	27.07
	Odontophora	77.76	33.6	5.03	32.99
	Oncholaimellus	116.06	1.35	4.99	38.85
	Chromadora	77.91	3.68	4.72	44.4
	Microlaimus	52.66	33.07	3.93	53.78
2-4 & 4-6	*Microlaimus*	63.15	33.07	6.5	11.29
	Paramonohystera	101.71	47.54	6.32	22.27
	Odontophora	45.53	33.6	5.15	31.22
	Trefusia	17.39	21.42	3.18	36.73
	Daptonema	22.73	23.38	3.14	42.19
	Theristus	22.41	17.29	3.11	47.6
	Hypodontolaimus	25.76	4.66	2.11	51.26

c

Groups	Genera	Av.Abund	Av.Abund	Av.Diss	Cum.%
st11 & st12	*Paramonohystera*	160.8	135.44	8.09	13.03
	Odontophora	179.16	184.15	5.48	21.84
	Metalinhomoeus	49.73	120.55	5.15	30.14
	Anoplostoma	1.32	144.72	4.66	37.63
	Terschellingia	11.5	101.09	4.43	44.77
	Sabatieria	62.5	82.42	3.73	50.77
st11 & st13	*Metalinhomoeus*	49.73	271.8	12.14	15.56
	Paramonohystera	160.8	33.57	11.25	29.98
	Odontophora	179.16	158.13	8.24	40.53
	Daptonema	33	213.04	5.1	47.07
	Theristus	22.33	94.6	4.65	53.03
st11 & st14	*Metalinhomoeus*	49.73	938.63	28.11	32.32
	Metachromadora	6.86	440.38	6.37	39.65
	Paramonohystera	160.8	30.41	6.09	46.65
	Odontophora	179.16	121.58	5.59	53.08
st11 & st15	*Paramonohystera*	160.8	10.1	10.18	12.83
	Metalinhomoeus	49.73	201.11	8.32	23.3
	Metachromadora	6.86	313.92	7.39	32.61
	Odontophora	179.16	59.21	5.28	39.26
	Linhomoeus	41.99	97.17	4.99	45.54
	Theristus	22.33	175.48	4.97	51.8
st12& st13	*Metalinhomoeus*	120.55	271.8	11.95	16.26
	Paramonohystera	135.44	33.57	8.21	27.44
	Odontophora	184.15	158.13	5.5	34.92
	Terschellingia	101.09	9.45	5.05	41.79
	Daptonema	48.93	213.04	4.24	47.55
	Sabatieria	82.42	0	4.09	53.12
st12 & st14	*Metalinhomoeus*	120.55	938.63	23.8	31.34
	Metachromadora	112.93	440.38	4.44	37.19
	Paramonohystera	135.44	30.41	4.21	42.74
	Odontophora	184.15	121.58	3.94	47.93
	Ptycholaimellus	77.37	321.87	3.81	52.94
st12 & st15	*Metalinhomoeus*	120.55	201.11	8.96	12.23
	Paramonohystera	135.44	10.1	7.49	22.46
	Metachromadora	112.93	313.92	4.57	28.7
	Linhomoeus	1.33	97.17	4.49	34.83
	Terschellingia	101.09	41.73	4.16	40.51
	Odontophora	184.15	59.21	3.95	45.9
	Theristus	77.04	175.48	3.8	51.09
st13 & st14	*Metalinhomoeus*	271.8	938.63	25.75	35.83
	Metachromadora	96.7	440.38	4.51	42.1
	Odontophora	158.13	121.58	3.95	47.59
	Ptycholaimellus	96.99	321.87	3.6	52.61
st13 & st15	*Metalinhomoeus*	271.8	201.11	11.28	16.78
	Linhomoeus	49.17	97.17	5.71	25.27
	Theristus	94.6	175.48	5.63	33.65

Groups	Genera	Av.Abund	Av.Abund	Av.Diss	Cum.%
	Metachromadora	96.7	313.92	5.39	41.67
	Odontophora	158.13	59.21	4.29	48.04
	Daptonema	213.04	169.08	4.07	54.09
st14 & st15	*Metalinhomoeus*	938.63	201.11	23.74	34.61
	Metachromadora	440.38	313.92	3.33	39.47
	Ptycholaimellus	321.87	76.36	3.24	44.19
	Theristus	73.97	175.48	3.12	48.75
	Odontophora	121.58	59.21	2.81	52.84
0-2 & 2-4	*Metachromadora*	525.52	36.11	9.26	12.24
	Odontophora	272.81	120.07	6.12	20.33
	Daptonema	262.96	30.65	6.12	28.42
	Ptycholaimellus	310.99	7.68	4.81	34.77
	Metalinhomoeus	299.73	277.86	4.81	41.13
	Chromadora	283.24	6.66	4.66	47.29
	Oncholaimellus	87.57	4.06	3.56	52
0-2 & 4-6	*Metachromadora*	525.52	20.85	9.73	11.99
	Odontophora	272.81	28.45	7.41	21.13
	Daptonema	262.96	40.4	6.22	28.79
	Metalinhomoeus	299.73	371.5	6.1	36.3
	Ptycholaimellus	310.99	25.06	4.77	42.18
	Oncholaimellus	87.57	0.26	4.76	48.05
	Chromadora	283.24	7.58	4.7	53.84
2-4 & 4-6	*Metalinhomoeus*	277.86	371.5	13.02	20.62
	Odontophora	120.07	28.45	7.09	31.86
	Paramonohystera	110.85	89.27	6.78	42.59
	Linhomoeus	60.82	38.32	3.86	48.71
	Theristus	56.33	56.28	3.77	54.68

Table S2.4. Result of distance-based linear model (DistLM) analyses showing the influence of environmental parameters on nematode density at two spatial scales (a, b: mesoscale: c, d: microscale). With the cumulative proportion of variation (adjusted R2) in nematode density that is explained by fitting variables within sets sequentially using step-wise selection, and conditional tests using 9999 permutations of residuals under a reduced model. Values in bold indicate P < 0.05.

a: mesoscale-marginal test

	0-2cm				2-4cm				4-6cm			
Variable	SS(trace)	Pseudo-F	P	Prop.	SS(trace)	Pseudo-F	P	Prop.	SS(trace)	Pseudo-F	P	Prop.
chl*a*	1686500	2.4	0.1271	6%	1147100	6.0	0.0172	14%	50565	1.4	0.2326	4%
fuco	2282700	3.3	0.0772	8%	726950	3.6	0.0617	9%	11770	0.3	0.5727	1%
pheophorbide	638610	0.9	0.3607	2%	328300	1.6	0.2152	4%	3118	0.1	0.7663	0%
log(pheophorbide+0.1)												
pheo *a*									153900	4.7	***0.0386***	11%
pyropheo *a*									152360	4.6	***0.0426***	11%
log(pheo *a*+0.1)	2865800	4.3	***0.0425***	10%	1110200	5.8	***0.0194***	13%				
log(pyropheo *a*+0.1)	3758300	5.8	***0.0186***	13%	995600	5.1	***0.0291***	12%				
log(PAP+0.1)	1435300	2.0	0.1675	5%								
PAP					243970	1.1	0.2868	3%	62715	1.8	0.1851	4%
peridinin	767140	1.1	0.3181	3%	13317	0.1	0.8263	0%	4564	0.1	0.7104	0%
log(diadino+0.1)	259680	0.4	0.5583	1%								
diadino					329880	1.6	0.2079	4%	45849	1.3	0.2557	3%
diato	1839200	2.6	0.1131	6%	4247500	39.1	***0.0001***	51%	189550	5.9	***0.0183***	13%
chl c2	20559	0.0	0.8638	0%	78204	0.4	0.55	1%	13837	0.4	0.5353	1%
									1031	0.0	0.8649	0%
log(a-car+0.1)	1208100	1.7	0.2006	4%	1219	0.0	0.948	0%				
b-car	3685700	5.7	***0.0247***	13%	3981500	34.5	***0.0001***	48%	174070	5.4	***0.0248***	12%
TOM	1909000	2.7	0.1046	7%	219250	1.0	0.3111	3%	19549	0.5	0.4385	1%
MGS	1703500	2.4	0.1319	6%	308210	1.5	0.24	4%	64602	1.8	0.1745	5%
Inundation	119430	0.2	0.6866	0%	745190	3.7	0.0597	9%	115090	3.4	0.073	8%
Silt	2289200	3.3	0.07	8%	477510	2.3	0.131	6%	100180	2.9	0.095	7%

b: mesoscale sequential test

layer	Variable	Adj R^2	SS(trace)	Pseudo-F	P	Prop.	Cumul.
0-2cm	**+log(pyropheo a+0.1)**	**0.11**	**3758300**	**5.8176**	**0.0206**	**13%**	**13%**
	+b-car	0.13	1177300	1.8638	0.1822	4%	17%
	+chl C2	0.24	3532400	6.4099	0.0157	12%	30%
	+log(pheo a+0.1)	0.30	1947600	3.8099	0.0598	7%	37%
	-log(pyropheo a+0.1)	0.31	74052	0.14486	0.7102	0%	37%
	+diato	0.33	837310	1.711	0.1966	3%	39%
	+pheophorbide	0.34	842420	1.7587	0.192	3%	42%
	+log(PAP+0.1)	0.38	1417300	3.1457	0.0847	5%	47%
	-log(pheo a+0.1)	0.38	428880	0.95187	0.337	2%	46%
	+TOM	0.41	1049800	2.4316	0.1274	4%	50%
	+log(pyropheo a+0.1)	0.41	598490	1.4031	0.2484	2%	52%
	+Inundation	0.42	575890	1.3656	0.256	2%	54%
2-4cm	**+diato**	**0.49**	**4247500**	**39.133**	**0.0001**	**51%**	**51%**
	+b-car	**0.61**	**1023400**	**12.21**	**0.0015**	**12%**	**63%**
	+chla	0.61	115230	1.3892	0.2401	1%	64%
4-6cm	**+diato**	**0.11**	**189550**	**5.9073**	**0.0203**	**13%**	**13%**
	+pheo a	0.13	54021	1.7152	0.1942	4%	17%
	+peridinin	0.13	34222	1.0892	0.2603	2%	20%

c: microscale marginal test

Variable	0-2cm SS(trace)	Pseudo-F	P	Prop.	2-4cm SS(trace)	Pseudo-F	P	Prop.	4-6cm SS(trace)	Pseudo-F	P	Prop.
chl*a*	11412000	2.94	0.1043	14%	329000	0.91	0.3436	5%	38354	0.10	0.7955	1%
fuco	11398000	2.94	0.1006	14%	291220	0.80	0.379	4%	1209	0.00	0.9589	0%
pheophorbide	8706200	2.16	0.1502	11%					388820	1.02	0.3422	5%
log(pheophorbide+0.1)					42282	0.11	0.7374	1%				
log(pheo a+0.1)	35404000	13.90	***0.0019***	44%	28582	0.08	0.789	0%				
log(pyropheo a+0.1)	31408000	11.34	***0.0037***	39%	1490.9	0.00	0.9519	0%				
log(PAP+0.1)	9875400	2.49	0.1316	12%								
pheo a									73085	0.18	0.6747	1%
pyropheo a									36581	0.09	0.7634	1%
PAP					317440	0.88	0.3622	5%	2120.3	0.01	0.9446	0%
peridinin	125020	0.03	0.8805	0%	35407	0.09	0.7617	1%	102420	0.26	0.6162	1%
diadino					14477	0.04	0.8448	0%	210400	0.54	0.5187	3%
log(diadino+0.1)	251.3	0.00	0.9957	0%								
diato	27095000	9.01	***0.0068***	33%	336890	0.94	0.3497	5%	323280	0.84	0.3702	4%
chl c2	1499600	0.34	0.5555	2%	18880	0.05	0.826	0%	1023600	2.97	0.105	14%
log(a-car+0.1)	19698000	5.76	***0.0244***	24%	38664	0.10	0.7598	1%				
a-car									1231300	3.69	0.071	17%
b-car	37006000	15.06	***0.0006***	46%	0.004609	0.00	1	0%	602970	1.64	0.2151	8%
TOM	9504900	2.38	0.1376	12%	420250	1.18	0.2916	6%	211670	0.54	0.4656	3%
MGS	21024000	6.28	***0.0209***	26%	277410	0.76	0.3962	4%	57.524	0.00	0.9905	0%
Silt	24693000	7.85	***0.0119***	30%	96546	0.26	0.611	1%	41427	0.1	0.759	6%

d: microscale sequential test

layer	Variable	Adj R^2	SS(trace)	Pseudo-F	P	Prop.	Cumul.
0-2cm	**+b-car**	**0.43**	**37006000**	**15.055**	**0.0008**	**46%**	**46%**
	+log(pheo a+0.1)	0.46	4669500	2.0058	0.1743	6%	51%
	+peridinin	0.54	8027400	4.0711	0.0659	10%	61%
	+diato	0.58	4821600	2.7061	0.1275	6%	67%
	-b-car	0.60	351120	0.19706	0.657	0%	67%
	+log(diadino+0.1)	0.69	7260500	5.4955	0.0335	9%	76%
	+fuco	0.72	3066100	2.5625	0.134	4%	79%
	+pheophorbide	0.73	1520800	1.2981	0.2737	2%	81%
2-4cm	+TOM	0.01	420250	1.1839	0.2876	6%	6%
	+diato	0.06	676970	2.0147	0.1725	10%	16%
4-6cm	+a-car	0.12	1231300	3.6946	0.0707	17%	17%
	+MGS	0.18	718790	2.3143	0.141	10%	27%

Table S2.5. Result of distance-based linear model (DistLM) analyses showing the influence of environmental parameters on nematode community composition at two spatial scales (a, b: mesoscale: c, d: microscale). With the cumulative proportion of variation (adjusted R^2) in nematode community that is explained by fitting variables within sets sequentially using step-wise selection, and conditional tests using 9999 permutations of residuals under a reduced model. Values in bold indicate $P < 0.05$. Bolded values were italic marked in sequential test when the variable was detected as insignificant in marginal test.

a: mesoscale marginal test

	0-2				2-4				4-6			
Variable	SS(trace)	Pseudo-F	P	Prop.	SS(trace)	Pseudo-F	P	Prop.	SS(trace)	Pseudo-F	P	Prop.
chl*a*	8222	3.3	***0.0104***	8%	7426	2.7	***0.0054***	7%	3822	1.3	0.1723	3%
fuco	9111	3.7	***0.005***	9%	6020	2.1	***0.0177***	5%	2613	0.9	0.5335	2%
pheophorbide	10055	4.2	***0.0011***	10%					2909	1.0	0.4297	3%
log(pheophorbide +0.1)					5580	2.0	***0.029***	5%				
log(pheo a+0.1)	14011	6.1	***0.0004***	14%	8871	3.2	***0.0007***	8%				
pheo									11808	4.4	***0.0002***	10%
pyro									10598	3.9	***0.0002***	9%
log(pyropheo a+0.1)	9206	3.8	***0.0027***	9%	8286	3.0	***0.0018***	7%				
log(PAP+0.1)	11587	4.9	***0.0005***	11%								
PAP					5118	1.8	***0.0444***	5%	8033	2.9	***0.0017***	7%
peridinin	11303	4.7	***0.0005***	11%	3764	1.3	0.1777	3%	2147	0.7	0.7443	2%
log(diadino+0.1)	4920	1.9	0.0709	5%	5668	2.0	***0.0247***	5%				
diadino									4155	1.5	0.126	4%
diato	5668	2.2	***0.0397***	6%	12574	4.8	***0.0001***	11%	5658	2.0	***0.0281***	5%
chl c2	5113	2.0	0.0691	5%	5464	1.9	***0.0325***	5%	4261	1.5	0.1124	4%
log(a-car+0.1)	5625	2.2	***0.0473***	6%	4283	1.5	0.1051	4%				
a-car									4365	1.5	0.084	4%
b-car	9497	3.9	***0.0043***	9%	14763	5.7	***0.0001***	13%	10761	4.0	***0.0006***	10%
TOM	10886	4.5	***0.0016***	11%	4552	1.6	0.0828	4%	3840	1.3	0.1683	3%
MGS	11385	4.8	***0.001***	11%	4544	1.6	0.0848	4%	7016	2.5	***0.0055***	6%
Inundation	7022	2.8	***0.0207***	7%	5408	1.9	***0.0392***	5%	4668	1.6	0.0752	4%
Silt	12309	5.2	***0.0004***	12%	6220	2.2	***0.0188***	6%	8321	3.0	**0.002**	7%

b: mesoscale sequential test

layer	Variable	Adj R^2	SS(trace)	Pseudo-F	P	Cumul.	res.df
0-2cm	**+log (pheo a+0.1)**	**0.115**	**14011**	**6.1**	**0.0002**	**14%**	38
	+log (a-car+0.1)	**0.151**	**5751**	**2.6**	**0.0175**	**19%**	37
	+chl C2	**0.190**	**5885**	**2.8**	**0.0135**	**25%**	36
	+chl*a*	**0.220**	**4879**	**2.4**	**0.0304**	**30%**	35
	+diato	**0.262**	**5811**	**3.0**	**0.0093**	**36%**	34
	+Silt	**0.292**	**4504**	**2.4**	**0.0282**	**40%**	33
	+pheophorbide	0.310	3362	1.9	0.0796	43%	32
	+peridinin	0.329	3315	1.9	0.0766	47%	31
	+b-car	0.344	2925	1.7	0.1057	50%	30
	+Inundation	0.359	2838	1.7	0.1039	52%	29
	+fuco	0.369	2382	1.4	0.164	55%	28
	+MGS	0.373	1931	1.2	0.2766	57%	27
	+log (PAP+0.1)	0.374	1728	1.1	0.3433	58%	26
	-log (pheo a+0.1)	0.374	1612	1	0.3941	57%	25
2-4cm	**+b-car**	**0.108**	**14763**	**5.7**	**0.0001**	**13%**	38
	+diato	**0.137**	**5704**	**2.3**	**0.0071**	**18%**	37
	+Silt	**0.168**	**5743**	**2.4**	**0.0065**	**23%**	36
	+MGS	***0.198***	***5435***	***2.3***	***0.0052***	***28%***	35
	+chl C2	**0.221**	**4620**	**2.0**	**0.0129**	**32%**	34
	+log(a-car+0.1)	0.232	3348	1.5	0.094	35%	33
	+log(pheophorbide+0.1)	0.243	3170	1.4	0.1143	38%	32
	+PAP	0.248	2724	1.3	0.2273	40%	31
	+diadino	0.261	3275	1.5	0.0905	43%	30
	+log(pheo a+0.1)	0.274	3202	1.5	0.0999	46%	29
	+Inundation	0.281	2697	1.3	0.186	48%	28
	+log(pyropheo a+0.1)	0.292	2908	1.4	0.128	51%	27
4-6cm	**+pheo a**	**0.081**	**11808**	**4.4**	**0.0003**	**10%**	38
	+a-car	**0.103**	**5031**	**1.9**	**0.0203**	**15%**	37
	+Inundation	***0.134***	***5746***	***2.3***	***0.0043***	***20%***	36
	+chl C2	0.145	3610	1.5	0.113	23%	35
	+chl*a*	0.162	4172	1.7	0.0458	27%	34
	+fuco	0.185	4673	2.0	0.0155	31%	33
	+MGS	0.205	4162	1.8	0.0295	35%	32
	+Silt	0.213	3057	1.3	0.1567	37%	31
	-pheo a	0.214	2217	1.0	0.4819	36%	32
	+peridinin	0.222	2980	1.3	0.1856	38%	31
	+b-car	0.226	2576	1.2	0.3107	40%	30
	+TOM	0.226	2242	1	0.454	42%	29

c: microscale marginal test

MARGINAL TESTS	0-2cm				2-4cm				4-6cm			
Variable	SS(trace)	Pseudo-F	P	Prop.	SS(trace)	Pseudo-F	P	Prop.	SS(trace)	Pseudo-F	P	Prop.
chl*a*	3361	1.4	0.1846	7%	1964	0.7	0.7412	4%	1592	0.6	0.8334	3%
fuco	3516	1.5	0.1688	8%	2094	0.8	0.6877	4%	1550	0.5	0.8293	3%
pheophorbide	2798	1.2	0.2634	6%					3356	1.2	0.2976	6%
log(pheophorbide+0.1)					2717	1.0	0.434	5%				
log(pheo a+0.1)	5191	2.3	***0.047***	11%	4133	1.6	0.0991	8%				
pheo									2120	0.7	0.6341	4%
pyro									2158	0.8	0.6345	4%
log(pyropheo a+0.1)	7466	3.5	***0.0085***	16%	3909	1.5	0.1282	8%				
log(PAP+0.1)	6028	2.7	***0.0118***	13%								
PAP					2572	0.9	0.4895	5%	2102	0.7	0.6548	4%
peridinin	825	0.3	0.9833	2%	3350	1.3	0.2374	6%	1515	0.5	0.8488	3%
log(diadino+0.1)	4173	1.8	0.1007	9%								
diadino					3623	1.4	0.173	7%	2755	1.0	0.4692	5%
diato	5003	2.2	0.0507	11%	4131	1.6	0.1041	8%	2139	0.7	0.6183	4%
chl c2	2784	1.2	0.2956	6%	3041	1.1	0.3219	6%	2598	0.9	0.4688	5%
log(a-car+0.1)	13945	7.9	***0.0003***	30%	4651	1.8	0.0566	9%				
a-car									7585	3.0	***0.0082***	14%
b-car	10384	5.3	***0.0002***	23%	4864	1.9	***0.0427***	9%	9647	4.0	***0.0023***	18%
TOM	6762	3.1	***0.0132***	15%	2748	1.0	0.4259	5%	4682	1.7	0.103	9%
MGS	10220	5.2	***0.0007***	22%	1692	0.6	0.8435	3%	2774	1.0	0.4109	5%
Silt	11082	5.7	***0.001***	24%	2276	0.8	0.6087	4%	5342	2.0	0.065	10%

d: microscale sequential tests

	Variable	Adj R^2	SS(trace)	Pseudo-F	P	Cumul.	res.df
0-2cm	**+log (a-car+0.1)**	**0.266**	**13945**	**7.9**	**0.0002**	**30%**	18
	+diato	***0.357***	***5519***	***3.6***	***0.0039***	***42%***	17
	+chla	***0.414***	***3727***	***2.6***	***0.0071***	***51%***	*16*
	+chl C2	0.448	2659	2.0	0.0534	56%	15
	+log (diadino+0.1)	0.488	2669	2.2	0.0358	62%	14
	+log (PAP+0.1)	0.545	3012.6	2.7	0.0069	69%	13
	+b-car	0.558	1489.5	1.4	0.1928	72%	12
	+fuco	0.570	1377.9	1.3	0.2365	75%	11
	+log (pheo a+0.1)	0.585	1396	1.4	0.2163	78%	10
2-4cm	**+b-car**	**0.044**	**4864**	**1.9**	**0.046**	**9%**	18
	+peridinin	0.083	4376	1.8	0.0747	18%	17
	+log (pheo a+0.1)	0.126	4371	1.8	0.0536	26%	16
	+chl C2	0.147	3230	1.4	0.1732	33%	15
4-6cm	**+b-car**	**0.135**	**9647**	**4**	**0.0032**	**18%**	18
	+TOM	0.161	3697	1.6	0.1257	25%	17
	+a-car	0.168	2677	1.1	0.3085	30%	16
	+PAP	0.171	2467	1.1	0.3687	35%	15

Chapter 3 Natural stable isotope ratios and fatty acid profiles of estuarine tidal flat nematodes reveal very limited niche overlap among co-occurring species

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Chapter 3 Natural stable isotope ratios and fatty acid profiles of estuarine tidal flat nematodes reveal very limited niche overlap among co-occurring species

Abstract

The high local-scale species diversity of marine meiofauna, and of nematodes in particular, has puzzled ecologists for decades. Both pronounced niche differentiation and neutral dynamics have been suggested as mechanisms underlying that high diversity. Differential resource use is the most plausible basis for niche differentiation, yet the vast majority of studies demonstrating that this is prominent in marine nematodes are based on laboratory experiments on single species or highly simplified assemblages. Only a small number of studies have investigated resource differentiation under natural conditions. Here we use natural stable-isotope ratios of carbon and nitrogen, as well as fatty-acid profiles, to assess differential resource use and trophic structure in nine abundant estuarine tidal flat nematode species, comprising different presumed feeding modes (deposit feeders, epistratum feeders, predators, …) and resource guilds (herbivores, carnivores, …). We demonstrate that resource differentiation is pronounced among as well as within feeding modes and resource guilds. Nematodes comprise up to three different trophic levels (from primary to tertiary consumers), yet with the exception of some herbivores, omnivory is prominent. Bivariate isotopic niche spaces were of similar size among most species, irrespective of their trophic level. Herbivory importantly contributes to the nutrition of herbivores as well as carnivores; it mainly targets diatoms in some species, yet prominently includes dinoflagellates in others. Bacteria, in contrast, appear to be of limited nutritional importance. *Odontophora setosus* is identified as a predator/omnivore with a trophic level in between that of secondary and tertiary consumers.

Key words: stable isotopes, fatty acids, biomarkers, marine nematodes, tidal flat, trophic niche, microphytobenthos, predation, omnivory

3.1 Introduction

Estuarine tidal flat sediments are highly productive ecosystems, the productivity of which can be driven by a broad range of organic matter inputs, including settled phytoplankton and particulate detritus of both terrestrial and marine origin, as well as of macroalgae, seagrasses and/or salt marsh vegetation (Heip et al., 1995; Middelburg et al., 1996; Herman et al., 2001). In most cases, however, the *in situ* productivity of microbial biofilms, i.e. complex consortia of benthic microalgae and heterotrophs embedded in a biogenic polymer matrix (Decho, 1990; Stal, 2010), fuels a major part of the secondary production on estuarine intertidal flats (Heip et al., 1995; Herman et al., 1999), and thus

forms an important basis of estuarine food webs that support commercially relevant fish and shellfish stocks as well as migratory bird populations (Cook et al., 2009). They play a pivotal role in carbon and nitrogen fluxes across the sediment-water interface, thereby affecting coastal eutrophication and water quality (Hochard et al., 2010). Biofilms also stabilize tidal flat sediment surfaces, thus reducing erosion (Paterson and Black, 1999; Stal, 2010). Nevertheless, several unknowns still exist about the complex interplay between microphytobenthos (MPB), benthic consumers and sediment properties.

Several studies have provided compelling evidence that MPB is the main basal resource fueling both a part of the macro- (Herman et al., 1999, 2001) and the majority of the meiofauna (mainly nematodes and copepods) (Moens et al., 2002, 2005a; Rzeznik-Orignac et al., 2008; Moens et al., 2014; Cnudde et al., 2015) on estuarine intertidal flats. Nevertheless, whereas meiofauna from sandy intertidal sediments can have almost purely MPB-based isotopic signatures, there are typically at least some representatives in muddy sediments which appear influenced by deposited phytoplankton or detritus, more so when mudflats are more sheltered or have features that enhance deposition of suspended particulate organic matter, such as the presence of vegetation (Moens et al., 2002, 2005a; Cnudde et al., 2015). Freshly settled phytoplankton can in some cases also contribute substantially to the diet of meiobenthos from sandy intertidal sediments (Maria et al., 2011; Evrard et al., 2012).

The high abundances and generally high biomass turnover rates of meiofauna, mainly nematodes (Moens et al., 2013), have caused many speculations about their importance in tidal flat sediments. The ecological importance of meiofauna to soft-bottom marine ecosystems can be manifold (Schratzberger and Ingels, 2018): they can microbioturbate sediments, thereby influencing fluxes of oxygen and nutrients and affecting organic matter decomposition and biogeochemical cycles (Cullen, 1973; Aller and Aller, 1992; Nascimento et al., 2012; Bonaglia et al., 2014). Their grazing and non-trophic interactions may affect the activity and community structure of both MPB and of sediment bacteria (De Mesel et al., 2003; De Mesel, 2004; Hubas et al., 2010; D'Hondt et al., 2018) and thus probably also some of the ecosystem processes mediated by these micro-organisms. Their grazing rates may on average amount to 1 % of MPB and bacterial biomass per hour (Montagna et al., 1995), but large variation around that average has been reported. Finally, benthic meiofauna can be an important food source for higher trophic levels, not only quantitatively, but also qualitatively because of the presence of high amounts of polyunsaturated fatty acids (PUFA) (Leduc and Probert, 2009; Leduc et al., 2015), thus forming a potentially important link between primary producers and higher trophic levels (Coull, 1999; Danovaro et al., 2007).

While it is generally accepted that at the higher-taxon level, marine nematodes can consume a broad array of resources, including prokaryotes, auto/mixo- and heterotrophic protists, and various benthic

invertebrates (Jensen, 1987; Moens and Vincx, 1997; Moens et al., 2004), information on feeding ecology and resource partitioning at the species level remains very scant (Moens et al., 2004). As an example, while MPB is undoubtedly a pivotal carbon source for many intertidal nematodes (Moens et al., 2005a; Rzeznik-Orignac et al., 2008), the pathways through which carbon and energy from MPB are transferred to nematodes are not always very clear. For example, there is debate whether nematode species obtain the MPB carbon directly through herbivory or indirectly, for instance through bacteria and/or herbivorous protists that feed on MPB and its extracellular polymeric substances (EPS) (Moens et al., 2005a, 2014).

There is a widespread habit in marine nematode ecology to assign nematodes to a limited number of feeding types, largely based on the morphology of the feeding apparatus (Wieser, 1953; Jensen, 1987; Moens and Vincx, 1997). Not only do such feeding-type classifications funnel the high species diversity of marine nematodes into a very limited trophic diversity, they also act as black boxes, ignoring the possibility that nematodes may shift from one feeding type to another depending on food availability and/or competitive interactions with other benthic invertebrates (Moens and Vincx, 1997; Moens et al., 2004).

Equally problematic from an ecosystem functioning point of view, is that the feeding guilds largely reflect feeding mode rather than resources (Moens et al., 2004). For example, both deposit feeders and epistratum feeders probably graze on (the same?) benthic microalgae, but in different ways. Predators/omnivores are capable of predation on other benthic invertebrates and/or heterotrophic protists (Moens and Vincx, 1997; Hamels et al., 1998), but at least some of these species may be very flexible feeders that can switch to herbivory (Franco et al., 2008b; Moens et al., 2014) or bacterivory (Moens et al., 1999b), depending on resource availability.

A direct consequence of our lack of species-level knowledge on nematode feeding ecology, is that the role of resource selectivity as a driver of the often species-rich local assemblages remains a matter of debate (Moens and Beninger 2018). Indeed, although it has been suggested that most marine nematodes may be relatively flexible feeders (Moens et al., 2004), it is unclear to what extent species within and among feeding groups compete for resources. It is equally unclear whether those that utilize MPB as a resource, do so selectively or rely primarily on particular components of the MPB.

Studying trophic interactions between animals and their resources is important to understand their fundamental characteristics (e.g. individual growth, population dynamics) and ecosystem functioning. A combination of dual stable isotope and fatty acid profiles has proven its use in examining food-web interactions and in tracing an animal’s diet (Neubauer and Jensen, 2015). Natural stable isotope ratios of carbon and nitrogen can provide good indication of the basal resources fuelling food webs, as well

as of the trophic level of consumers (Peterson and Fry, 1987; Vander Zanden and Rasmussen, 2001; Post, 2002). On the other hand, this technique has limitations, for instance with respect to identifying which exact primary producers act as a basal resource, because different primary producers (for instance in tidal flat biofilms) often have only very limited isotopic differences (Mutchler et al., 2004). Fatty acid (FA) profiles of consumers and their resources may offer complementary information that can allow to further disentangle food-web links (Neubauer and Jensen, 2015), for instance because certain primary producers (e.g. diatoms and dinoflagellates) with overlapping stable-isotope signatures have distinct FA biomarkers. Combined use of stable isotopes and FA in marine nematodes has nevertheless remained rare (but see Leduc, 2009; Leduc and Probert, 2009; Van Gaever et al., 2009; Guilini et al., 2013; Braeckman et al., 2015; Leduc et al., 2015; Van Campenhout and Vanreusel, 2016).

Against the background of several published papers which have convincingly demonstrated that nematodes on estuarine tidal flats are largely fuelled by MPB carbon (Carman and Fry 2002; Moens et al. 2002, 2005, 2014; Rzeznik-Orignac et al. 2008), the present paper determined natural stable carbon and nitrogen isotopes as well as fatty acid profiles of nine abundant nematode species, representing different feeding guilds to address the following questions and hypotheses. Firstly, we evaluated the hypothesis that nematodes belonging to the often microalgae-consuming feeding types deposit feeders and epistratum feeders, and species belonging to the feeding types facultative predators and predators represent clearly separate trophic levels, i.e. primary and secondary consumers, respectively. To this end, we assessed the trophic level of several nematode species which are presumed to be mainly consumers of MPB and of others which are known as facultative or strict carnivores. Secondly, by comparing trophic level and resource use of multiple species of presumed primary and secondary consumers, we tested the degree of resource partitioning among nematode species with supposedly similar feeding ecology. We used isotopic niche spaces as well as multivariate analysis of fatty acid profiles to assess this concept. Thirdly, we used fatty acid biomarkers to investigate the contribution, if any, of hitherto poorly documented resources such as dinoflagellates and zooplankton (dead and/or faecal pellets) in the diet of intertidal nematodes. In addition to these main aims, we also assessed the following more specific hypotheses: a) microalgal grazers which ingest their prey whole are more likely to co-ingest bacteria and EPS, and will therefore have higher contributions of bacterial biomarkers in their diet; b) omnivory is common in nematodes with presumed predatory ecology.

3.2 Materials and Methods

3.2.1 Sampling site, sampling procedure and collection of nematodes

Sampling was conducted at the Paulina intertidal flat (Gallucci et al., 2005; Cnudde et al., 2015) in the polyhaline reach of the Schelde Estuary, SW Netherlands. This tidal flat is characterised by a high heterogeneity in sediment types, which range from muddy in the more downstream parts to medium sandy at the most upstream portion of the tidal flat. Moreover, there is a salt marsh bordered by muddy sediments in the downstream part of this intertidal area.

Our samples for stable isotope analyses were collected in a transition zone with a dynamic mosaic of patches of different sediment compositions (Gallucci et al., 2005; Cnudde et al., 2015) in an area of ca. 200 x 200 m (stations 1 and 6, Fig. 3.1). Whereas the nematode assemblages inhabiting the extremes of the sedimentary gradient from muddy to sandy are very different (Wu, unpubl.; Bezerra, unpubl.; Gallucci et al. 2005), within the transition zone, patches which differ more subtly in granulometry have different yet partly overlapping assemblage compositions. Based on prior knowledge of the area (Bezerra, unpubl.; Wu, unpubl.), we *a priori* identified 8 genera (Table 3.1) that are typically abundant in fine- to medium-sandy sediments with a relatively low silt content (≤ 15 %) at this tidal flat. A ninth genus (Table 3.1) that only occurred in silty sediment was included here because of its hitherto completely unresolved feeding ecology. We sampled two sites (st1, st6) in the above-mentioned transitional area and an additional one in a silty gully of the salt marsh (st16), where some of our target species also reach high abundances. Samples for stable isotope analysis (SIA) were collected from the two sites in the transitional area only, except for the ninth nematode species *Odontophora setosus* (see below), whereas samples for fatty acid analysis (FAA) originated from either the transitional area or the salt marsh gully or both. Samples for SIA and FAA were collected in the same season (late spring, June) but in different years: 2010 for the SI samples and 2014 for the FA samples. Samples for stable isotope analysis were stored frozen at -20°C. One sample at a time was taken from the freezer, thawed and washed using ludox to separate nematodes from sediment. We processed a sample within max. two days; during this period, we always took a subsample to work on, whilst the bulk of the sample was maintained at 4°C in the fridge to slow down any decomposition.

Sediment samples for the extraction of nematodes for FAA were collected in a non-quantitative way by scraping the top 1-2 cm of sediment off using a small shovel and pooling it per site into a bucket. The collected sediment was hand-mixed in the field and – upon return to the lab – incubated overnight at environmental temperature with a thin layer of habitat water on top. During this incubation, many nematodes move from deeper layers towards the surface, hence even fairly small subsamples from the surface layer in the buckets tend to yield high abundances of live nematodes. Nematodes were

extracted alive by simple, repeated decantation over a 63-µm or a 125-µm mesh size sieve after vigorous stirring of samples with a jet of tap water, as our targeted nematodes have relatively large body sizes (Table 3.1), and using a larger pore diameter thus resulted in removal of more detrital and very fine sediment particles, whilst retaining most specimens of the nematode species used here. This procedure facilitates release of the nematodes from the sediments (Somerfield et al., 2005) and was repeated 5 to 10 times. The nematodes were then harvested from the sieve using a small volume of sterile artificial seawater (ASW (Dietrich and Kalle, 1957)) of ambient salinity and stored in the dark at 4°C until further processing.

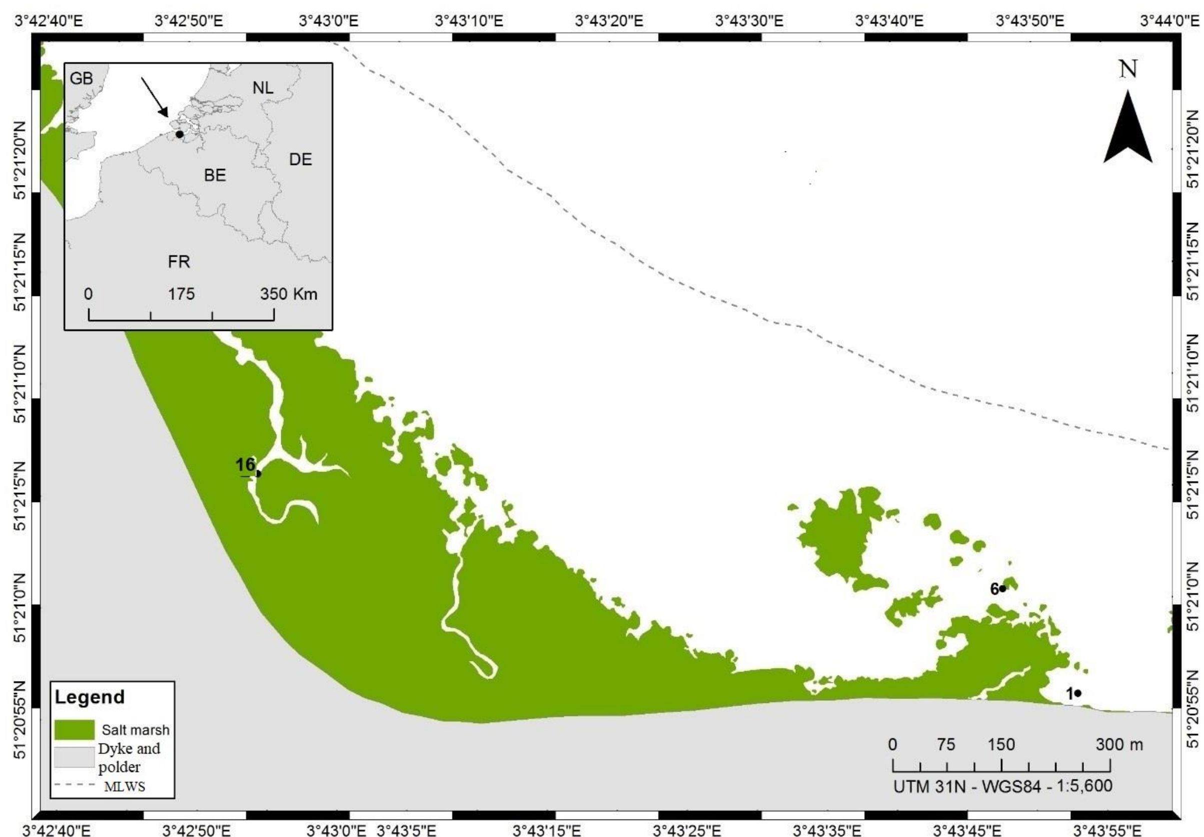

Fig. 3.1 1. Map of sampling locations at the Paulina polder intertidal flat, The Netherlands. Numbers indicate the different sampling stations; mean low water spring tide level was indicated by MLWS, high water spring tide level coincided with the position of the dyke.

3.2.2 Selection of nematode taxa for stable-isotope and fatty-acid analyses

We chose a selection of locally abundant nematode genera (Table 3.1) that encompass a variety of traits, including different body sizes, feeding habits and – presumably – trophic levels.

Theristus acer is a deposit feeder that ingests diatoms, other microalgae and perhaps other unicellular organisms, particle size determining the upper limit of food items that can be ingested (Moens and Vincx, 1997). *Daptonema hirsutum* belongs to the same family and feeding type (deposit feeders *sensu* Moens and Vincx (1997), or non-selective deposit feeders *sensu* Wieser (1953)), yet is considerably larger and wider than *T. acer*, and hence may be expected to be capable of ingesting a broader range of food particles. In both *Theristus* and *Daptonema*, diatom frustules can commonly be observed in the gut, confirming their contribution to the diet of these nematodes (Nehring et al., 1990; Moens and Vincx, 1997).

Table 3.1. Nematode characteristics, including body width and length, feeding type according to Moens and Vincx (1997), and numbers of replicate samples for stable isotope (SI) and Fatty Acid Methyl Esters (FAMEs) analysis. DF = deposit feeder, EF = epigrowth feeder, P = predator, FP = facultative predator. The feeding guild of *Odontophora* is unknown.

Species	width(μm)	length(μm)	feeding type	Replicate number SI	Replicate numbers FAME(st1,st16,st6)
Theristus acer	44±3	1780±67	DF	1	3,3,0
Daptonema hirsutum	72±17	1640±97	DF	3	0,0,4
Praeacanthonchus punctatus	73±8	1822±102	DF/EF	4	2,4,4
Metachromadora remanei	59±12	1275±70	EF	6	3,4,0
Enoploides longispiculosus	118±5	3020±194	P/FP	7	2,0,0
Adoncholaimus fuscus	165±17	4934±30	FP	7	3,0,0
Oncholaimus oxyuris	62±2	3800±120	FP	1	3,0,0
Enoplus brevis	176±1	7000±800	P	3	0,3,0
Odontophora setosus	34±1	3050±351	?	2	0,0,3

Praeacanthonchus punctatus has been classified as an epistratum feeder because of the presence of buccal armature (Wieser, 1953). However, observations indicate that it mostly swallows whole prey, much like the above-mentioned deposit feeders. Either way, this species has been demonstrated to very actively graze on diatoms and is therefore generally considered as a herbivore (Moens et al., 2014). Herbivory has also been proposed as the main feeding strategy of *Metachromadora remanei* (Moens et al., 2005a), although this genus was initially classified as a predator based on its strong tooth and very muscular pharynx (Wieser, 1953). *M. remanei* does not ingest its food whole but pierces diatom cells with its tooth, then sucks out their contents (Moens et al., 2005a).

The four species mentioned thusfar are considered primary or secondary consumers (as bacterivory may occur, particularly – though not exclusively – in the deposit feeders), although in a stable isotope study on the feeding ecology of nematodes in a *Zostera* seagrass bed, the genera *Metachromadora* and *Daptonema* did not stand out as grazers of microphytobenthos or epiphytic microalgae, but rather of fungi and/or bacteria associated with decomposing *Zostera* detritus (Vafeiadou et al., 2014).

The remaining species are considered secondary or higher-order consumers. *Enoploides longispiculosus* was long considered a strict predator of other nematodes (Moens et al., 2000), ciliates (Hamels et al., 1998) and other small benthic invertebrates (Moens and Vincx, 1997), but it has meanwhile been shown to also graze on microalgae (Franco et al., 2008b, Moens et al., 2014). It is therefore undoubtedly an omnivore. Oncholaimidae such as *Adoncholaimus fuscus* and *Oncholaimus oxyuris* are capable of predation on other nematodes, but probably have other feeding strategies as well, perhaps including bacterivory (Moens et al., 1999a). Microalgae are only rarely seen in their intestines. They have been classified as facultative predators, where strategies other than predation are poorly understood, although they may encompass some form of deposit feeding (Meyers et al., 1970). *Enoplus brevis* is a generalist feeder which is capable of ingesting a range of prey, from cyanobacteria over microalgae to many benthic invertebrates (Hellwig-Armonies et al., 1991). Finally, *Odontophora setosus* strongly resembles genera that are commonly believed to be deposit feeders. They are long and very slender nematodes with fairly narrow mouth openings, yet they do possess a buccal cavity with cuticularised walls and a ring of six odontia, which could point to a predatory feeding strategy; their assignment to any feeding type therefore remains dubious (Austen et al., 1998). We only encountered this species in silty sediments in front of the salt marsh, but decided to include it as it is a common genus in many coastal nematode assemblages, yet empirical information on its feeding ecology is totally lacking. Henceforth, we refer to these nine species by their genus name.

3.2.3 Preparation of nematode samples for stable-isotope and fatty-acid analyses

After decantation (see above, section 'Sampling site, sampling procedure and collection of nematodes'), nematodes were maintained in sterile ASW with a salinity of 25 (psu) in the fridge until further sample processing. This sample processing was performed within 2 days after field sampling. Nematodes were hand-picked one by one on the tip of a tungsten wire under a Leica M5 binocular (20-40X) and transferred to sterile ASW to rinse off adhering particles, then – in the case of nematodes collected for SIA – transferred again one by one to precombusted (4 h at 500 °C) 2.5 x 6 mm aluminium cups (Elemental Microanalysis Ltd) with a few drops of milliQ water. These cups were kept upright in a multiwell plate and allowed to dry for 3 h at 60 °C, after which they were pinch closed with sterile forceps, and kept under dry atmosphere until isotopic analysis (Moens et al., 2005a, 2014). Sufficient individuals were pooled per cup to ensure that enough biomass was available for reliable C and N analysis (≥ 5 µg of each element). Given the large differences in nematode size and biomass, this implies that very different numbers of specimens were pooled for different species.

Nematodes for FA analysis were hand-sorted in much the same way as for SIA. However, instead of transferring them into aluminium cups, they were stored in 2.5-ml GC vials with ASW. Immediately after transfer of the last nematode, a vial was centrifuged for 6 min at 1800 g and the supernatant

ASW replaced by milliQ water for rinsing during a final centrifugation step, after which most of the supernatant milliQ water was gently siphoned off and the pellet with the nematodes was immediately stored frozen at -80 °C and later freeze-dried.

3.2.4 Stable isotope analysis

The aluminium cups containing nematodes were combusted in a ThermoFinnigan 1112 elemental analyser coupled online through a Conflo III interface to a ThermoFinnigan Delta Plus XL isotope ratio mass spectrometer for the simultaneous analysis of C and N isotopes. Isotope ratios are expressed as δ values in units of ‰ relative to the conventional standards, i.e. Vienna Peedee Belemnite for C and atmospheric N_2 for N, δ being equal to $(R_{sample}/R_{standard} - 1) \times 1000$ (Fry, 2007). In this formula, R is the ratio of the heavy to the light isotope. IAEA (International Atomic Energy Agency) standards CH6 (sucrose) and N1 (ammonium sulphate) were used as external standards, with at least one standard being measured after every 10 regular samples.

All $\delta^{13}C$ values so obtained were corrected for possible carbon contamination of sample cups according to the procedure described in Moens et al. (2014). No such correction was required for $\delta^{15}N$ data.

3.2.5 Fatty acid analysis

The freeze-dried nematode samples were subjected to a slightly adapted (in terms of reagent volumes) version of the protocol by (Masood et al., 2005) to extract lipids and prepare methyl esters. Fatty Acid Methyl Esters (FAMEs) were analyzed, identified and quantified following (De Troch et al., 2012). In short, we performed gas chromatography-mass spectrometry in splitless mode with a Hewlett Packard 6890N gas chromatograph coupled to an HP 5973 mass spectrometer, using the same injection and running time parameters as De Troch et al. (2012). FAMEs so obtained were identified by comparing their retention times and mass spectra with those of authentic standards and available ion spectra in WILEY mass spectral libraries and analysed with the software MSD ChemStation (Agilent Technologies), using external standards (Supel-coTM 37 Component FAME Mix, Supelco # 47885, Sigma-Aldrich Inc., USA) for individual FAME quantification (De Troch et al., 2012). FA concentrations were determined by reference to the internal standard C19:0 (Fluka 74208, Sigma Aldrich, USA). Fatty acid notation is in the form of A:BωX, where A represents the number of carbon atoms, B gives the number of double bonds and X is the position of the double bond closest to the terminal methyl group (Guckert et al., 1985).

3.2.6 Fatty acid biomarkers

Although the usefulness of some fatty acid biomarkers depends on habitat and environmental conditions (Parrish et al., 2000), we applied fatty acid biomarkers which have repeatedly been used in temperate estuarine environments (Kelly and Scheibling, 2012).

Diatoms, which usually form by far the main component of microphytobenthos on tidal flats in the polyhaline reach of the Schelde Estuary (Sabbe and Vyverman, 1991; Hamels et al., 1998), were indicated by the concentration of C16:1ω7 (Dalsgaard et al., 2003) as well as by the ratio of C16:1/C16:0 (Claustre et al., 1988). Longer-chain FA like eicosapentaenoic acid (EPA) are abundant in, but not unique to, diatoms. Docosohexaenoic acid (DHA) only occurs in limited abundance in diatoms, but is prominently present in dinoflagellates, which can also form an important part of MPB. Hence, we applied the ratio EPA/DHA as a measure of the relative importance of diatoms *vs* dinoflagellates, lower values indicating a higher prominence of dinoflagellates (Parrish et al., 2000; Kelly and Scheibling, 2012). When concentrations of C_{18} PUFA (polyunsaturated fatty acids) are low (≤ 3%), the contributions of SFA (saturated fatty acids) (C14:0 + C16:0 + C18:0) can be used as indicators of feeding on dinoflagellates and prymnesiophytes such as *Phaeocystis* (Dalsgaard et al., 2003; Braeckman et al., 2015).

We used the sum of FA C15:0 and C17:0 to indicate feeding on prokaryotes in general (Parrish et al., 2000; Kelly and Scheibling, 2012), whereas C18:1ω7 was used as a marker of chemoautotrophic bacteria (Van Gaever et al., 2009; Cnudde et al., 2015).

Other sources, such as salt marsh vascular plants and green algae, were indicated by C18:1ω9 (Kelly and Scheibling, 2012), whereas vascular plant detritus of terrestrial origin was indicated by a sum of LC-SFA (C20-C24)(Douglas et al., 1970; Cnudde et al., 2015). Microzooplankton was indicated by arachidonic acid (ARA, 20:4ω6) (Parrish et al., 1995) and zooplankton by a sum of C20:1 and C22:1 (Parrish et al., 2000). Finally, we used the ratio of PUFA/saturated FA (PUFA/SFA) and the abundance of 20:1ω9 as indicators of carnivory (Cripps and Atkinson, 2000).

3.2.7 Data analysis

3.2.7.1 Dual stable isotope data

We visually inspected dual (C + N) isotope plots as a first pointer to major carbon sources and to the trophic level of nematode taxa. Given the existence of previous studies highlighting the predominant contribution of microphytobenthos to nematodes at this (and other) tidal flat(s) (Moens et al., 2002, 2005a, 2014), our goal was not to assess in detail the contributions of different carbon sources to the

diets of nematodes, but rather to reconstruct the nematode part of a benthic food chain from MPB to higher trophic levels and to assess resource overlap between different nematode taxa. We used the formula

$$TL = (\delta^{15}N_{consumer} - \delta^{15}N_{baseline})/FF + TL_{baseline}$$

to estimate trophic level, where TL = trophic level, baseline is an organism of known trophic level, and FF is the N fractionation factor at trophic transfer (Post et al., 2000; Post, 2002). Given the variability of the FF (McCutchan et al., 2003), we used two scenarios, one with the often proposed FF of 3.4 (Minagawa and Wada, 1984), the other with an FF value of 2.5 ‰ as proposed by Vander Zanden and Rasmussen (2001). This comparison allowed us to assess if, and to what extent, different FF scenarios affect the main conclusions on nematode trophic level. Each of these two scenarios was run for two different baseline organisms: one with microphytobenthos as a primary producer at trophic level 1, the other with *Metachromadora remanei* as a herbivore at trophic level 2 (Moens et al., 2005a). The latter was done because the present and a previous study found a large offset in $\delta^{15}N$ (close to 5) between MPB and the nematodes with lowest $\delta^{15}N$ (see Moens et al., 2014, for possible explanations). Unpublished dual stable-isotope data from seven consecutive samplings on a bimonthly basis at Paulina in 2010-2011 demonstrate that all-year long, *M. remanei* consistently had (one of) the lowest δ15N of all nematode species analysed. It is therefore plausible that this species is a first-order consumer which feeds primarily as a herbivore on MPB (Moens et al. 2005; Bezerra and Moens unpubl.).

We further used our stable-isotope data to calculate two descriptive metrics that assess the niche width of consumers, i.e. convex hull volumes (CHV) (Layman et al., 2007) and standard ellipse areas (SEA) (Jackson et al., 2011). While CHV provide a suitable representation of niche width, they are rather sensitive to small sample sizes (Jackson et al., 2011), an issue which is less important for SEA, which use Bayesian inference and allow robust comparisons with data sets comprising different sample sizes. When sample size is generally low, as is the case in our study, a corrected SEA (SEAc) is calculated which leads to a slightly larger ellipse but with the same geometrical shape as SEA (Jackson et al., 2011). The SEAc, containing ~40% (default value in SIBER) of the data (centred on the mean and SDs of the bivariate data as semi-axes), and convex hulls were used to delineate isotopic niche spaces per nematode species. Differences in niche area between species, as well as niche overlap among the ellipses of different species, were derived using Bayesian inference based on 10,000 posterior probabilities drawn from the SEAc model. These isotope-based metrics were analysed in the SIBER package in R (Jackson et al., 2011).

3.2.7.2 Fatty acid composition and biomarker concentrations

All analyses were done in Primer (v6.0) with PERMANOVA add-on (Anderson et al., 2008).

We determined the total amount of FA (TFA) in our nematode samples, and identified different major FA classes based on the degree of their saturation: SFA (saturated FA), PUFA (polyunsatured FA), HUFA (highly unsaturated FA), MUFA (mono-unsaturated FA), as well as different PUFA classes based on the position of ω: ω3PUFA and ω6PUFA. Differences in the concentrations of these FA classes between nematode species were examined with one-way PERMANOVA based on a Euclidean distances matrix, in which all samples of a given species were considered replicates, irrespective of their station of origin. P values were obtained from 999 permutations. However, when a limited number of unique permutations (< 100) were possible, as occurred sometimes in pairwise tests, Monte Carlo permutational p values were chosen (Anderson and Robinson, 2003).

Secondly, non-metric multidimensional scaling (nMDS) was used to visualize differences in the multivariate fatty acid compositions of nematode species; we chose a Bray-Curtis similarity matrix on the basis of the relative fatty acid concentrations. Each individual nematode sample was plotted separately in the nMDS.

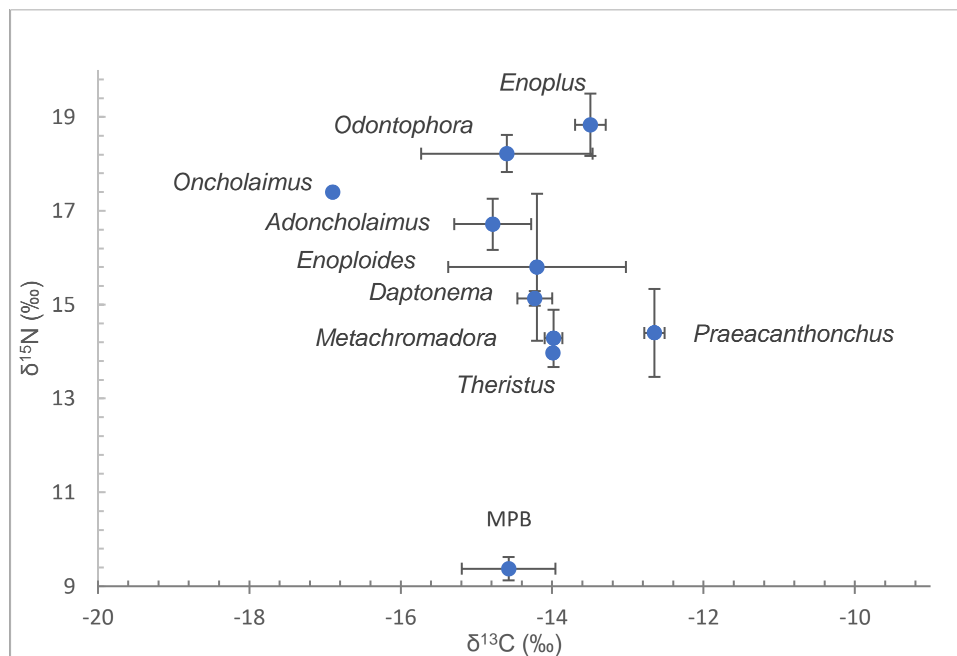

Fig. 3.2. Dual stable isotope data ($\delta^{13}C$ and $\delta^{15}N$) of nematode species and of microphytobenthos. Data are means of the numbers of replicates listed in table 3.1 with standard deviation. Nematode species are indicated by their genus name. Note that for *Oncholaimus* and *Theristus*, only a single measurement was available.

PERMANOVA was then used to formally identify statistically significant differences in the FA composition or in the concentrations of specific FA biomarkers or in biomarker ratios between nematode species. Firstly, a one-way PERMANOVA was performed on the whole dataset, to assess differences in FA composition/concentration/ratio between nematode species. In this analysis, all samples of a given genus were considered replicates, irrespective of their station of origin. To address the possibility of station differences within a nematode species, a two-way PERMANOVA was performed with factors species (three levels: *M. remanei, P. opheliae, T. acer*) and station (two levels: st1: sandy, st16: silty) on a dataset composed of all data of genera that were collected from more than one location. Pairwise tests were done on significant factor(s) or interaction terms. Because PERMANOVA is sensitive to heterogeneity of variances (dispersion effect), PERMDISP was used to test whether significant differences were due to treatment (location) or to variance effects.

SIMPER (Similarity Percentage Analysis) was conducted to identify which fatty acids contributed most to the dissimilarity among species.

3.3 Results

3.3.1 Trophic level and resources of nematodes based on SIA

Nematode $\delta^{13}C$ values exhibited a small range, from -12.6 ± 0.13 to -16.9 ‰ (Fig. 3.1, Table 3.2). Omission of *Oncholaimus* further reduced that range to -12.6 ± 0.13 to -14.8 ± 0.51 ‰. These values largely correspond to measured and previously published data on MPB on this and other intertidal areas (Moens et al., 2002, 2005, 2014).

Table 3.2. Natural stable carbon and isotope ratios of nine nematode species from a temperate tidal flat. Nematode trophic level (TL) was calculated from the $\delta^{15}N$ according to 4 scenarios: with a trophic-level fractionation of 3.4 (TLa) and one of 2.5‰ (TLb), and for both fractionation factors, one with MPB as the reference trophic level (TL = 1) and one with *Metachromadora* as the reference level (TL = 2) (Moens et al., 2005a; Bezerra and Moens unpubl.).

Genus	$\delta^{15}N$	$\delta^{13}C$‰	TLa_MPB	TLb_MPB	TLa_M	TLb_M
Enoplus	18.83 ± 0.67	-13.49 ± 0.20	3.8	4.8	3.3	3.8
Odontophora	18.22 ± 0.40	-14.60 ± 1.13	3.6	4.5	3.2	3.6
Oncholaimus	17.4	-16.88	3.4	4.2	2.9	3.2
Adoncholaimus	16.71 ± 0.55	-14.79 ± 0.51	3.2	3.9	2.7	3.0
Enoploides	15.84 ± 0.36	-13.63 ± 0.36	2.9	3.6	2.5	2.6
Daptonema	15.13 ± 0.15	-14.23 ± 0.23	2.7	3.3	2.3	2.3
Praeacanthonchus	14.4 ± 0.94	-12.65 ± 0.13	2.5	3.0	2.0	2.0
Metachromadora	14.28 ± 0.61	-13.98 ± 0.12	2.4	3.0	2.0	2.0
Theristus	13.97	-13.99	2.4	2.8	1.9	1.9
MPB	9.38 ± 0.25	-14.58 ± 0.62	1.0	1.0		

$\delta^{15}N$ of nematodes also spanned a fairly narrow range between 14.0 ‰ in *Theristus* and 18.9 ± 0.67 ‰ in *Enoplus*. Depending on the trophic fractionation factor and trophic baseline used, nematodes occupied trophic levels from 2 up to almost 5 (Table 3.2). Specifically, when using MPB as a baseline (TL = 1) and a FF of 3.4 ‰, trophic level varied between 2.4-2.5 for *Theristus, Metachromadora* and *Praeacanthonchus* to 3.8 for *Enoplus*, with a majority of species clustering at TL's between 2.7 and 3.4. Still with MPB as a baseline but with a FF of 2.5 ‰, this range expanded from a TL close to 3 for *Theristus, Metachromadora* and *Praeacanthonchus* to values in excess of 4.5 for *Enoplus* and *Odontophora*, with a majority of species having a TL of 3.3 – 4.

When using *Metachromadora* as a baseline and a FF of 3.4 ‰, nematode TL ranged from close to 2 for *Theristus* and *Praeacanthonchus* to in between 3 and 3.5 for *Enoplus* and *Odontophora*. With a FF of 2.5, the corresponding TL's remained unaltered for *Theristus* and *Praeacanthonchus*, but increased to values in between 3.5 and 4 for *Enoplus* and *Odontophora* (Table 3.2).

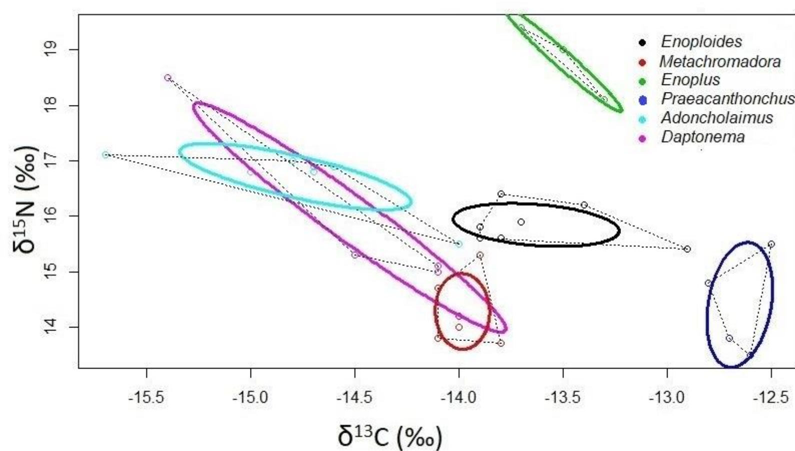

Fig. 3.3. Variation in $\delta^{13}C$ and $\delta^{15}N$ per species, considering all replicate samples of each species, irrespective of the exact sampling station in the Paulina. Thick coloured lines and dotted grey lines: ~40% CI (default value in SIBER) bivariate ellipses and convex hulls, respectively, demonstrating isotopic niche partitioning among the six nematode species. Species are indicated by their genus name.

Isotopic niches based on the stable isotopes of carbon and nitrogen exhibited no overlap whatsoever between *Enoploides, Enoplus* and *Praeacanthonchus* nor between any of these three species and the remaining three for which sufficient replicate data were available (Fig. 3.3). Moreover, *Metachromadora*'s isotopic niche only overlapped with that of *Daptonema* (proportion of overlap = 0.14), and only *Daptonema* and *Adoncholaimus* exhibited a somewhat more pronounced isotopic niche overlap (proportion of overlap = 0.33) (Fig. 3.3). *Daptonema* also had the largest standard ellipse area, followed by *Enoplus* and *Praeacanthonchus* (Fig. 3.4), but only the difference in isotopic niche breadth between *Daptonema* and *Enoploides* stood out as statistically significant in pairwise tests (with probability = 0.96).

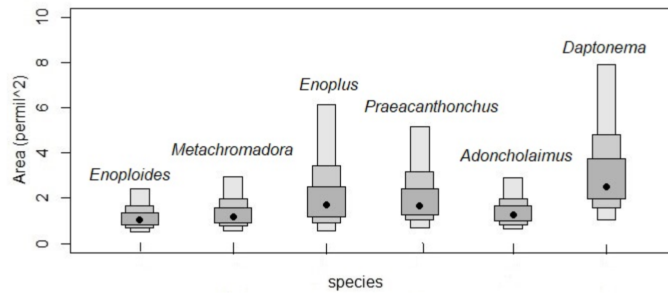

Fig. 3.4. Surface area measurements of the isotopic standard ellipse areas per nematode species. Measures of uncertainty and central tendency (black circles = mode) of standard ellipses are given (SEAc). Boxes show 95, 75 and 50 % credibility intervals from light to dark grey, respectively. Species are indicated by their genus names in the figure.

3.3.2 Fatty acid composition

The fatty acid content and composition of nematodes can be found in supplementary table S3.1. In short, total fatty acid (TFA) content ranged from 40 ± 5 ng/ind in *Theristus* to 1403 ± 213 ng/ind in *Enoplus*, generally exhibiting a clear correlation with individual nematode biomass (Table S3.1). TFA standardized per unit nematode body mass differed by a factor of 3, with the lowest value in *Enoplus* and the highest in *Oncholaimus* (Table S3.1). Generally, most nematode species had substantial amounts of PUFA (38 % to 64 %), with HUFA (34 % to 64 %) and ω3 PUFA (36 % to 59 %) being dominant, whereas MUFA (17 % to 36 %), SFA (12 % to 28 %) and ω6 PUFA (1 % to 6 %) were present in lower abundances. Among PUFA, EPA and/or DHA dominated, the sum of these two PUFA ranging from 30 % to 54 % of total FA. The relative abundance of all these FA classes differed among species (Table S3.2).

Patterns of fatty acid compositions among nematode species and stations were visualised in nMDS ordination (Fig. 3.5), where the relative distances between samples in the ordination reflect their variation in terms of fatty acid composition. Most pairs of species were differentiated and exhibited limited within-species variability. Species with a presumed partial or main predatory feeding ecology (*Adoncholaimus, Oncholaimus, Enoplus, Enoploides, Odontophora*) had mutually non-overlapping positions in the ordination and were all situated in the lower part of the ordination plot. The two confamiliar xyalid species, *Daptonema* and *Theristus*, had slightly overlapping FA compositions, different from those of all other species, including the other supposed MPB feeders, i.e. *Metachromadora* and *Praeacanthonchus*. The latter species exhibited by far the largest intraspecific variability, but still had limited overlap with other species (only partly with *Metachromadora*), whereas all except one sample of the former species formed a separate cluster from all other species. Of the three species (*Metachromadora, Praeacanthonchus and Theristus*) that were obtained from more than one location, only the FA composition of *Theristus* exhibited a slight separation between stations.

The pattern of the nMDS was confirmed by a one-way PERMANOVA with factor nematode species (Table S3.4) (df = 8, Pseudo-F = 16, p = 0.001), which was highly significant (but note a significant PERMDISP ($p < 0.05$)) and exhibited significant pairwise differences ($p < 0.05$) among all pairs of species. A two-way PERMANOVA with species and station, using only data of three species which were sampled at two stations (st1, st16), revealed no effect of station nor of station x species, whereas species again had a highly significant effect (p = 0.001) with significant differences between all pairs of species. Note, however, that there was a significant dispersion effect, calling for a cautionary interpretation of this species effect.

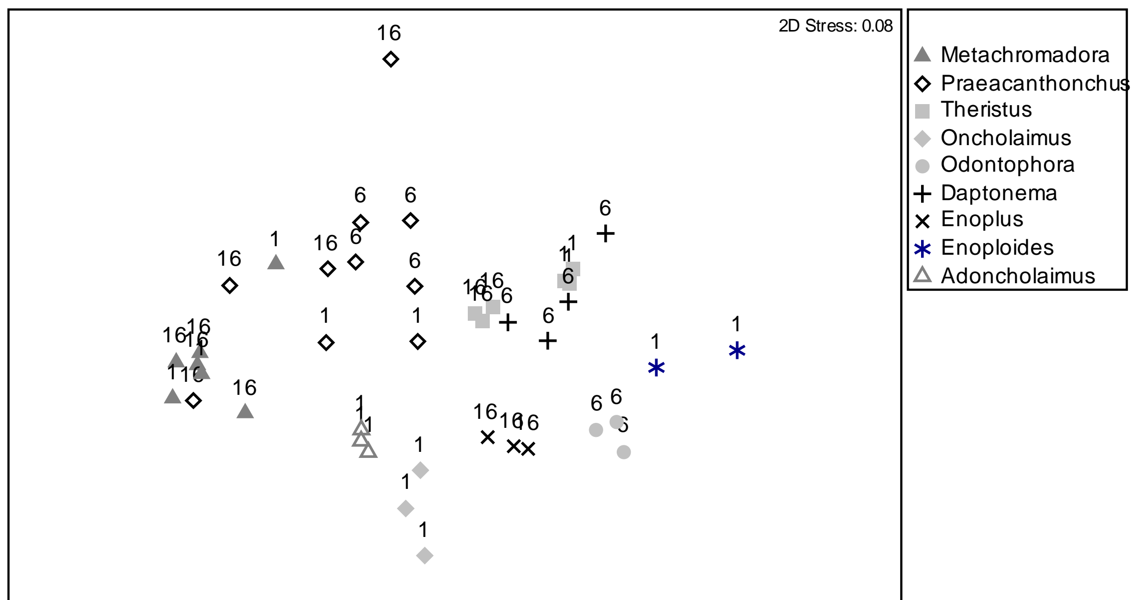

Fig. 3.5 nMDS ordination of nematode fatty acid composition on the basis of a Bray-Curtis similarity matrix of relative abundances of FAMEs (as % of total fatty acids). Numbers indicate sampling stations (1, 6 and 16).

SIMPER analyses revealed the main FA that contributed to the dissimilarity among species (Table S3.3). Among the expected MPB feeders, *Metachromadora* was differentiated from *Praeacanthonchus, Daptonema* and *Theristus* mainly by a higher concentration of C16:1ω7 and a lower concentration of DHA. Similarly, *Praeacanthonchus* differed from the Xyalidae *Daptonema* and *Theristus* by a lower level of DHA and higher concentrations of EPA, C16:1ω7 and C16:0. *Theristus* had slightly higher concentrations of EPA and C22:1ω9 compared to *Daptonema*, the latter being characterized by a slightly higher concentration of DHA and the presence of C24:1ω9 (Table S3.3). Presumed MPB feeders differed in many different, species-specific ways from other nematodes, the only nearly consistent difference being the usually higher EPA concentrations and the absence or lower concentrations of C22:5ω3 in MPB feeders. Some of the other nematodes also had higher concentrations of C18:0 and of ARA (Table S3.3).

Among these other nematode species, all presumed (partly) predatory, *Oncholaimus* and *Adoncholaimus* both had elevated concentrations of C16:1ω7 and of C16:0 and lower concentrations of DHA compared to most other species (Table S3.3). *Odontophora* had higher C20:1 and DHA concentrations than other predatory nematodes, except *Enoploides* which had higher DHA than *Odontophora*. Indeed, *Enoploides* differed from all other presumed predators by its higher levels of DHA. There were no consistent differences between *Enoplus* and other presumed predators.

3.3.3 Fatty acid markers of nematode diet

Variation in FA biomarkers among the nine nematode species can be found in table S3.2. In short, significant differences were observed in most biomarkers, except the bacterial marker C15:0 + C17:0, C24:0, and between multiple pairs of nematode species. PERMDISP values were non-significant for most biomarkers.

Among the diatom biomarkers, EPA concentrations were generally lower in nematodes with presumed predatory feeding than in the presumed MPB feeders *Daptonema, Theristus*, *Metachromadora* and *Praeacanthonchus*; *Praeacanthonchus* had the highest EPA level (30.02 ± 5.38 %). Concentrations of C16:1ω7 did not show a similar separation, but the highest concentration was found in the presumed diatom feeder *Metachromadora*, followed by *Adoncholaimus*. Both species had significantly higher C16:1ω7 concentrations than all other species. The ratio of C16:1ω7 to C16:0 followed a similar pattern, *Metachromadora* having significantly higher values than all other species, again followed by *Adoncholaimus*. The EPA/DHA ratio was again significantly higher in *Metachromadora* than in all other species. *Praeacanthonchus* and *Adoncholaimus* in turn had significantly higher EPA/DHA ratios than the remaining species.

Whereas *Metachromadora* thus consistently scored high values of diatom-related FA biomarkers, it had the significantly lowest concentration of the dinoflagellate marker DHA of all nine species. Highest values for DHA were found in the deposit feeders *Daptonema* and *Theristus* and in the predator/omnivore *Enoploides*. The sum of C14, C16 and C18 was highest in the supposedly predatory/omnivorous *Oncholaimus* and *Adoncholaimus*, followed by *Enoploides* and *Metachromadora*. The significantly lowest values were present in the deposit-feeding species *Daptonema* and *Theristus* and in *Odontophora*.

Most species had negligible concentrations (< 2.5 %) of C_{18}PUFA, indicating limited if any contribution of vascular plant detritus to the nematode diet. Only *Metachromadora* had a C_{18}PUFA concentration > 2%, while this marker was completely absent from *Enoploides*.

The bacterial biomarker C15:0+C17:0 ranged from 2.95 ± 0.63 % in *Enoploides* to 9.8 ± 3.24 % in *Oncholaimus*, but without a significant species effect. Similarly, no significant differences were observed among species in the concentration of C24:0.

Markers of carnivory did not reach high relative abundances, but did differ significantly between species. C20:1ω9 always comprised < 4 % of TFA, with highest values in *Enoploides* and *Enoplus* and lowest in *Oncholaimus*. The former two species and *Odontophora* generally had significantly higher levels of this FA than all other species (Table S3.2). The ratio of PUFA/SFA was lowest in *Metachromadora* but highest in the two Xyalidae, rather than in any presumed predatory species. Still, differences between the Xyalidae, *Enoploides, Enoplus* and *Odontophora* were not statistically significant (Table S3.2).

When focusing on the two-way comparison of stations (2 levels) and species (3 levels) (Table S3.5), no significant differences were observed in the relative abundance of EPA among stations, species or their interaction (see Table S3.5). Another diatom marker, C16:1ω7, only differed among species, while the ratio of C16:1ω7/ C16:0 was significantly affected by the interaction of species x station: *Metachromadora* and *Praeacanthonchus* had higher values of this ratio at st16 than at st1, while *Theristus* showed the opposite pattern. The ratio EPA/DHA was highest in *Metachromadora*, followed by *Praeacanthonchus* and *Theristus*. It was also significantly higher in st16 than in st1 (Table S3.1, S3.5).

The relative concentration of DHA and of the bacterial marker C15:0 + C17:0 did not differ between stations nor species (Table S1, S3.5).

3.4 Discussion

3.4.1 Carbon sources of tidal flat nematodes

As in previous studies on this and other estuarine tidal flats and coastal beaches (Moens et al., 2002, 2005, 2014; Carman and Fry, 2002; Rzeznik-Orignac et al., 2008; Maria et al., 2011), microphytobenthos appeared to be the predominant basal carbon source for the majority of nematode species in this study. This is evidenced by the relatively 'heavy' carbon isotopic signatures of all species except *Oncholaimus*, values which fell well within published values for estuarine tidal flat MPB (Moens et al., 2002). Whilst the $\delta^{13}C$ of *Oncholaimus* was still within that same range of published MPB values, it was relatively depleted compared to our own MPB measurements, suggesting some contribution of other resources. In the polyhaline reach of the Scheldt Estuary, these most likely include settled phytoplankton or – more generally – suspended particulate matter (Hellings et al., 1999; Boschker et al., 2005), although other sources like macroalgae cannot be excluded (Moens et al., 2002). It is interesting, in this respect, that *Oncholaimus* had the largest contribution of arachidonic

acid, a fatty acid that has been used as an indicator of microzooplankton (Parrish et al., 1995). Given its ability to prey on small invertebrates (Moens and Vincx, 1997) and to scavenge on dead animals (Jensen, 1987), it is possible that dead zooplankton contributed to the diet of this species. In fact, substantial quantities of zooplankton of marine origin enter the Schelde Estuary at each high tide and die there, yielding ca 1500 tonnes dry weight of dead zooplankton of marine origin which decays in the estuary per year, mostly so in the polyhaline reaches, where our study site was located (Soetaert et al., 1994); much of this dead zooplankton ends up in Schelde sediments, so it is conceivable that this would contribute to the nutrition of some benthic animals. On the other hand, this was only partly reflected in the concentrations of the FA's C20:1 and C20:2, indicators of feeding on (macro)zooplankton in estuaries (Parrish et al., 2000), in *Oncholaimus* (3.96 ± 0.41 %). These two FA's were consistently present in all our nematode species, but always at relative contributions < 7 % and without any clear correlations with the expected feeding types of the nematodes. Since the $\delta^{13}C$ of *Oncholaimus* is based upon a single sample, albeit composed of several tens of individuals, we cannot draw firm conclusions for this species at this field site. It is nevertheless noteworthy that the pigment pyropheophytin, which is commonly used as an indicator of zooplankton faecal pellets, was an important driver of both total nematode abundance and nematode genus composition at the Paulina intertidal flat (Wu et al., chapter 2 of this thesis), suggesting that the potential of zooplankton-related inputs as a resource to estuarine nematodes deserves further investigation.

MPB biofilms on tidal flats in the Schelde Estuary are commonly dominated by diatoms (Sabbe and Vyverman, 1991; Hamels et al., 1998). Our fatty-acid data nevertheless suggest variable but often substantial contributions of other microalgae, particularly dinoflagellates, to the diets of nematodes. The significance of dinoflagellates is evidenced by DHA concentrations that sometimes rivalled the concentrations of EPA, and by EPA/DHA ratios close to, or lower than 1 in five out of the nine nematode species studied here, comprising both presumed microalgal grazers and predators. Similar results were obtained at the Paulina tidal flat for two harpacticoid copepod species, albeit only at specific stations and seasons (Cnudde et al., 2015). In this context, it is tempting to explain the relatively heavy $\delta^{13}C$ of *Praeacanthonchus* as an indication that it may utilize different components of the MPB than other nematodes, such as dinoflagellates, which at station 1 regularly form a significant component of biofilms (Moens, unpubl.). However, there was no obvious correlation in our data between $\delta^{13}C$ and fatty-acid based proxies of the relative contribution of dinoflagellates to nematode diet. A study of the horizontal variability in nematode assemblages at the Paulina tidal flat found that only a very small portion of the observed variability could be linked to peridinin, a light-harvesting pigment characteristic of dinoflagellates (Wu et al., chapter 2 of this thesis). While it is plausible that resource differentiation, for instance, based on microalgal cell size, occurs among nematode species

feeding on MPB (Rzeznik-Orignac et al., 2008; Moens et al., 2014), our data do not allow to pinpoint the underlying mechanisms or the preferred components of the MPB.

Whilst natural stable-isotope data alone cannot decisively discriminate between the direct use of microalgae and the consumption of bacteria (Boschker and Middelburg, 2002), both major components of MPB biofilms, our FA data provide evidence against the idea that bacteria would contribute a major share to the diet of any of these nematode species, since the bacterial markers C15:0 and C17:0 together always comprised < 4 % of their total FA; including C18:1ω7 slightly changes the picture, with the sum of these three bacterial biomarker FA contributing from 5.1 (*Enoploides*) to 11.8 % (*Oncholaimus*) of total nematode FA, compared to, for instance, a range of 38 to 57 % for the sum of the microalgal markers EPA, DHA and C16:1ω7. Much of these bacterial FA may actually reflect various kinds of nematode-bacteria cohabitations (such as gut bacteria) which, together with ingested bacteria, collectively form a nematode's microbiome (Derycke et al., 2016; Wu et al., chapter 4 of this thesis). The bacterial marker FA contributions also did not differ substantially between species with different feeding modes. Whereas we expected higher bacterial contributions in nematodes that ingest whole particles rather than piercing them and sucking out the contents, we found rather the opposite: higher contributions of bacterial markers to the diet of the epistrate feeding *Metachromadora* than in the deposit feeders *Daptonema, Theristus* and *Praeacanthonchus* and the omnivore *Enoploides*. Similarly low contributions of bacterial marker FA's to the total FA pool were also found in nine out of eleven harpacticoid copepod species on the same tidal flat. Two other copepod species, however, had more elevated concentrations of these bacterial FA (Cnudde et al., 2015). Unlike in these harpacticoid copepods, the contribution of C18:1ω7 largely outweighed that of C15:0 and C17:0 in our nematodes. C18:1ω7 has also been proposed as a marker of chemoautotrophic bacteria (Van Gaever et al., 2009). While it is known that chemoautotrophic processes, mainly related to the sulphur cycle, can be important in estuarine intertidal sediments, particularly in and nearby salt marshes (e.g. Howarth, 1984), they are commonly reflected in moderately to heavily depleted carbon isotope ratios (Alperin and Hoehler, 2009). No such depleted isotope signatures were found in any of the nematode species in our study, suggesting that chemoautotrophic bacteria do not contribute to their diets. One copepod species from the same sampling area did have such strongly depleted $\delta^{13}C$, but its concentrations of bacteria-specific FA were extremely low (Cnudde et al., 2015). Such discrepancies between isotope and FA data indicate that we should remain cautious when drawing conclusions about the (lack of) importance of bacteria (including chemoautotrophs) in the diet of tidal-flat meiofauna.

3.4.2 The nematode part of the benthic food web comprises more than two trophic levels and a substantial degree of omnivory

When looking at the stable nitrogen isotopic ratios of MPB and nematodes, it immediately becomes clear that MPB is not always directly consumed by all nematode species. This is not a novel result, yet the trophic structure of this study's small 'food web' reveals some striking features.

First of all, the idea that most nematodes are either primary consumers, grazing on MPB, or predators foraging on primary consumers, is too simple. Trophic-level calculations based on different scenarios for fractionation and with different baseline organisms, rather suggest that the nematodes studied here span up to three trophic levels. Under the assumption that *Metachromadora* is a primary consumer (i.e. TL = 2), *Enoplus* has a TL of 3.3 or 3.8 in case of a fractionation factor of 3.4 or 2.5 ‰, respectively. *Odontophora* follows with respective TL's of 3.2 and 3.6. Detailed observations of the gut contents of the same species of *Enoplus* from a salt marsh in the North Sea revealed that this 'giant' nematode species is a generalist feeder, capable of ingesting prey ranging from cyanobacteria and diatoms all the way up to rotifers and oligochaetes, predation being the predominant strategy in adults, while grazing on bacteria and microalgae is crucial for juveniles (Hellwig-Armonies et al., 1991). The elevated $\delta^{15}N$ of the species in this and a previous study (Moens et al., 2005a) suggest that it obtains a dominant share of its diet from preying on a combination of species belonging to the second and third trophic level. However, as we further point out below, several nematode species in our study had non-integer TL's, and the $\delta^{15}N$ of *Enoplus* and *Odontophora* might also reflect a predominant predation on omnivorous prey species, which in turn fed on a combination of MPB and MPB grazers. In any case, our results underline the presence of multiple trophic levels in estuarine nematode assemblages, thus largely invalidating whole-assemblage estimates of trophic level, which have been relatively common because of the difficulty in obtaining sufficient nematode biomass for species- or genus-level analyses. They also convincingly demonstrate that *Odontophora* is not a deposit feeder, but ranks among the highest TL's in estuarine nematode assemblages.

A second obvious conclusion from our $\delta^{15}N$ results is that omnivory is common in estuarine nematodes. With few exceptions, estimated TL's of nematodes had non-integer values (note that we treat small deviations as not different from an integer value), indicating that they obtain resources from more than one trophic level. Notable exceptions were *Metachromadora, Praeacanthonchus* and *Theristus* in three out of the four scenarios, *Adoncholaimus* when applying a TL fractionation of 2.5 ‰ (irrespective of whether MPB or *Metachromadora* was used as a baseline), and *Enoploides* and *Oncholaimus* in one scenario each.

The trophic position of *Praeacanthonchus*, which was the same as that of *Metachromadora* and *Theristus*, differs from its omnivorous position in between the latter two nematode species and species at higher trophic levels in a previous study at almost the same location (Moens et al., 2014). This suggests that *Praeacanthonchus* may be an opportunistic feeder which can temporarily switch resources depending on their availability and/or on competitive interactions. An even much more pronounced variability in trophic level within a species was also observed in the giant nematode *Deontostoma tridentum* from deep-sea sediment, the variation in TL of which spanned 1-3 units, reflecting a high degree of variability in its diet (Leduc et al., 2015). However, because of its much larger size, the results on *Deontostoma* were obtained on single individuals and thus represent interindividual variation, whereas our *Praeacanthonchus* samples were composed of many tens of specimens at a time.

Depending on the precise scenario, *Oncholaimus, Adoncholaimus, Enoploides* and *Daptonema*, in order of decreasing TL, together spanned almost one trophic level above the three abovementioned primary consumers, pointing at omnivorous feeding strategies with different relative contributions of predation vs primary consumption. *Daptonema* is closely related to *Theristus*, but at least in this species (*D. hirsutum*) characterized by a larger body and mouth size than the latter, potentially allowing it to access resources that are unavailable to *Theristus*. While *Daptonema* has often been observed with diatom frustules in its intestine (Nehring, 1991; Moens and Vincx, 1997), it is also capable of swallowing small nematodes, including juveniles of its own, in a foraging strategy which appears mainly based on selection of particles using size and shape as the principal criteria (Moens and Vincx, 1997). *Enoploides* has been listed as a 'strict' carnivore (Moens and Vincx, 1997), based on its voracious predation on prey ranging from ciliates to nematodes and oligochaetes (Moens and Vincx, 1997, 2000; Hamels et al., 2001; Gallucci et al., 2005), yet it is now clear that it is also capable of ingesting microalgae such as benthic and settled planktonic diatoms (Franco et al., 2008b; Moens et al., 2014). This is corroborated by the present TL results, which indicate that *Enoploides* at the time and site of our study obtained roughly equal amounts of carbon from MPB and from predation on MPB grazers. Both oncholaimid species were classified as facultative predators (Moens and Vincx, 1997) or scavengers (Jensen, 1987), probably complementing their carnivorous diets with other, mostly unknown resources. In the case of *Adoncholaimus*, its high scores for the diatom markers C16:1ω7 and C16:1ω7/C16:0 (second only to *Metachromadora*) and intermediate value for EPA suggest that it too may obtain part of its food by grazing on MPB diatoms, and/or by preying on MPB grazers. Based on their FA compositions, *Adoncholaimus* and *Oncholaimus* were the secondmost similar pair of species (only just surpassed by the two Xyalidae, *Daptonema* and *Theristus*), with a similarity of 85 %. The two species differed mainly in their concentrations of the diatom markers

C16:1ω7 and EPA (higher in *Adoncholaimus*), of arachidonic acid and of C22:1ω9 (both indicative of feeding on zooplankton and higher in *Oncholaimus*). These slightly more 'diatom-oriented' and 'carnivory-oriented' FA compositions in *Adoncholaimus* and *Oncholaimus*, respectively, are in accordance with the slightly higher TL of the latter species.

Moens et al. (2014) discussed the large trophic fractionation between MPB and presumed MPB grazers, suggesting that this could represent a real value (although fractionation factors tend to be lower at lower TL's (McCutchan et al., 2003) or might alternatively indicate that part of the MPB carbon is obtained through a trophic intermediate. Our current data suggest that bacteria are unlikely to be that intermediate, mainly because bacterial markers FA's were present in only limited abundances in all nematode species (see above). Certain heterotrophic protists might provide an alternative explanation (see also Leduc, 2009), but in the absence of good protozoan biomarkers, we can only speculate on this.

Thirdly, the isotopic niche size of nematodes did not clearly correlate with trophic level nor with presence and prominence of omnivory. The only significant difference in bivariate standard ellipse areas occurred between *Daptonema* (largest SEA) and *Enoploides* (smallest SEA), two species which in the present study exhibited substantial omnivory and had relatively similar TL's. Hence, our data indicate that most nematode species utilized different resources, and that the degree of resource variability did not spectacularly differ between species.

Finally, neither the ratio of PUFA/saturated FA nor the abundance of 20:1ω9 appeared reliable indicators of carnivory, since they did not correlate with trophic level. PUFA/SFA values were highest in the two species of Xyalidae, which both ranked among the species with low TL. 20:1ω9 was highest in carnivorous/omnivorous species, mainly *Enoplus* and *Enoploides*, suggesting that it may be a useful marker in some cases, but it had its lowest values in the two species of Oncholaimidae, which exceeded *Enoploides* in trophic level.

3.4.3 Resource differentiation among nematode species is prominent

Elucidating the factors that maintain and structure the high local (alpha) species diversity of meiofauna remains a challenge. On the one hand, the large spatiotemporal variation in disturbances acting at sometimes small/short scales, combined with the mostly passive and short-distance dispersal of most meiofauna (Derycke et al., 2013), allows neutral dynamics to play a significant role (Snyder and Chesson, 2003). In a small-scale laboratory experiment with deep-sea nematodes, Gallucci et al. (2008) demonstrated that the precise species composition of assemblages that colonize vacant patches was largely unpredictable, underlining the potential role of neutral processes. The same experiment,

however, also demonstrated that the overall structural properties of the colonizer assemblages were quite consistent, indicating that more deterministic, niche-based factors are also important.

In intertidal flat sediments, the diversity and extreme small-scale patchiness of resources, as well as the temporal variation in their availability, combined with species-specific feeding preferences, offer a profound basis for resource-driven niche differentiation (Pace and Carman, 1996; Azovsky et al., 2005). We prefer the term differentiation over specialization here, because the latter suggests a more fixed/constant resource use over time, whereas the former merely implies that different species avoid major mutual overlap. Since the present study covered only a single time point, and since resource niches of meiofaunal species can vary over time (e.g. Mascart et al., 2018), what we observed was pronounced resource differentiation, which became apparent in two independent approaches, SIA and FAA.

Because of lack of sufficient replicates for some species, we could only determine bivariate core isotopic niche areas for six nematode species, which were *a priori* assigned as predators/omnivores (three species: *Enoplus, Enoploides, Adoncholaimus*), deposit feeders (two species: *Daptonema, Praeacanthonchus*) and epistratum feeders (*Metachromadora*). It is important here to stress that the deposit feeders and epistratum feeders both feed on microalgae and therefore generally belong to a single guild ('unicellular eukaryote feeders') in an alternative feeding-type classification which is more based on food source than on feeding mode (Moens et al., 2004). We obviously expected 'carnivorous' nematodes to differ in their core isotopic niche from 'herbivorous' species, but had no solid *a priori* basis to expect such differences among herbivores, given that we lack isotopic signatures of different components of the MPB. Nevertheless, the core isotopic niches of all three species differed profoundly: that of *Praeacanthonchus* was completely separate from both other species, whereas there was limited overlap between the core isotopic niches of *Daptonema* and *Metachromadora*. Even though core isotopic niche spaces do not depict the entire niche space, this result convincingly demonstrates that these three species differ significantly in their resource use. Different size fractions of diatom biofilms can exhibit different isotopic signatures (Rzeznik-Orignac et al., 2008), which would obviously be reflected in the isotopic niches of their consumers, and food-particle size has repeatedly been demonstrated to be an important driver of feeding selectivity in meiofauna (De Troch et al., 2006; Moens et al., 2014). However, in a dedicated lab experiment, neither *Daptonema* nor *Praeacanthonchus* exhibited pronounced size selectivity, even though on average they consumed less carbon from the smallest diatoms (Moens et al., 2014). Indeed, such deposit-feeding nematodes tend to ingest cells as long as these are not too large to be swallowed (Moens and Vincx, 1997). *Daptonema* and *Praeacanthonchus* did, however, have very different EPA/DHA and C16:1ω7/C16:0 ratios, both indicating that *Praeacanthonchus* fed more on diatoms, whereas dinoflagellates appear to have

contributed substantially to the diet of *Daptonema* at the time of our sampling. Although no other FA contributed profoundly to the dissimilarities in FA compositions among these three nematode species, the slightly higher trophic level and bivariate core ellipse area of *Daptonema* suggest that this species has additional feeding strategies which *Praeacanthonchus* and *Metachromadora* lack, likely including some degree of carnivory. The FA composition of *Metachromadora* supports a preference for diatoms even more than in *Praeacanthonchus*; in the cell-size experiment by Moens et al. (2014), *Metachromadora* was not included, yet the only epistratum feeder that was included, exhibited a very pronounced preference for cells of larger size, which could be one explanation for the niche differentiation with *Praeacanthonchus*. In addition, *Metachromadora* from an intertidal site with *Zostera marina* vegetation obtained a substantial part of its carbon from *Zostera* biomass, probably by feeding on associated bacteria and/or fungi. It is possible that this species also scrapes off bacteria from microalgal cells or sediment grains in biofilms; this would be consistent with the fact that *Metachromadora* had the second highest proportion of bacterial marker FA of all nine species in this study, after *Oncholaimus*.

Much as for the herbivores, supposedly carnivorous nematode species had non-overlapping core standard isotope ellipse areas. *Enoplus* was mostly separated from *Enoploides* and *Adoncholaimus* by its higher trophic level, wheras the latter two species were mainly differentiated by different core carbon isotope signatures, suggesting they utilize at least partly different resources. *Enoploides* was involved in half of the six most dissimilar pairwise fatty acid composition comparisons, including the one with *Adoncholaimus* (dissimilarity = 35 %). These differences were always to a large extent explained by an exceptionally high proportion of DHA and low levels of C16:1ω7 in *Enoploides*, indicating that at the time of sampling, this species used dinoflagellates as an important food source. *Enoploides* is undoubtedly an opportunistic feeder, as it is known as a voracious predator of other nematodes, oligochaetes and ciliates (Moens and Vincx, 1997; Moens et al., 2000; Hamels et al., 2001), but also as a grazer of diatoms (Moens et al., 2014) and of fresh detritus of phytoplanktonic origin (Franco et al., 2008b). *Adoncholaimus* had an isotopic niche space that substantially overlapped with that of *Daptonema* rather than with other carnivorous species, but with a higher mean trophic level. Based on FA compositions, these two species were also mainly differentiated by a stronger diatom signal in *Adoncholaimus* vs a more pronounced dinoflagellate imprint in *Daptonema*.

Fatty acid compositions could be compared among all nine nematode species used in this study. Pairwise dissimilarities ranged from a mere 14 to 15 % between the two species of Xyalidae (*Daptonema* and *Theristus*) and the two Oncholaimidae (*Oncholaimus* and *Adoncholaimus*), respectively, to 49 % between *Enoploides* and *Metachromadora*. An nMDS ordination essentially separated the supposedly carnivorous species in the lower half of the plot from the other species.

Most striking, however, was the exceptionally low overlap between species, with the two Xyalidae on the one hand, and *Metachromadora* and *Praeacanthonchus* on the other, forming the only two species pairs which exhibited some mutual overlap. This again confirms the strong degree of resource differentiation among species. Moreover, whereas no confamilial species were included in the isotope-based niche analysis, the limited overlap between the two confamilial species pairs mentioned above demonstrates that resource overlap also occurs between closely related species, in agreement with isotope-based data from an intertidal seagrass bed (Vafeiadou et al., 2014), but also with microbiome-based data on congeneric nematodes from macroalgal wrack (Derycke et al., 2016).

In general, our data demonstrate the importance of resource differentiation among both distantly and closely related nematode species as a mechanism that can potentially contribute to the maintenance of a high species diversity of meiofauna at a local scale. They also highlight the limits of traditional black-box approaches, in which most meiofaunal species are considered primary consumers, and of feeding-guild classifications, which appear to create at least partly artificial groupings of species which in reality have a substantially different feeding ecology.

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Supplementary information of Chapter 3

Table S3.1. Concentrations of total fatty acids (TFA: ng/nematode; TFA/biomass: ng/µm^3), relative concentrations of specific fatty acids or FA classes (%), and values of some FA ratios with biomarker value in nine estuarine tidal flat nematode species. *M, P, T, D, On, Od, Enoplu, Enoplo* and *A* indicate the nematode species: *Metachromadora remanei, Praeacanthonchus punctatus, Theristus acer, Daptonema hirsutum, Oncholaimus oxyuris, Odontophora setosus, Enoplus brevis, Enoploides longispiculosus,* and *Adoncholaimus fuscus*, respectively.

Variables	*M*	*P*	*T*	*D*	*On*	*Od*	*Enoplu*	*Enoplo*	*A*
TFA	61±19	85±56	40±5	70±22	255±30	53±6	1403±213	362±100	1089±89
TFA/ biomass	206.8	131.7	174.6	123.9	262.6	226.1	97.3	129.5	121.9
PUFA	39.5±4.7	51.5±6.9	60.8±3.5	60.8±4	44.8±3.9	56.5±2.7	55.6±1.2	59.7±2.3	44.1±0.7
HUFA	34.6±4.7	48.1±8.5	60.5±3.4	60.7±4	43.9±3.9	55.6±2.7	53.4±1.1	58.1±2.2	41.5±0.6
MUFA	34.9±5.4	23±6.5	23.6±1	24.1±3.4	27±4.1	24.5±1	23.1±0.1	17.2±1.5	29.2±0.4
SFA	25.7±1.3	25.5±4.7	15.7±3.7	15.1±3	28.2±2.4	19.1±2.8	21.3±1.2	23.2±0.7	26.8±1.1
ω3pufa	37±4.7	49.1±7.6	56.9±2.9	57.4±3.6	36.5±2.9	49.9±2.3	47.6±0.9	55±3.4	37±0.4
ω6pufa	2.5±0.6	2.3±1.8	3.8±0.7	3.5±0.7	7.9±0.9	6.6±0.4	6.5±0.2	3.1±1.2	6.3±0.4
C18PUFA	2.2±0.7	1.3±0.8	0.3±0.2	0.1±0.3	0.5±0	0.9±0.1	0.7±0.1	-	1.2±0.1
EPA	25.5±2.6	30±5.4	26.8±0.6	24.2±2.3	18.3±1.5	17.7±0.8	19.6±0.6	17±1.4	21.2±0.7
DHA	7.4±1.7	14.2±3.7	24.6±2.7	28.5±5.5	11.7±0.8	21.1±0.9	16.1±0.9	28±3.4	10.1±0.5
EPA/DHA	3.5±0.4	2.3±0.6	1.1±0.1	0.9±0.3	1.6±0.1	0.8±0	1.2±0.1	0.6±0.1	2.1±0.1
C16:1ω7	18.5±4.2	8.4±5.3	4.8±0.6	4±1.4	8.2±1.3	4±0.5	5.4±0.3	2.3±1.2	12.8±0.4
C16:1ω7/c16:0	1.5±0.3	0.8±0.4	0.6±0.1	0.6±0.2	0.6±0.1	0.7±0	0.6±0.1	0.3±0.1	0.9±0
C14:0+C16:0+C18:0	20.1±1.2	19.3±4	14.5±3.9	13.3±2.9	26.1±2.4	13.3±0.8	18.3±1.2	20.8±0.6	24.2±1.2
C15:0+C17:0	3.2±1.4	2.5±2	0.9±0.1	0.9±0.1	2±0.2	1.2±0	2.6±0.2	2.2±0.1	2±0.1
C18:1ω7	8.1±0.9	6.5±1.2	6.1±1	7.7±0.4	9.8±3.2	6±0.5	7.9±0.1	3±0.6	7.7±0.3
C20:1+C22:1	2.5±0.8	2.3±0.9	6.5±1	4±0.1	4±0.4	5±0.4	2.5±0.1	5.7±0.2	2.9±0.1
ARA	0.4±1	0.6±1.1	3.3±0.4	3.2±0.1	7.5±0.9	5.7±0.5	5.3±0.3	2.8±0.8	4.7±0.3
C24:0	0.1±0.1	0.1±0.1	-	-	-	0.1±0	-	-	-
C18:1ω9	0.2±0.6	0.5±0.8	3.4±0.2	2.7±0.4	4±2.7	4.1±0.1	2.3±0.3	0.5±0.1	3.2±0.2
LC-SCF(C20-24)	2.4±0.7	3.6±0.6	0.3±0.2	1±0.3	-	4.6±2.9	0.5±0	0.3±0	0.6±0
c18:2ω6	1.3±0.3	0.9±0.6	0.3±0.2	0.1±0.3	0.5±0	0.9±0.1	0.7±0.1	-	0.9±0.1
20:3ω3	-	0.1±0.2	0.6±0.1	0.9±0	1.5±0.2	1.8±0.2	2.4±0.2	1.6±0.1	0.7±0.9
20:3ω6	0.1±0.2	0.1±0.2	0.2±0.2	0.2±0.3	-	-	0.6±0.1	0.3±0.5	0.2±0
C14:0	1.6±0.2	1.5±0.6	1±0.2	0.8±0.2	1.5±0.1	1.1±0.1	1.2±0.2	0.7±0.2	1.6±0.2
C15:0	2.4±1.3	1.5±1.8	-	-	0.8±0.1	0.3±0	1.5±0.1	1.1±0	1.3±0.1
C15:1ω5	0.7±0.9	0.8±1.5	0.7±0.2	0.6±0.1	1±0.1	0.5±0.1	-	-	-
C16:0	12.7±1	11±2.7	9±2.5	7.1±1.5	14.2±0.5	5.5±0.5	9.1±1	6.7±0.7	13.6±0.1
C16:2ω6	0.7±0.4	0.7±0.5	-	-	-	-	-	-	0.6±0.1
C16:3ω3	1.9±0.9	1.4±0.9	-	-	-	-	-	-	0.4±0.1
C17:0	0.8±0.2	1±0.3	0.9±0.1	0.9±0.1	1.2±0.1	0.9±0	1±0	1.1±0.1	0.7±0
C17:1ω7	2.7±1.5	2.4±4.6	0.2±0.2	0.3±0.4	-	0.3±0.1	0.5±0	0.1±0	0.3±0
C18:0	5.8±0.5	6.8±1.3	4.6±1.3	5.5±1.3	10.4±2	6.6±0.4	8±1.2	13.3±0.2	9±1.2
C18:2ω6cis	0.6±0.3	0.4±0.4	-	0.1±0.3	-	0.5±0	-	-	0.3±0
C18:2ω6tr	0.8±0.4	0.5±0.5	0.3±0.2	-	0.5±0	0.4±0.1	0.7±0.1	-	0.6±0.1
C18:3	0.1±0.1	0.1±0.1	-	-	-	-	-	-	0.3±0
C18:4ω3	0.8±0.4	0.3±0.5	-	-	-	-	-	-	-
C20:0	1±0.2	1.7±0.4	0.3±0.2	0.6±0.1	-	3.3±2.9	0.3±0	0.3±0	0.2±0
C20:1	0.6±0.1	0.3±0.3	1.3±0.3	1.7±0.1	0.9±0.1	4.7±0.1	1.6±0.1	1±0	1.7±0.1
C20:2	-	-	-	-	0.4±0.4	-	1.4±0.1	1.6±0.1	0.5±0.1
C21:0	0.7±0.1	0.2±0.3	-	-	-	-	-	-	-
C22:0	0.7±0.4	1.7±0.5	-	0.4±0.2	-	1.2±0	0.2±0	-	0.4±0
C22:1ω9	2±0.7	2.1±1	5.2±0.7	2.3±0.2	3.1±0.4	0.3±0.3	0.9±0	4.7±0.2	1.2±0
C22:5ω3	1.3±0.5	3.1±1	4.9±0.4	3.7±1.2	5±0.5	9.2±0.5	9.5±0.7	8.4±1.5	4.6±0.2
C24:1ω9	0.1±0.3	0.3±0.4	0.1±0.3	2.7±2.8	-	1.6±0.1	0.9±0.1	2.3±0.1	0.4±0.4
C16:2+C16:3	2.7±1.2	2.1±1.2	-	-	-	-	-	-	1±0.1
C16:1ω7+C18:1ω7	26.6±4.9	14.9±6.3	10.9±1.5	11.6±1.7	18±2	10±1	13.3±0.3	5.2±1.8	20.5±0.2
C20:1ω9	2±0.1	1.9±0.7	1.9±0.2	2.2±0.1	0.1±0.2	2.9±0.1	3.6±0.3	3.4±0.1	1.8±0.2
DHA/EPA	3.5±0.4	2.3±0.6	1.1±0.1	1.2±0.3	0.6±0	1.2±0	0.8±0.1	1.7±0.3	0.5±0
PUFA/SFA	1.5±0.2	2.1±0.7	4.1±1.2	4.2±1	1.6±0.2	3±0.6	2.6±0.2	2.6±0.2	1.7±0.1

values are reported as averaged values of all replicates, with values lower than 1 % marked with -.

Table S3.2. Results of PERMANOVA on dataset of different FA classes and biomarkers in nine species of nematodes. Species are indicated by their genus name; *a* presents the results of the main tests and of PERMDISP tests, while *b* shows the results of pairwise tests.

a

Variables	df	SS	MS	Pseudo-F	P(perm)	p_PERMDISP
TFA	8	7150500	893820	190.2	***0.001***	0.022
PUFA	8	0.24	0.03	13.404	***0.001***	0.356
HUFA	8	0.34	0.04	14.457	***0.001***	0.163
MUFA	8	0.09	0.01	5.7036	***0.002***	0.23
SFA	8	0.08	0.01	9.9993	***0.001***	0.701
ω3PUFA	8	0.26	0.03	13.087	***0.001***	0.057
ω6PUFA	8	0.015	0.0019	15.674	***0.001***	0.377
C18PUFA	8	0.002	0.0003	9.5388	***0.001***	0.481
EPA	8	0.08	0.01	9.9	***0.001***	***0.008***
DHA	8	0.21	0.03	29.7	***0.001***	0.131
EPA/DHA	8	35.6	4.5	28.7	***0.001***	0.397
C16:1ω7	8	0.11	0.01	11.63	***0.001***	0.533
C16:1ω7/C16:0	8	4.3	0.5	10	***0.001***	0.657
C14:0+C16:0+C18:0	8	0.06	0.007	8.8	***0.001***	0.473
C15:0+C17:0	8	0.003	0.0004	2.4	0.059	0.695
C18:1ω7	8	0.008	0.001	7.3	***0.001***	***0.024***
C20:1+C22:1	8	0.01	0.001	21.8	***0.001***	***0.018***
ARA	8	0.02	0.003	40.5	***0.001***	0.595
C24:0	8	0.000002	0.0000003	0.4	0.9	0.8
C18:1ω9	8	0.009	0.0012	15.8	***0.001***	***0.003***
LC_SFA	8	0.01	0.001	17.8	***0.001***	***0.006***
C18:2ω6	8	0.0007	0.00008	6	***0.001***	0.066
C20:1ω9	8	0.0025673	0.0003209	21	***0.001***	0.789
PUFA/SFA	8	39.7	4.97	10	***0.001***	***0.011***

Significant p values are marked in bold and italics.

b

Metachromadora	*Praeacanthonchus*	*Theristus*	*Daptonema*	*Oncholaimus*	*Odontophora*	*Enoplus*	*Enoploides*	*Adoncholaimus*
TFA	0.351	***0.013***	0.468	***0.012***	0.569	***0.011***	***0.001***	***0.011***
PUFA	***0.005***	***0.001***	***0.001***	0.104	***0.013***	***0.008***	***0.001***	0.151
HUFA	***0.004***	***0.001***	***0.006***	***0.024***	***0.01***	***0.008***	***0.001***	0.07
MUFA	***0.005***	***0.003***	***0.013***	***0.041***	***0.032***	***0.025***	***0.003***	0.137
SFA	0.927	***0.001***	***0.006***	***0.056***	***0.015***	***0.009***	***0.039***	0.236
ω3PUFA	***0.004***	***0.001***	***0.002***	0.912	***0.007***	***0.019***	***0.002***	0.989
ω6PUFA	0.805	***0.004***	***0.038***	***0.006***	***0.008***	***0.008***	0.265	***0.008***
C18PUFA	***0.019***	***0.001***	***0.001***	***0.011***	***0.026***	***0.007***	***0.006***	***0.053***
EPA	0.06	0.303	0.466	***0.002***	***0.007***	***0.013***	***0.003***	***0.03***
DHA	***0.002***	***0.001***	***0.003***	***0.013***	***0.013***	***0.008***	***0.001***	***0.031***
EPA/DHA	***0.001***	***0.002***	***0.001***	***0.015***	***0.011***	***0.009***	***0.001***	***0.008***
C16:1ω7	***0.004***	***0.002***	***0.003***	***0.007***	***0.009***	***0.011***	***0.002***	0.057
C16:1ω7/C16:0	***0.004***	***0.002***	***0.003***	***0.009***	***0.01***	***0.007***	***0.002***	***0.03***

Metachromadora	*Praeacanthonchus*	*Theristus*	*Daptonema*	*Oncholaimus*	*Odontophora*	*Enoplus*	*Enoploides*	*Adoncholaimus*
C14:0+C16:0+C18:0	0.79	***0.003***	***0.009***	***0.008***	***0.003***	0.053	0.486	***0.008***
C15:0+C17:0	0.526	***0.004***	***0.023***	0.252	0.086	0.481	0.359	0.235
C18:1ω7	***0.014***	***0.011***	0.403	0.228	***0.007***	0.812	***0.001***	0.608
C20:1+C22:1	0.651	***0.001***	***0.015***	***0.042***	***0.012***	0.877	***0.004***	0.48
ARA	0.594	***0.001***	***0.001***	***0.001***	***0.001***	***0.001***	***0.012***	***0.001***
C18_1ω9	0.462	***0.001***	***0.001***	***0.009***	***0.001***	***0.002***	0.587	***0.001***
LC_SFA	***0.003***	***0.004***	***0.002***	***0.001***	0.067	***0.011***	***0.005***	***0.012***
C18:2ω6	0.087	***0.001***	***0.002***	***0.004***	0.072	***0.029***	***0.017***	***0.044***
C20:1ω9	0.774	0.085	0.102	***0.001***	***0.011***	***0.01***	***0.001***	0.067
PUFA/SFA	***0.022***	***0.006***	***0.005***	0.685	***0.004***	***0.01***	***0.001***	0.295
Praeacanthonchus		*Theristus*	*Daptonema*	*Oncholaimus*	*Odontophora*	*Enoplus*	*Enoploides*	*Adoncholaimus*
TFA		0.067	0.671	***0.004***	0.359	***0.004***	***0.001***	***0.008***
PUFA		***0.011***	***0.035***	0.131	0.282	0.354	0.13	0.117
HUFA		***0.004***	***0.013***	0.431	0.174	0.331	0.13	0.168
MUFA		0.854	0.784	0.357	0.694	0.988	0.252	0.154
SFA		***0.001***	***0.002***	0.368	***0.042***	0.199	0.53	0.621
ω3PUFA		***0.041***	***0.068***	***0.03***	0.869	0.682	0.316	***0.029***
ω6PUFA		***0.051***	0.196	***0.005***	***0.005***	***0.008***	0.555	***0.007***
C18PUFA		***0.005***	***0.01***	0.133	0.438	0.282	***0.053***	0.796
EPA		0.176	0.06	***0.004***	***0.004***	***0.008***	***0.013***	***0.017***
DHA		***0.001***	***0.001***	0.294	***0.012***	0.438	***0.001***	0.109
EPA/DHA		***0.003***	***0.003***	0.097	***0.005***	***0.031***	***0.008***	0.687
C16:1ω7		0.097	0.127	0.955	0.183	0.368	***0.14***	***0.2***
C16:1ω7/C16:0		0.273	0.387	0.454	0.937	0.502	0.168	0.381
C14:0+C16:0+C18:0		***0.036***	***0.019***	***0.031***	***0.019***	0.663	0.628	0.084
C15:0+C17:0		***0.021***	0.102	0.8	0.245	0.988	0.817	0.803
C18:1ω7		0.474	0.069	***0.014***	0.534	0.057	***0.002***	0.123
C20:1+C22:1		***0.001***	***0.01***	***0.019***	***0.005***	0.845	***0.001***	0.381
ARA		***0.001***	***0.002***	***0.001***	***0.001***	***0.001***	***0.039***	***0.001***
C18:1ω9		***0.001***	***0.001***	***0.002***	***0.001***	***0.005***	0.978	***0.001***
LC_SFA		***0.001***	***0.003***	***0.004***	0.251	***0.005***	***0.001***	***0.001***
C18:2ω6		***0.037***	0.051	0.266	0.945	0.674	0.082	0.993
C20:1ω9		0.986	0.409	***0.005***	***0.015***	***0.004***	***0.011***	0.911
PUFA/SFA		***0.001***	***0.004***	0.25	0.072	0.297	0.425	0.297
Theristus			*Daptonema*	*Oncholaimus*	*Odontophora*	*Enoplus*	*Enoploides*	*Adoncholaimus*
TFA			***0.012***	***0.001***	***0.011***	***0.001***	***0.001***	***0.001***
PUFA			0.954	***0.002***	0.111	0.067	0.715	***0.002***
HUFA			0.865	***0.017***	0.054	***0.014***	0.433	***0.001***
MUFA			0.782	0.089	0.265	0.522	***0.002***	***0.001***
SFA			0.816	***0.001***	0.21	0.055	***0.037***	***0.002***
ω3PUFA			0.764	***0.001***	***0.009***	***0.003***	0.409	***0.001***
ω6PUFA			0.431	***0.001***	***0.001***	***0.002***	0.329	***0.001***
C18PUFA			0.396	***0.205***	***0.003***	***0.014***	0.121	***0.002***
EPA			***0.016***	***0.001***	***0.001***	***0.001***	***0.001***	***0.001***
DHA			0.167	***0.001***	0.061	***0.002***	0.219	***0.001***
EPA/DHA			0.159	***0.001***	***0.012***	0.203	***0.003***	***0.001***

Theristus	*Daptonema*	*Oncholaimus*	*Odontophora*	*Enoplus*	*Enoploides*	*Adoncholaimus*
C16:1ω7	0.215	***0.001***	0.095	0.127	***0.007***	***0.001***
C16:1ω7/C16:0	0.996	***0.856***	***0.048***	0.681	0.055	***0.001***
C14:0+C16:0+C18:0	0.566	***0.004***	0.607	0.129	0.072	***0.005***
C15:0+C17:0	0.891	***0.001***	***0.001***	***0.001***	***0.001***	***0.001***
C18:1ω7	***0.037***	***0.024***	0.916	***0.023***	***0.008***	***0.04***
C20:1+C22:1	***0.009***	***0.007***	***0.048***	***0.001***	0.321	***0.002***
ARA	0.567	***0.001***	***0.001***	***0.001***	0.205	***0.001***
C18:1ω9	***0.015***	***0.553***	***0.002***	***0.001***	***0.001***	0.268
LC_SFA	***0.008***	***0.077***	***0.008***	0.197	0.83	0.091
C18:2ω6	0.404	***0.157***	***0.008***	***0.011***	0.126	***0.003***
C20:1ω9	***0.022***	***0.001***	***0.001***	***0.001***	***0.001***	0.821
PUFA/SFA	0.914	***0.011***	0.184	0.092	0.156	***0.009***

Daptonema	*Oncholaimus*	*Odontophora*	*Enoplus*	*Enoploides*	*Adoncholaimus*
TFA	***0.001***	0.244	***0.002***	***0.003***	***0.001***
PUFA	***0.005***	0.166	0.09	0.751	***0.003***
HUFA	***0.002***	0.116	***0.023***	0.423	***0.001***
MUFA	0.353	0.851	0.672	0.072	***0.05***
SFA	***0.004***	0.131	***0.022***	***0.027***	***0.002***
ω3PUFA	***0.001***	***0.034***	***0.014***	0.486	***0.001***
ω6PUFA	***0.002***	***0.002***	***0.002***	0.651	***0.003***
C18PUFA	0.085	***0.009***	***0.019***	0.533	***0.001***
EPA	***0.013***	***0.005***	***0.026***	***0.025***	0.08
DHA	***0.005***	0.085	***0.011***	0.927	***0.005***
EPA/DHA	***0.003***	0.772	0.096	0.317	***0.004***
C16:1ω7	***0.014***	0.963	0.163	0.225	***0.002***
C16:1ω7/C16:0	0.894	0.133	0.767	0.144	***0.017***
C14:0+C16:0+C18:0	***0.003***	0.974	***0.046***	***0.028***	***0.002***
C15:0+C17:0	***0.001***	***0.008***	***0.001***	***0.001***	***0.001***
C18:1ω7	0.228	***0.003***	0.264	***0.001***	0.773
C20:1+C22:1	0.989	***0.002***	***0.001***	***0.002***	***0.001***
ARA	***0.002***	***0.001***	***0.001***	0.281	***0.002***
C18:1ω9	0.371	***0.004***	0.18	***0.005***	0.099
LC_SFA	***0.002***	***0.041***	0.063	***0.032***	***0.07***
C18:2ω6	0.103	***0.006***	***0.011***	0.535	***0.008***
C20:1ω9	***0.001***	***0.002***	***0.001***	***0.001***	***0.014***
PUFA/SFA	***0.007***	0.158	0.054	0.105	***0.012***

Oncholaimus	*Odontophora*	*Enoplus*	*Enoploides*	*Adoncholaimus*
TFA	***0.001***	***0.001***	0.159	***0.001***
PUFA	***0.011***	***0.016***	***0.02***	0.736
HUFA	***0.016***	***0.017***	***0.025***	0.335
MUFA	0.362	0.152	0.058	0.412
SFA	***0.013***	***0.017***	0.067	0.429
ω3PUFA	***0.004***	***0.001***	***0.006***	0.773
ω6PUFA	***0.092***	***0.076***	***0.015***	0.052
C18PUFA	***0.003***	***0.004***	***0.002***	***0.001***

Oncholaimus	*Odontophora*	*Enoplus*	*Enoploides*	*Adoncholaimus*
EPA	0.595	0.225	0.408	***0.045***
DHA	***0.001***	***0.004***	***0.006***	0.057
EPA/DHA	***0.001***	***0.009***	***0.003***	***0.001***
C16:1ω7	***0.01***	***0.02***	***0.015***	***0.008***
C16:1ω7/C16:0	0.064	0.763	0.08	***0.004***
C14:0+C16:0+C18:0	***0.001***	***0.008***	0.053	0.293
C15:0+C17:0	***0.005***	***0.03***	0.537	0.819
C18:1ω7	0.109	0.386	0.069	0.328
C20:1+C22:1	***0.038***	***0.003***	***0.017***	***0.008***
ARA	***0.049***	***0.02***	***0.009***	***0.007***
C18:1ω9	0.954	0.328	0.191	0.63
LC_SFA	***0.054***	***0.001***	***0.001***	***0.001***
C18:2ω6	***0.003***	***0.003***	***0.001***	***0.004***
C20:1ω9	***0.001***	***0.001***	***0.002***	***0.002***
PUFA/SFA	***0.011***	***0.005***	***0.014***	0.742
Odontophora		*Enoplus*	*Enoploides*	*Adoncholaimus*
TFA		***0.001***	***0.013***	***0.001***
PUFA		0.635	0.256	***0.004***
HUFA		0.261	0.345	***0.001***
MUFA		0.067	***0.011***	***0.003***
SFA		0.271	0.146	***0.008***
ω3PUFA		0.201	0.158	***0.001***
ω6PUFA		0.891	***0.015***	0.435
C18PUFA		***0.034***	***0.003***	***0.03***
EPA		***0.031***	0.467	***0.007***
DHA		***0.004***	***0.031***	***0.001***
EPA/DHA		***0.008***	0.052	***0.001***
C16:1ω7		***0.016***	0.089	***0.001***
C16:1ω7/C16:0		***0.042***	***0.015***	***0.003***
C14:0+C16:0+C18:0		***0.005***	***0.001***	***0.001***
C15:0+C17:0		***0.001***	***0.002***	***0.001***
C18:1ω7		***0.007***	***0.006***	***0.007***
C20:1+C22:1		***0.001***	0.12	***0.002***
ARA		0.292	***0.009***	***0.035***
C18:1ω9		***0.002***	***0.001***	***0.003***
LC_SFA		0.06	0.148	0.083
C18:2ω6		***0.033***	***0.002***	0.683
C20:1ω9		***0.019***	0.011	***0.001***
PUFA/SFA		0.313	0.38	***0.015***
Enoplus			*Enoploides*	*Adoncholaimus*
TFA			***0.005***	0.088
PUFA			0.075	***0.001***
HUFA			***0.041***	***0.001***
MUFA			***0.003***	***0.001***
SFA			0.167	***0.009***

Enoplus	*Enoploides*	*Adoncholaimus*
ω3PUFA	***0.023***	***0.001***
ω6PUFA	***0.011***	0.403
C18PUFA	***0.001***	***0.001***
EPA	0.06	***0.042***
DHA	***0.01***	***0.001***
EPA/DHA	***0.014***	***0.002***
C16:1ω7	***0.013***	***0.001***
C16:1ω7/C16:0	***0.042***	***0.002***
C14:0+C16:0+C18:0	0.081	***0.005***
C15:0+C17:0	0.059	***0.004***
C18:1ω7	***0.002***	0.24
C20:1+C22:1	***0.001***	***0.007***
ARA	***0.01***	0.074
C18:1ω9	***0.005***	***0.009***
LC_SFA	***0.006***	0.053
C18:2ω6	***0.002***	***0.045***
C20:1ω9	0.407	***0.001***
PUFA/SFA	0.876	***0.001***
Enoploides		*Adoncholaimus*
TFA		***0.002***
PUFA		***0.003***
HUFA		***0.003***
MUFA		***0.002***
SFA		***0.032***
ω3PUFA		***0.003***
ω6PUFA		***0.028***
C18PUFA		***0.002***
EPA		***0.011***
DHA		***0.003***
EPA/DHA		***0.001***
C16:1ω7		***0.002***
C16:1ω7/C16:0		***0.006***
C14:0+C16:0+C18:0		***0.032***
C15:0+C17:0		0.184
C18:1ω7		***0.001***
C20:1+C22:1		***0.001***
ARA		***0.024***
C18:1ω9		***0.001***
LC_SFA		***0.004***
C18:2ω6		***0.001***
C20:1ω9		***0.002***
PUFA/SFA		***0.005***

Significant p values are marked in bold and italics.

Table S3.3. Results of similarity percentage analysis. Dissimilarity in the FA compositions between pairs of nematode species, and the main fatty acids responsible for these dissimilarities (listed here up to a cumulative contribution of ca 50 % of the total dissimilarity) as detected using SIMPER (similarity percentage analysis), with relative concentration of FA expressed as fractions of 1 (1=100 %).

Compare groups		AV Diss	FA	Relative Concentration		Av.Diss±SD	Contrib%
Species 1	Species 2	%		Species 1	Species 2		
Metachromadora	*Praeacanthonchus*	24	C16:1ω7	0.19	0.08	5.4±2.1	22.8
			DHA	0.07	0.14	3.5±2	14.7
			EPA	0.26	0.3	3±1.5	12.7
Metachromadora	*Theristus*	34	DHA	0.07	0.25	8.6±5.8	25
			C16:1ω7	0.19	0.05	6.9±3.5	20
			C16:0	0.13	0.09	1.9±1.6	5.5
Metachromadora	*Daptonema*	36	DHA	0.07	0.29	10.5±4.1	29.3
			C16:1ω7	0.19	0.04	7.3±3.5	20.2
Metachromadora	*Oncholaimus*	32	C16:1ω7	0.19	0.08	5.2±2.5	16.4
			EPA	0.26	0.18	3.6±2.6	11.5
			ARA	0	0.07	3.6±5.9	11.3
			C18:0	0.06	0.1	2.3±2.7	7.4
			DHA	0.07	0.12	2.1±2.5	6.7
Metachromadora	*Odontophora*	43	C16:1ω7	0.19	0.04	7.3±3.6	16.7
			DHA	0.07	0.21	6.8±7.6	15.7
			C22:5ω3	0.01	0.09	4±12.5	9.1
			EPA	0.26	0.18	3.9±3	9
Metachromadora	*Enoplus*	35	C16:1ω7	0.19	0.05	6.6±3.3	18.9
			DHA	0.07	0.16	4.3±4.8	12.4
			C22:5ω3	0.01	0.1	4.1±11.5	11.9
			EPA	0.26	0.2	3±2.3	8.5
Metachromadora	*Enoploides*	49	DHA	0.07	0.28	10.3±6.9	21.1
			C16:1ω7	0.19	0.02	8.1±3.9	16.7
			EPA	0.26	0.17	4.3±3.1	8.8
			C18:0	0.06	0.13	3.8±14.4	7.7
Metachromadora	*Adoncholaimus*	22	C16:1ω7	0.19	0.13	3.3±3.4	15
			EPA	0.26	0.21	2.2±1.7	9.9
			ARA	0	0.05	2.2±4.6	9.8
			C22:5ω3	0.01	0.05	1.7±6.8	7.6
			C18:0	0.06	0.09	1.6±2.9	7.4
Praeacanthonchus	*Theristus*	26	DHA	0.14	0.25	5.2±2.4	20
			EPA	0.3	0.27	2.6±1.7	10.1
			C16:1ω7	0.08	0.05	2.1±0.9	8
			C16:0	0.11	0.09	1.6±1.4	6.2
			C22:1ω9	0.02	0.05	1.6±2.8	5.9
Praeacanthonchus	*Daptonema*	29	DHA	0.14	0.29	7.2±2.4	24.8
			EPA	0.3	0.24	3.3±1.5	11.4
			C16:1ω7	0.08	0.04	2.4±1	8.3
			C16:0	0.11	0.07	2.3±2.6	7.8
Praeacanthonchus	*Oncholaimus*	30	EPA	0.3	0.18	5.9±2.2	19.5
			ARA	0.01	0.07	3.4±5.2	11.4

Compare groups		AV Diss	FA	Relative Concentration		Av.Diss±SD	Contrib%
Species 1	Species 2	%		Species 1	Species 2		
			DHA	0.14	0.12	2±2.3	6.7
			C16:1ω7	0.08	0.08	2±1.1	6.5
			C18:0	0.07	0.1	1.8±1.7	5.9
Praeacanthonchus	*Odontophora*	34	EPA	0.3	0.18	6.1±2.4	18
			DHA	0.14	0.21	3.5±1.9	10.2
			C22:5ω3	0.03	0.09	3±5.9	8.9
			C16:0	0.11	0.05	2.9±3	8.5
			ARA	0.01	0.06	2.5±4.4	7.4
Praeacanthonchus	*Enoplus*	28	EPA	0.3	0.2	5.2±2	18.4
			C22:5ω3	0.03	0.1	3.2±5.9	11.2
			ARA	0.01	0.05	2.3±4.2	8.2
			C16:1ω7	0.08	0.05	1.9±0.9	6.8
			C16:0	0.11	0.09	1.4±1.9	5.1
Praeacanthonchus	*Enoploides*	39	DHA	0.14	0.28	6.9±3.2	17.7
			EPA	0.3	0.17	6.5±2.5	16.7
			C18:0	0.07	0.13	3.2±5	8.3
			C16:1ω7	0.08	0.02	3.1±1.2	7.9
Praeacanthonchus	*Adoncholaimus*	25	EPA	0.3	0.21	4.4±1.7	17.8
			C16:1ω7	0.08	0.13	3±2	12.1
			DHA	0.14	0.1	2.5±2.2	9.9
			ARA	0.01	0.05	2±3.7	8.1
			C18:1ω9	0	0.03	1.4±3.5	5.4
Theristus	*Daptonema*	14	DHA	0.25	0.29	3±2	21.9
			C22:1ω9	0.05	0.02	1.5±4.5	10.8
			EPA	0.27	0.24	1.4±1.4	10
			C24:1ω9	0	0.03	1.3±1.1	9.9
Theristus	*Oncholaimus*	27	DHA	0.25	0.12	6.5±4.9	23.7
			EPA	0.27	0.18	4.2±6.2	15.5
			C18:0	0.05	0.1	2.9±2.9	10.6
Theristus	*Odontophora*	23	EPA	0.27	0.18	4.5±10.5	19.9
			C22:1ω9	0.05	0	2.4±7.2	10.8
			C22:5ω3	0.05	0.09	2.1±7.2	9.4
			DHA	0.25	0.21	1.8±1.4	7.9
			C16:0	0.09	0.05	1.7±1.5	7.7
Theristus	*Enoplus*	23	DHA	0.25	0.16	4.3±3.2	18.6
			EPA	0.27	0.2	3.6±9.2	15.5
			C22:5ω3	0.05	0.1	2.3±6.8	9.9
			C22:1ω9	0.05	0.01	2.2±6.8	9.3
Theristus	*Enoploides*	24	EPA	0.27	0.17	4.9±8.4	20.2
			C18:0	0.05	0.13	4.4±7.2	18.1
			DHA	0.25	0.28	2±1.6	8.4
			C22:5ω3	0.05	0.08	1.7±3	7.1
Theristus	*Adoncholaimus*	26	DHA	0.25	0.1	7.3±5.7	27.6
			C16:1ω7	0.05	0.13	4±12.6	15.2
			EPA	0.27	0.21	2.8±7	10.6

Compare groups		AV Diss	FA	Relative Concentration		Av.Diss±SD	Contrib%
Species 1	Species 2	%		Species 1	Species 2		
Daptonema	*Oncholaimus*	30	DHA	0.29	0.12	8.4±3.3	28
			C16:0	0.07	0.14	3.6±5.2	11.8
			EPA	0.24	0.18	3±2.4	9.8
Daptonema	*Odontophora*	22	DHA	0.29	0.21	3.9±1.7	17.4
			EPA	0.24	0.18	3.2±3	14.5
			C22:5ω3	0.04	0.09	2.7±4.6	12.3
			C20:1	0.02	0.05	1.5±19.3	6.8
Daptonema	*Enoplus*	23	DHA	0.29	0.16	6.2±2.5	27.5
			C22:5ω3	0.04	0.1	2.9±4.7	12.8
			EPA	0.24	0.2	2.3±2.1	10.1
Daptonema	*Enoploides*	24	C18:0	0.05	0.13	3.9±6.6	16.7
			EPA	0.24	0.17	3.6±3	15.3
			C18:1ω7	0.08	0.03	2.4±8.2	10
			C22:5ω3	0.04	0.08	2.3±3	9.9
Daptonema	*Adoncholaimus*	28	DHA	0.29	0.1	9.2±3.7	33.4
			C16:1ω7	0.04	0.13	4.4±6.7	16
Oncholaimus	*Odontophora*	29	DHA	0.12	0.21	4.7±8.9	16.3
			C16:0	0.14	0.05	4.4±14.3	15
			C22:5ω3	0.05	0.09	2.1±6.7	7.3
			C16:1ω7	0.08	0.04	2.1±3.4	7.2
			C20:1	0.01	0.05	1.9±29.4	6.6
Oncholaimus	*Enoplus*	20	C16:0	0.14	0.09	2.5±5.4	12.7
			C22:5ω3	0.05	0.1	2.3±6.3	11.3
			DHA	0.12	0.16	2.2±4.1	11
			C20:1ω9	0	0.04	1.8±11.4	8.8
			C16:1ω7	0.08	0.05	1.4±2.4	7
Oncholaimus	*Enoploides*	32	DHA	0.12	0.28	8.2±6	25.2
			C16:0	0.14	0.07	3.7±11	11.5
			C18:1ω7	0.1	0.03	3.4±2.3	10.6
			C16:1ω7	0.08	0.02	3±4	9.1
Oncholaimus	*Adoncholaimus*	15	C16:1ω7	0.08	0.13	2.3±3.9	15.6
			EPA	0.18	0.21	1.4±2.1	9.7
			ARA	0.07	0.05	1.4±3.3	9.4
			C18:1ω7	0.1	0.08	1±0.7	7.1
			C22:1ω9	0.03	0.01	0.9±5.2	6.3
			C18:1ω9	0.04	0.03	0.9±1.1	6.2
Odontophora	*Enoplus*	17	DHA	0.21	0.16	2.5±4.5	15.3
			C16:0	0.05	0.09	1.8±3.8	11
			C20:1	0.05	0.02	1.6±22	9.4
			C20:0	0.03	0	1.5±1.2	9.3
			C18:1ω7	0.06	0.08	1±4.7	5.8
Odontophora	*Enoploides*	23	DHA	0.21	0.28	3.4±2.5	14.6
			C18:0	0.07	0.13	3.3±18.6	14.2
			C22:1ω9	0	0.05	2.2±14.7	9.2
			C20:1	0.05	0.01	1.8±29.6	7.8
			C18:1ω9	0.04	0	1.8±48.4	7.8

Compare groups		AV Diss	FA	Relative Concentration		Av.Diss±SD	Contrib%
Species 1	Species 2	%		Species 1	Species 2		
Odontophora	*Adoncholaimus*	29	DHA	0.21	0.1	5.5±12.1	19.1
			C16:1ω7	0.04	0.13	4.4±15.1	15.3
			C16:0	0.05	0.14	4.1±17.7	14.1
			C22:5ω3	0.09	0.05	2.3±9.6	7.9
Enoplus	*Enoploides*	23	DHA	0.16	0.28	6±4.4	26.1
			C18:0	0.08	0.13	2.7±4.8	11.7
			C18:1ω7	0.08	0.03	2.5±10.1	10.9
			C22:1ω9	0.01	0.05	1.9±25.4	8.3
Enoplus	*Adoncholaimus*	18	C16:1ω7	0.05	0.13	3.7±17.7	20.5
			DHA	0.16	0.1	3±6.4	16.6
			C22:5ω3	0.1	0.05	2.4±8.3	13.5
Enoploides	*Adoncholaimus*	35	DHA	0.28	0.1	8.9±6.8	25.2
			C16:1ω7	0.02	0.13	5.3±10.7	14.8
			C16:0	0.07	0.14	3.4±12.8	9.7

Table S3.4. Result of PERMANOVA tests on the FA composition of nematodes. Results are shown of a one-way PERMANOVA with factor species and of a two-way PERMANOVA with factors species and station. The former included all nematode samples, while the latter only included information on three species that were present at both st1 and st16.

PERMANOVA	Source	df	SS	MS	Pseudo-F/t	P
main test	Species	8	12676	1585	16	***0.001***
pairwise test	*Metachromadora, Praeacanthonchus*				2.9	***0.007***
	Metachromadora, Theristus				7.7	***0.002***
	Metachromadora, Oncholaimus				5.1	***0.013***
	Metachromadora, Odontophora				7.6	***0.008***
	Metachromadora, Daptonema				6.2	***0.005***
	Metachromadora, Enoplus				6.1	***0.013***
	Metachromadora, Enoploides				6.9	***0.001***
	Metachromadora, Adoncholaimus				3.7	***0.01***
	Praeacanthonchus, Theristus				3.4	***0.001***
	Praeacanthonchus, Oncholaimus				2.8	***0.009***
	Praeacanthonchus, Odontophora				3.4	***0.006***
	Praeacanthochus, Daptonema				3.0	***0.001***
	Praeacanthochus, Enoplus				2.7	***0.005***
	Praeacanthochus, Enoploides				3.2	***0.001***
	Praeacanthochus, Adoncholaimus				2.3	***0.014***
	Theristus, Oncholaimus				6.2	***0.001***
	Theristus, Odontophora				5.9	***0.001***
	Theristus, Daptonema				2.4	***0.008***
	Theristus, Enoplus				6.3	***0.001***
	Theristus, Enoploides				5.1	***0.002***
	Theristus, Adoncholaimus				7.3	***0.001***
	Oncholaimus, Odontophora				6.2	***0.002***
	Oncholaimus, Daptonema				4.9	***0.003***

PERMANOVA	Source	df	SS	MS	Pseudo-F/t	P
	Oncholaimus, Enoplus				4.4	***0.011***
	Oncholaimus, Enoploides				5.4	***0.007***
	Oncholaimus, Adoncholaimus				3.2	***0.007***
	Odontophora, Daptonema				4.0	***0.003***
	Odontophora, Enoplus				5.8	***0.002***
	Odontophora, Enoploides				5.7	***0.006***
	Odontophora, Adoncholaimus				10.5	***0.001***
	Daptonema, Enoplus				4.3	***0.003***
	Daptonema, Enoploides				3.3	***0.013***
	Daptonema, Adoncholaimus				5.3	***0.003***
	Enoplus, Enoploides				6.2	***0.004***
	Enoplus, Adoncholaimus				7.8	***0.003***
	Enoploides, Adoncholaimus				10.0	***0.001***
main test	Species	2	3990	1995	14	***0.001***
	Station	1	176	176	1	0.274
	SpeciesxStation	2	307	154	1	0.35
pairwise test	*Metachromadora, Praeacanthonchus*				1.9	***0.035***
	Metachromadora, Theristus				8.6	***0.001***
	Praeacanthonchus, Theristus				3.0	***0.001***

Significant p values are marked in bold and italics.

Table S3.5. Concentration of six fatty acid markers in three nematode species and related PERMANOVA tests. Two-way PERMANOVA results (a) based on a Euclidean distance matrix of the relative abundances of individual fatty acid markers (b) in three nematode species (*Metachromadora remanei*, *Praeacanthonchus punctatus* and *Theristus acer*) at two stations (st1 and st16).

a

Markers	Factor	Source/Groups	df	SS	MS	Pseudo-F/t	P(MC)	p-PERMDISP
C16:1ω7		Sp	2	0.059	0.029	14.4	***0.002***	0.219
		St	1	0.002	0.002	0.8	0.407	0.253
		SpxSt**	2	0.001	0.000	0.2	0.779	
		Metachromadora, Praeacanthonchus				2.5	***0.033***	
		Metachromadora, Theristus				8.0	***0.001***	
		Praeacanthonchus, Theristus				1.9	0.091	
EPA		Sp	2	0.001	0.000	0.2	0.832	0.177
		St	1	0.002	0.002	1.3	0.259	0.92
		SpxSt	2	0.003	0.001	1.1	0.391	
EPA/DHA		Sp	2	18.4	9.2	74.6	***0.001***	***0.042***
		St	1	1.9	1.9	15.2	***0.004***	0.693
		SpxSt	2	1.1	0.5	4.5	***0.032***	
	Metachromadora	st1 vs st16				1.9	0.275	
	Praeacanthonchus	st1 vs st16				3.3	***0.034***	
	Theristus	st1 vs st16				11.1	***0.002***	
	st1	*Metachromadora, Praeacanthonchus*				5.0	***0.021***	
	st1	*Metachromadora, Theristus*				8.1	***0.002***	

Markers	Factor	Source/Groups	df	SS	MS	Pseudo-F/t	P(MC)	p-PERMDISP
	st1	*Praeacanthonchus, Theristus*				11.3	***0.004***	
	st16	*Metachromadora, Praeacanthonchus*				2.7	***0.025***	
	st16	*Metachromadora, Theristus*				16.5	***0.001***	
	st16	*Praeacanthonchus, Theristus*				4.9	***0.004***	
DHA		Sp	2	0.095	0.048	103.3	***0.001***	***0.017***
		St	1	0.006	0.006	14.0	***0.003***	0.38
		SpxSt	2	0.002	0.001	2.0	0.179	
		Metachromadora, Praeacanthonchus				4.1	***0.006***	
		Metachromadora, Theristus				22.6	***0.001***	
		Praeacanthonchus, Theristus				7.9	***0.001***	
		st1 vs st16				3.7	***0.003***	
C15:0+C17:0		Sp	2	0.002	0.001	3.8	0.052	0.188
		St	1	0.000	0.000	0.0	0.906	0.712
		SpxSt	2	0.001	0.000	1.5	0.273	
C18:1ω7		Sp	2	0.001	0.001	5.3	***0.022***	0.479
		St	2	0.000	0.000	1.3	0.269	0.724
		SpxSt**	2	0.000	0.000	0.1	0.91	
		Metachromadora, Praeacanthonchus				2.3	0.06	
		Metachromadora, Theristus				3.7	***0.006***	
		Praeacanthonchus, Theristus				0.4	0.675	

Significant p values are marked in bold and italics.

b

		C16:1ω7	EPA	EPA/DHA	DHA	C15:0+C17:0	C18:1ω7
Metachromadora	st1	0.163592	0.25614	3.312311	0.080111	0.039441	0.078018
	st16	0.201582	0.254604	3.647944	0.069994	0.026169	0.082755
Praeacanthonchus	st1	0.09811	0.238184	1.462478	0.163041	0.017153	0.061451
	st16	0.108687	0.292128	2.824593	0.10826	0.03688	0.066434
Theristus	st1	0.044532	0.265362	0.980465	0.270653	0.008603	0.055935
	st16	0.05142	0.269549	1.214655	0.221955	0.008643	0.065388

Chapter 4 Characterization of marine nematode associated microbiomes by high-throughput sequencing

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Chapter 4 Characterization of marine nematode associated microbiomes by high-throughput sequencing

Abstract

Invertebrate microbiomes may contain information that is relevant to the feeding ecology, fitness, and symbiotic relationships of their hosts. The present study characterizes the spatial (i.e. two stations with contrasting sediment granulometry) and temporal (i.e. three consecutive seasons) variation in the microbiomes of three microphytobenthos biofilm-associated marine nematode species (*Metachromadora remanei, Praeacanthonchus punctatus, Theristus acer*) in relation to the microbiomes of the nematodes' substrates. Only 5 % of the prokaryotic OTUs found in sediments were ever encountered in nematode microbiomes, and only up to 20 % of OTUs from nematode microbiomes were present in sediments. There was also no link between the proportional abundance of specific bacterial taxa in sediments and in nematodes, demonstrating that nematode microbiomes are distinct from those of sediments. Moreover, only just less than half of the OTUs that were shared between nematodes and sediments were also common to all three nematode species, suggesting selective relationships between nematode species and sediment bacteria. These relationships probably involve selective feeding; no clear indications were found for the presence of prominent species-specific nematode-bacteria symbioses. Differences in nematode microbiomes were mostly prominent between *M. remanei* on the one hand and *T. acer* and *P. punctatus* on the other, which likely reflects known differences in their mode of feeding. The microbiomes of sediments and nematodes were strongly context-dependent, differing among stations as well as seasons. A substantial portion (61 %) of the variation in sediment microbiomes, but a much smaller portion of the variation in nematode-associated microbiomes (7-23 %), could be explained by the spatiatemporal variation in sediment granulometry and in biomass and composition of the microphytobenthos.

Keywords: microbiomes, marine nematodes, intertidal flat, microphytobenthos, trophic relationships, symbiotic relationship

4.1 Introduction

Marine benthic communities are complex networks in which primary producers, prokaryotes and their respective grazers, including a variety of invertebrate taxa, interact in multiple ways and with diverse consequences for a range of ecosystem functions (Gerbersdorf et al., 2009; Hubas et al., 2010; Passarelli et al., 2014; Stock et al., 2014; Van Colen et al., 2014). Many knowledge gaps still hamper our understanding of the dynamics of these communities and their functioning. These gaps range from

the quantification of the importance of direct trophic interactions, such as bulk and selective metazoan grazing on microalgae and prokaryotes, to our understanding of much more intricate interaction networks in which microalgae, bacteria and invertebrates affect each other's fitness and interactions with the other players in the network.

One element in these interaction networks that may affect the fitness and functional performance of a broad range of marine organisms, from diatoms (Amin et al., 2012; Sison-Mangus et al., 2014) over micro-invertebrates (Gerdts et al., 2013; Derycke et al., 2016; Schuelke et al., 2018) all the way up to fish and other vertebrates (Schmidt et al., 2015), is their microbiome. In animals, the microbiome comprises both a microbiome *sensu stricto*, i.e. all bacteria living in or on an animal host, from pathogens to mutualists, and a microbiome *sensu lato*, i.e. the bacteria taken up as food or attached to food organisms (Derycke et al., 2016; Schulenburg and Félix, 2017). Microbiomes thus contain information that can be relevant to the feeding ecology, fitness, and symbiotic relationships of their hosts (Cabreiro and Gems, 2013; Schulenburg and Félix, 2017), and have therefore been receiving increasing attention in a variety of host organisms (see, e.g., the special issue of the journal Molecular Ecology on 'The host-associated microbiome' in 2018).

Nematodes are by far the most abundant metazoans in many terrestrial, freshwater and marine soils and sediments (Traunspurger, 2000; Yeates et al., 2009; Moens et al., 2013). A range of nematode species may also feed on bacteria. Nematode microbiomes have hitherto mostly been studied in the model organism *Caenorhabditis elegans* (Dirksen et al., 2016; Samuel et al., 2016; Schulenburg and Félix, 2017; Zhang et al., 2017), a species from terrestrial environments. We know of only two papers which have studied the microbiomes of marine nematodes (Derycke et al., 2016; Schuelke et al., 2018), the former focusing on species-specific microbiome differences in three intertidal-living congeneric bacterivores that belong to the same nematode family as *C. elegans*, whereas the latter included a broad range of species from very different marine habitats. Neither of these studies investigated temporal variability in nematode microbiomes, nor did they compare nematode microbiomes to those of the substrate the nematodes inhabit.

Prokaryotes are the most important decomposers of organic matter in tidal flat sediments (Henrichs and Doyle, 1986; Rusch et al., 2001), yet *in situ* labeling studies suggest that their most important fate is mortality (Herman et al., 2001; Van Oevelen et al., 2006b), e.g. through viral lysis, whilst transfer of prokaryotic biomass up the food chain would be limited (Van Oevelen et al., 2006a). The results of one such *in situ* pulse-chase experiment have indicated that meiobenthos (with nematodes as the dominant component) may graze ca 3 % of prokaryotic carbon production, while prokaryotic biomass in turn accommodates no more than 6 % of the nematodes' carbon requirements (Van Oevelen et al., 2006a, 2006b). However, such estimates are community-based, hence it is plausible that at least some

nematode species would rely more heavily on prokaryotic biomass. Moreover, such estimates may also be context-dependent. As an example, the genera *Metachromadora, Daptonema* and *Theristus* from tidal flat sediments in the Schelde estuary relied prominently on microphytobenthos (Moens et al., 2005a, 2014; Wu and Moens, chapter 3 of this thesis), whilst the same genera in a mudflat sparsely vegetated with seagrass depended primarily on detritus-associated micro-organisms (Vafeiadou et al., 2014). In addition to bacterivory, other, non-trophic interactions between nematodes and bacteria exist. These include both ecto- and endosymbioses, which are well-documented for a few marine nematode species (Polz et al., 1992; Musat et al., 2007), but also the existence of a gut microflora, the roles of which for their nematode hosts may be manifold (Cabreiro and Gems, 2013). This gut microflora can be highly species-specific and can contribute a large portion of the bacterial OTUs that are found inside bacterivorous marine nematodes (Derycke et al., 2016).

Here, we use a metagenetic approach to document nematode-bacteria relationships in tidal flat sediments. We deep-sequence the 16S ribosomal RNA gene of multiple specimens of three abundant nematode species from two stations on a tidal flat in the Schelde estuary, sampled in three consecutive seasons, to reconstruct their microbiomes and address the following questions and hypotheses. (1) Given that even the microbiomes of 'strict' bacterivore nematode species comprise a substantial portion of non-food related bacteria (Derycke et al., 2016; Dirksen et al., 2016), we expected that the microbiomes of our nematode species would significantly differ from those of their surrounding sediment. (2) We also expected to find significant species-specific differences in microbiomes, which would be in part linked to differences in their feeding ecology. Based on observations of their feeding behavior, 'deposit feeders' such as *Theristus acer* are expected to co-ingest bacteria along with microalgae, detrital or sediment particles, whereas epistratum feeders such as *Metachromadora remanei* selectively pierce and suck out food particles (Moens and Vincx, 1997; Moens et al., 2004). *Praeacanthonchus punctatus* may take an intermediate position; it is generally considered an epistratum feeder, but it often ingests prey whole in much the same way as deposit feeders do (Moens and Vincx, 1997; Moens et al., 2014). Under the assumption that bacterivory is a major source of these nematodes' microbiomes, we expected the microbiome of *T. acer* to better resemble that of its substratum (sediment with microphytobenthos (MPB) biofilm) and to reflect a clearer signature of bacterivory than would be the case for *M. remanei* (3) Given the trophic dependence of the three nematode species used here on microphytobenthos biofilms as a basal carbon source (Moens et al., 2005a, 2014; Wu and Moens, chapter 3 of this thesis), we expected to find spatial and seasonal variation in nematode microbiomes that would be closely coupled to the spatiotemporal variability in microphytobenthos biomass and composition. Alternatively, it is currently unknown whether species-specific (feeding or other) associations between particular marine

nematodes and bacterial strains are prominent. If they are, then we should expect to find these associations largely independently of the environmental context.

4.2 Material and Methods

4.2.1 Sampling and pre-treatment of samples

Sampling was conducted at two stations on the Paulina intertidal flat (Fig. 4.1) in the Scheldt Estuary, SW Netherlands, in three consecutive seasons: winter (December 2014), spring (April 2015) and summer (June 2015), to investigate the microbiomes of different 'hosts' (three nematode species and sediment) in relation to environmental variables such as sediment granulometry, phytopigment concentrations and total organic matter content (Table 4.1). Station 1 (st1) was located on bare, fine sandy sediment just upstream of the easternmost border of a salt marsh. Station 2 (st2) was situated in one of the main drainage gullies of the marsh, the sediment being characterized mainly by silt and very fine sand.

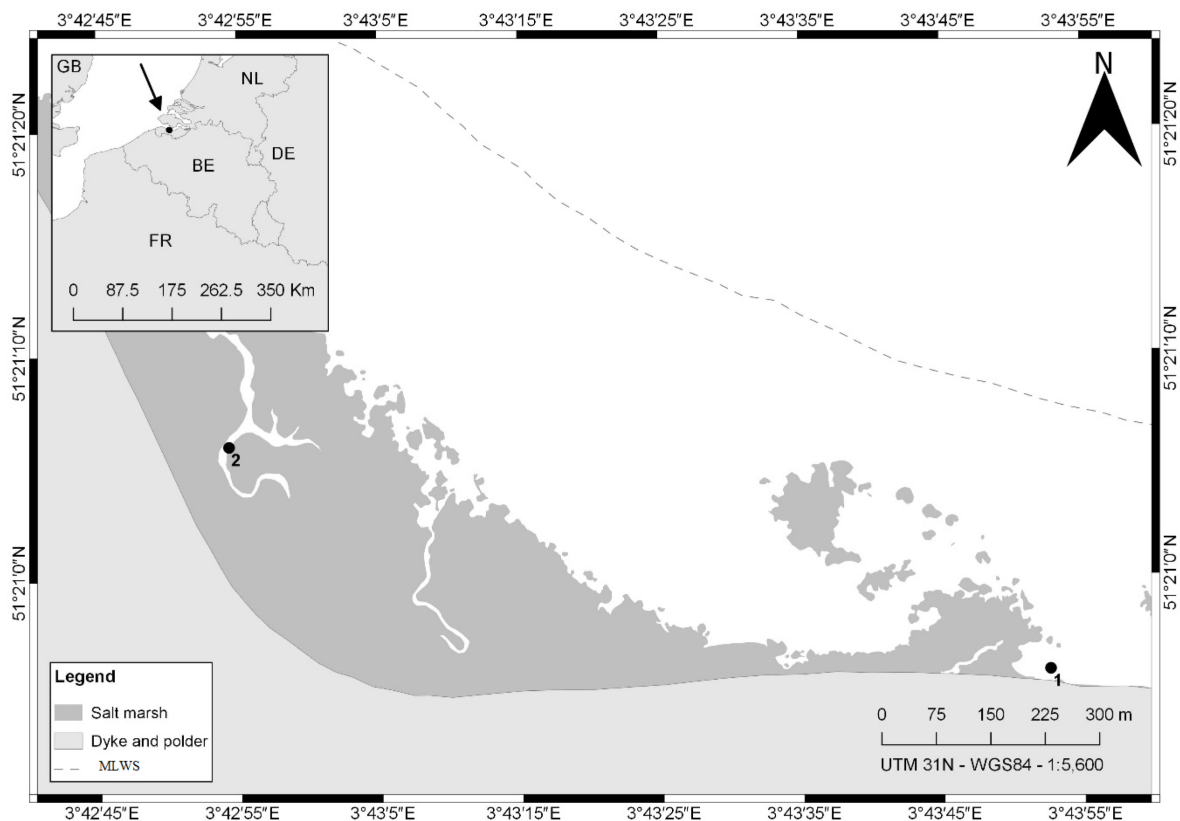


Fig. 4.1. Location of our sampling stations (stations 1 and 2) at the Paulina tidal flat, Schelde Estuary, The Netherlands. MLWS is mean low water spring tide level; high water spring tide level coincided with the position of the dyke. St 2 corresponds to station 16 in chapter 3.

The top-2 cm of sediment was collected using a spoon and pooled into a bucket until an area of ca 0.25 m^2 had been collected. This amount of sediment was then well mixed by hand and subsampled four times each for environmental variables and for nematodes. Samples for phytopigment concentrations were taken with a cut-off syringe with an inner diameter of 1.5 cm down to a depth of 2 cm. A 144-cm^2 Petri dish was filled with sediment for grain size and total organic matter (TOM) analysis. In the field, samples for phytopigments, TOM and granulometry were immediately stored in the dark in a cooling box. Back in the lab, they were preserved at -80 °C (phytopigment samples) or -20 °C (granulometry + TOM samples) until analysis. The remaining sediment from the buckets was used for the collection of nematodes for microbiome analysis. It was transported without any preservation to the lab, where nematodes were elutriated and hand-sorted alive before being preserved in worm lysis buffer (WLB) (Williams et al., 1992).

Three replicate sediment samples for sediment microbiome analysis were taken in the field using a 3.5-cm inner diam. perspex hand corer to a depth of 2 cm and immediately preserved in DESS (Yoder et al., 2006), with a DESS:sediment ratio of 2:1 (vol:vol).

Table.1 Origins of different phytopigments, based on Wright and Jeffrey (1997).

Variables	Abbr.	Major organisms	Additional organisms
Chlorophyll a	chl a	photosynthetic algae, higher plants	
Chlorophyll b	chl b	chlorophytes, euglenophytes	
Pheophytin a	pheo	higher plants, and algal detritus	
Pyropheophytin a	pyro	zooplankton faecal pellets	
Peridinin	peri	photosynthetic dinoflagellates	
Diadinoxanthin	diadino	diatoms, prymnesiophytes	chrysophytes, dinoflagellates
Diatoxanthin	diato	diatom, prymnesiophytes	chrysophytes, dinoflagellates
Chlorophyll c1,c2	c1c2	chromophytes, brown seaweeds	
β-Carotene	b-car	cryptophytes, prochlorophytes, rhodophytes	
Fucoxanthin	fuco	diatoms, prymnesiophytes, brown seaweeds, raphidophytes	dinoflagellates with endosymbionts
Lutein	lutein	chlorophytes, prasinophytes, plant	
Zeaxanthin	zea	cyanobacteria	prasinophytes, cryptophytes, prochlorophytes
pheo/(chl a+pheo)	PAP ratio	higher values indicate more degraded algal matter	
Chl a/TOM	Chl *a/*TOM	the proportion of fresh photoautotrophic-derived organic matter in TOM	

4.2.2 Analysis of environmental samples

4.2.2.1 Measurements

Sediment samples for analysis of granulometry and TOM were first thawed and dried at 60 °C until reaching constant weight. Granulometry and median grain size (MGS) were analyzed using laser

diffraction on a Malvern Mastersizer Hydro 2000G, following a protocol from the Malvern supporter (https://www.malvern.com/en/support/product-support/mastersizer-range/mastersizer-2000). TOM content was calculated from the difference in the weight of sediment samples before and after combustion in a muffle furnace at 500 °C for 2 h.

Samples for phytopigment analysis were first lyophilized and homogenized, then extracted in 90 % acetone at 4 °C overnight, and separated by reversed-phase high-performance liquid chromatography (HPLC) with fluorescence detection according to Wright and Jeffrey (1997). Table 4.1 provides an overview of the different phytopigments (and phytopigment ratios) used as food-related environmental variables and of their origins. The ratio of chlorophyll a to TOM was used as an indicator of food quality, with high values indicating a high proportion of fresh autotrophic biomass in the sediment organic matter pool. The ratio of phaeopigments to the sum of Chl*a* + phaeopigments (PAP ratio) was calculated as an indicator of the freshness of primary producer-derived biomass in the sediment (Boon and Duineveld, 1997), with high values indicating that more primary producer biomass is in a degraded state.

4.2.2.2 Data analysis

Environmental data were first checked using a draftsman scatter plot to assess collinearity. After log transformation of skewed variables, highly correlated variables (r> 0.95, Pearson correlation) were removed. Principal component analysis (PCA) was then used to explore temporal and spatial patterns in environmental data.

4.2.3 Collection of nematodes for microbiome analysis

Nematodes were extracted from the sediment by repeated (three times) vigorous stirring followed by decantation of sediment aliquots with filtered (diam. 90 mm) seawater. After their elutriation from sediment, nematodes were kept alive in autoclaved artificial sea water (ASW) with a salinity of 23 at a temperature of 4 °C, and hand-picked one by one on the tip of a fine tungsten wire needle. Sorting was performed within 24 h after nematode elutriation from the sediment, using a Leica binocular microscope (Wild M10) (20-40X). At least eight individuals per species x station x sampling time were picked up. Nematodes were subsequently rinsed twice by transferring them into new embryo dishes with clean ASW (salinity of 23) to remove particles and loosely attached bacteria. Finally, each individual was transferred to 20 µl WLB (worm lysis buffer) in Eppendorf tubes (0.5 ml) for further genetic analysis. Each specimen was stored in a separate tube and preserved at -20 °C until DNA extraction.

We targeted three nematode species which were abundant at both field sites and easy to identify to species level under low magnification: *Metachromadora remanei* (M), *Praeacanthonchus punctatus*

(P) and *Theristus acer* (T) (microbiome host: M, T, P). We regularly and randomly sorted additional individuals in the same way and identified those under high magnification as a test of our accuracy in sorting the correct nematode species. Based on these tests, identification was flawless.

4.2.4 DNA extraction, amplification, and sequencing

Total DNA of sediment samples was extracted using the Power soil® DNA isolation kit (Mo Bio, Laboratories, Carlsbad, California, USA), with ca 0.3 g wet sediment used for each replicate. This was followed by the protocol of the Power soil® DNA isolation kit according to manufacturer's instructions.

DNA of individual nematodes was extracted by adding 1 µl proteinase K into the Eppendorf tube with a nematode in WLB, and incubated at 65 °C for 1 h, followed by an increase in temperature to 95 °C for 10 min (Derycke et al., 2007). Extracted DNA of sediments and nematodes was stored at -20 °C until further analysis.

A multiplexed DNA library was obtained through a single-step PCR (polymerase chain reaction) process where amplification and attachment of barcodes happened simultaneously, with target sequences being the V4 region of the 16*S rRNA*. The PCR mix (20-µl volumes) included 11.4 µl PCR water, 4 µl HFX buffer, 0.4 µl dNTP (10 mM), 1 µl forward primer (515F) (Table S4.1), 1 µl reverse primer (806R) (Table S4.1), 2 µl template DNA and 0.2 µl Phusion HiFi HS. The PCR cycle comprised the following steps: denaturation at 98 °C for 30 s, followed by 35 cycles of annealing (98 °C for 20 s, 50 °C for 30 s, and 72 °C for 30 s); elongation happened at 72 °C for 10 min. Different samples can be recognized by barcodes in each Miseq run, with primers and barcodes referenced from the earth microbiome project (http://press.igsb.anl.gov/earthmicrobiome/protocols-and-standards/16s/). Three 20-µl PCR technical replicates per DNA sample were conducted and PCR success was checked by electrophoresis (2 %), by reprocessing the samples with faint or weak bands. The three technical replicates were mixed and stored in the fridge until a final pool library was obtained and then stored at -20 °C until further processing.

Purification was completed through running E-gel size selection on a fragment size of 300 bp on 20 µl of PCR product/barcode. Based on Qubit analyses, no substantial differences in DNA concentration were found among purified samples. Hence, they were pooled in equal volumes (1 µl of PCR product/barcode) to prepare a final library. A Bioanalyzer was used to check DNA concentration and fragment length of this library, with a final DNA concentration of 10 nM. Paired-end sequencing was performed on an Illumina Miseq platform at the Genomics Core facility of the KULeuven, Belgium.

4.2.5. Bioinformatics

4.2.5.1 Sequence processing

Demultiplexed data was assembled and quality filtered by using Paired-End reAd mergeR (PEAR) (Zhang et al., 2014), with a minimum quality score of 25, a fragment length between 200 and 1000 bp, and removal of singleton sequences. Quality of sequences was subsequently checked using FastQC (Bioinformatics, 2011). Primer and adapters were trimmed using cutadapt (Martin, 2011) and quality was checked again with FastQC. The checked reads were used as input data. Downstream processing (OTU picking and clustering) was performed using QIIME (v 1.9.1) (Caporaso et al., 2010).

Taxonomy was assigned up to 7 levels (Kingdom, Phylum, Class, Order, Family, Genus, and Species), against 99 % taxonomy and OTU files of the Greengenes 13.8 reference database (DeSantis et al., 2006), using an open-reference OTU picking strategy (pick_open_reference_otus.py). We used default values of QIIME 1.9.1, except for percentage of failed sequences to include in the subsample to cluster de novo, which was set at 0.01 instead of 0.001. OTUs were defined at a 97 % sequence similarity level, or labelled as 'not available' (NA) when no hit was observed.

4.2.5.2 Downstream data analysis and statistics

Taxonomic profile

Taxonomic composition of the microbiomes was calculated on merged, non-rarefied datasets, using collapse_samples.py and summarize_taxa_through_plots.py in QIIME (Caporaso et al., 2010). To investigate microbiome composition in each nematode species and sediment, non-rarefied datasets merged per 'Host' were employed. To assess differences in microbiomes of particular hosts between seasons and sampling stations, a dataset merged per 'HostStationSeason' (HSS) was used. Relative frequencies (RF) of taxa were generated by summarize_taxa_through_plots.py; main taxa were defined as taxa with RF values ≥ 1 %, while taxa with values of RF < 1 % were pooled in a low-frequency group (LF). We restrict the description of the taxonomic composition of main taxa to the phylum level in each host (nematode species and sediment). In addition, to examine to what extent OTUs in each nematode species were also present in sediments, the numbers of OTUs, of shared OTUs among hosts, and of unique OTUs per host were calculated using collapse_samples.py, filter_otus_from_otu_table.py and shared_phylotypes.py in QIIME.

Nematode and sediment microbiome diversity and composition

We determined microbiome diversity indices: number of observed OTUs and Chao's first estimator as richness measures; Shannon-Wiener diversity as an index which combines aspects of richness and evenness; and the inverse of the Simpson Index (InvSimpson) as a measure of evenness. To account for differences in the numbers of sequences between samples, we used a dataset that was rarefied to

4600 sequences per sample, with the sequence depth being slightly smaller than the minimum sample read count (4656); rarefaction clusters samples more clearly than other normalisation approaches (Weiss et al., 2017). All indices were calculated using Phyloseq in R (McMurdie and Holmes, 2013).

Differences in diversity indices between hosts (4 levels, i.e. three nematode species and sediment), stations (2 levels: st1, st2) and seasons (3 levels, i.e. spring, summer, and winter) were analysed using a factorial design in PERMANOVA (PERmutational Multivariate ANalysis Of VAriance) (Anderson, 2005), because the data did not meet the assumption of normality required for parametric ANOVA. Euclidean distance was used as a similarity measure. Because PERMANOVA is sensitive to heterogeneity of variances (i.e. the 'dispersion effect'), PERMDISP was used to assess whether obtained significant differences could be explained by such dispersion effect or by real factor effects. Pairwise tests were done on significant factor(s) or interaction terms in PRIMER (Anderson et al., 2008). Due to the small number of replicates for sediment microbiome samples (3) and stations (2), we used Monte Carlo permutations for the pairwise tests with a limited number of unique permutations (< 100) (Anderson and Robinson, 2003).

Similarly, three-way PERMANOVA following the same factorial design as above was performed to examine differences in microbiome composition between hosts, seasons and stations, using generalised Unifrac (GuniFrac) distances (α=0.05) (Lozupone et al., 2011), with taxonomic relatedness being taken into account by these distances. We used Principal Coordinates Analysis (PCoA) included in the GUniFrac package (Lozupone et al., 2011) to visualise differences among microbiomes of different datasets.

Core microbiomes

Core microbiomes were determined on a non-rarefied dataset using the following script in QIIME: compute_core_microbiome.py. Only OTUs present in at least 80 % of all samples of a particular dataset were considered to belong to the core microbiome. The taxonomic profile of core OTUs was presented in the same way as the microbiome composition described above.

Heatmap plots were used to visualise the distribution of core microbiomes in the rarefied dataset using Phyloseq in R (McMurdie and Holmes, 2013). Linear discriminant analysis effect size (Lefse) with factor host was used to identify the most differentially abundant taxa of the core microbiomes of the three nematode species, and with factor StationSeason to detect the taxa that were present in all nematode hosts but with differences in relative abundances among stations and/or seasons. Lefse was conducted through the online website (http://mbac.gmu.edu:8080/). It was performed using a non-parametric Kruskal-Wallis rank sum test followed by linear discriminant analysis to examine the

effect size of those significant strains which are considered biomarker taxa (Segata et al., 2011). Default values of Lefse were used.

Differences in the above core microbiome composition were examined in the same way as microbiome composition mentioned above, with PERMANOVAs performed on the GuniFrac distances of the core microbiomes distributed in the rarefied dataset instead of on the 'total' microbiomes. PCoA was used to visualise differences among the core microbiomes.

Relation of microbiome composition with environmental variables

To examine the relationship between host microbiomes and environmental variables, a distance-based linear modeling (DistLM) routine was performed, which essentially uses a distance-based redundancy analysis approach (dbRDA) (Anderson et al., 2008). It performs a permutation test for the null hypothesis of no relationship between the data matrices of the microbiomes (GUniFrac distance) and of the environmental variables (marginal test). The tests implemented in DistLM allow straightforward interpretation of partial regression tests since they are based on the individual samples (Anderson et al., 2008). Marginal tests identify the individually significant predictor variables. Sequential tests reflect the relative importance of the variables after removal of strongly collinear ($r > 0.95$) variables, and examines whether adding a particular variable in a specified order contributes significantly to the explained variation, using a step-wise selection procedure (Anderson et al., 2008).

DistLM analysis was further conducted on multiple data subsets based on microbiomes of separate hosts or groups of hosts, to examine the correlation of each host with environmental variables. These data subsets were Nematoda, sediments (i.e. the two sediments together), *M. remanei*, *P. punctatus* and *T. acer*.

4.3 Results

4.3.1 Environmental variables

The first and second axis (PC1 and PC2) of a PCA explained 71.0 % and 16.1 %, respectively, of the variation in the environmental variables dataset (Fig. 4.2), with a large variability present mainly in st2. The first axis separated st1 and st2, mainly in relation to median grain size, organic matter content and several phytopigment concentrations (pheophytin *a*, chlorphyll *c*1*c*2 and fucoxanthin). In short, st1 was characterised by coarser sediment and a higher organic matter quality as indicated by the chl*a*/TOM ratio, whereas st2 had finer sediment with higher total organic matter and phytopigment concentrations.

Seasonal variation in environmental variables at st1 was limited and mainly separated winter and spring from summer samples along PC2. Samples from st2 were more clearly separated between

seasons, most of this variability also being manifest along PC2 in relation to concentrations of lutein, zeaxanthin (both with highest concentrations in winter and lowest in spring), diadinoxanthin and diatoxanthin (both with highest concentrations in spring and lowest in winter).

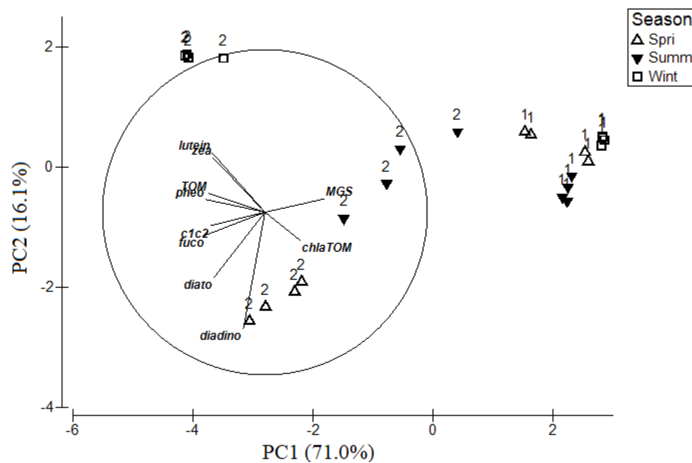


Fig. 4.2. Principal component analysis (PCA) showing the variation in sediment samples from two different stations (1, 2) in three seasons (Spri (spring), Summ (summer) and Wint (winter)) with respect to 10 variables related to sediment texture (median grain size, MGS) and food availability/quality (see Table 4.1 for abbreviations) after stepwise exclusion of highly collinear (r>0.95) variables: chl*a* (vs fuco, peridinin), β-carotene (vs lutein, pheo, PAP, pyro, chlb), chl b (vs lutein), peridinin (vs fuco), pyro (vs pheo) and PAP (vs pheo). The first and second axis of the PCA ordinations captured 71.0% (PC1) and 16.1% (PC2) of the variation in these sediment variables. Seasons are indicated by different shapes, while station is indicated by a number (1 or 2).

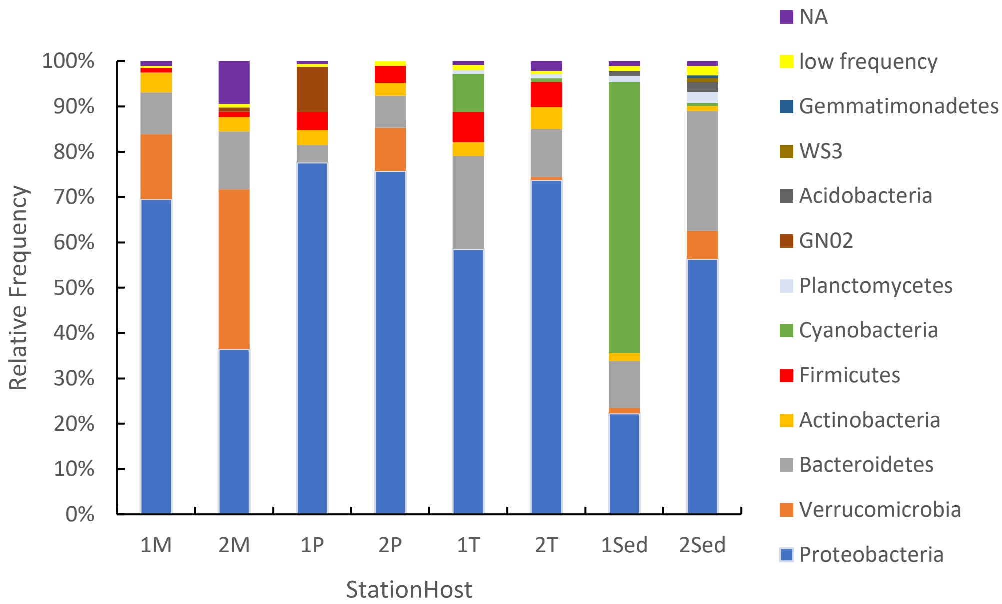
4.3.2 Microbiomes

4.3.2.1 Taxonomic profile

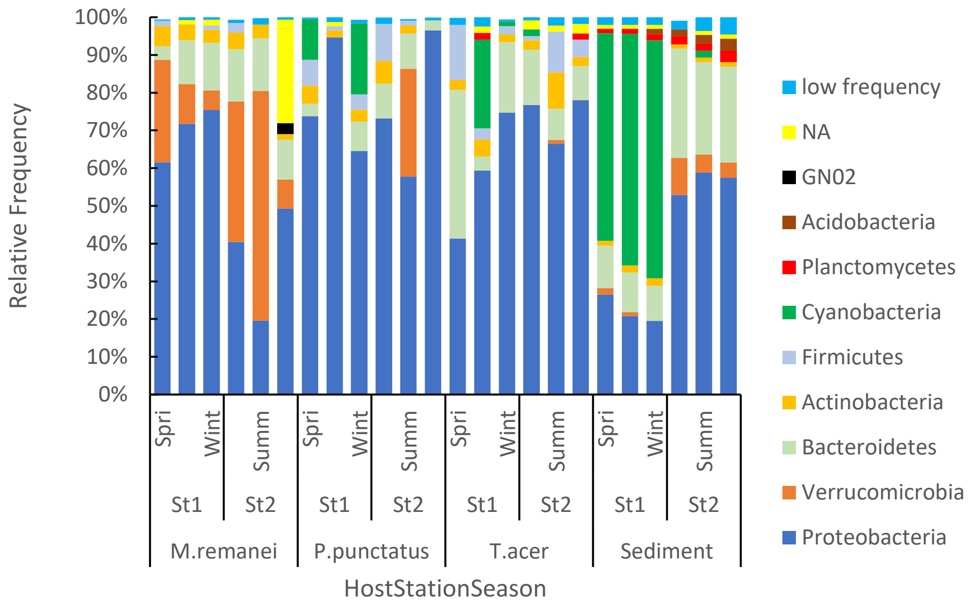
Microbiome prokaryotes comprised 329,469 OTUs, belonging to 67 phyla, 197 classes, 396 orders, 684 families and 1247 genera in 2 kingdoms (Bacteria and Archaea) and unassigned taxa group (NA) (S4.2).

An overview of microbiome phyla and observed OTUs per host and per 'HostStationSeason' can be found in supplementary file S4.2. There was considerable variability in OTU richness and composition among specimens of the same species but sampled at different stations or seasons; numbers of OTUs per specimen ranged from 90 – 200. Each host presented a different microbiome profile at two stations (Fig. 4.3a). Overall, these microbiomes were mainly composed of eleven known phyla, with the dominant phyla being Cyanobacteria in sediment at st1 (60 %) and Proteobacteria in sediment at st2 (56 %) and in nematodes (70 % (36 %), 78 % (76 %) and 58 % (74 %) for *M. remanei*, *P. punctatus* and *T. acer* at st1 (st2), respectively). The sum of the low-frequency phyla was below 2 % of total OTUs in sediment and in all three nematode species. Unassigned taxa (NA) made up 9 % of total OTUs in *M. remanei* at st2, but less than 2 % in other hosts at both st1 and st2 (Fig. 4.3a). The high abundance of NA in *M. remanei* was mainly because of the prominence of NA in winter at st2 (Fig. 4.3b). The top 10 genera per host were listed in figure 4.3c1-c4.

a



b



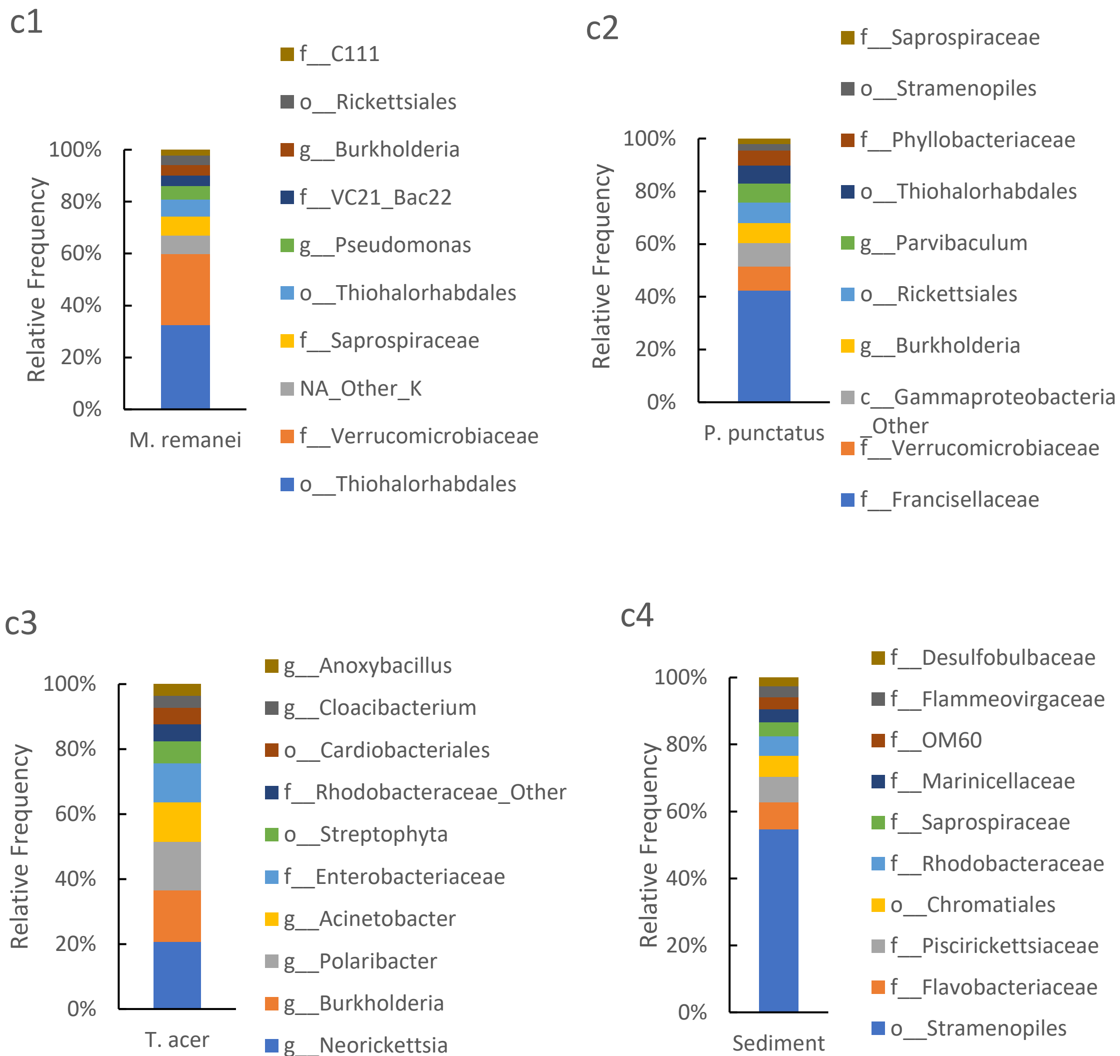
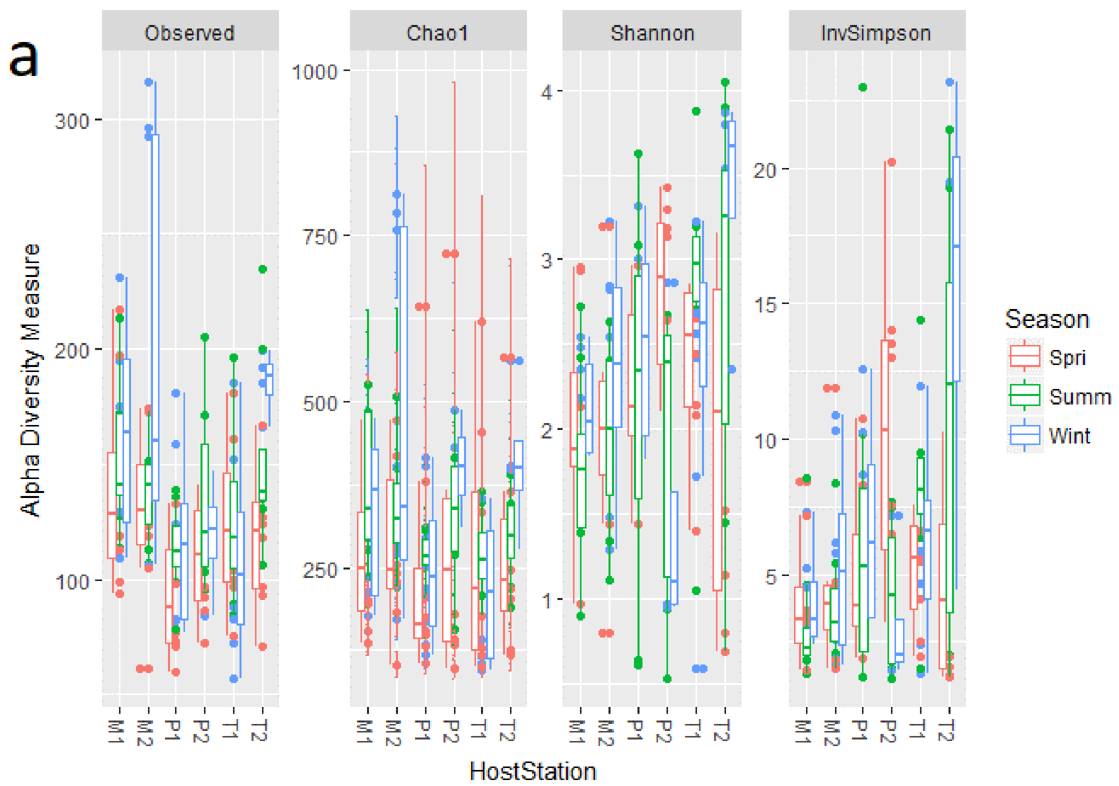


Fig. 4.3. Taxonomic assignment at the Phylum level of Miseq reads per microbiome host (M: *Metachromadora remanei*, P: *Praeacanthonchus punctatus*, T: *Theristus acer*, Sed: sediment) at each station (1, 2) (a) and per hoststationseason (b), and top 10 genus per host (c1-c4), unknown genus was showed to higher known taxa level (c1-c4), with "c__, o__, f__, g__" referring to class, order family and genus level, respectively (c1-c4). 'Low frequency' refers to the (sum of the) prokaryotic taxa which occurred in a relative abundance < 1 %. "NA" and "Other" are OTUs which could not be assigned to known taxa. NA_other_K indicates a genus from an unknown kingdom.

Cyanobacteria was the most dominant phylum at st1 in all three seasons in sediments, followed by Proteobacteria and Bacteroidetes; while at st2, Proteobacteria was the most dominant group across all three seasons, followed by Bacteroidetes and Verrucomicrobia (Fig. 4.3b). Proteobacteria was almost consistently the most dominant phylum in nematodes. The only exception was observed in *M. remanei* at st2 in summer, with Verrucomicrobia (61 %) being the most abundant phylum. Proteobacteria was almost consistently the most dominant phylum in nematodes (Fig. 4.3b). The second and third most dominant phyla varied between stations and seasons in each nematode species.



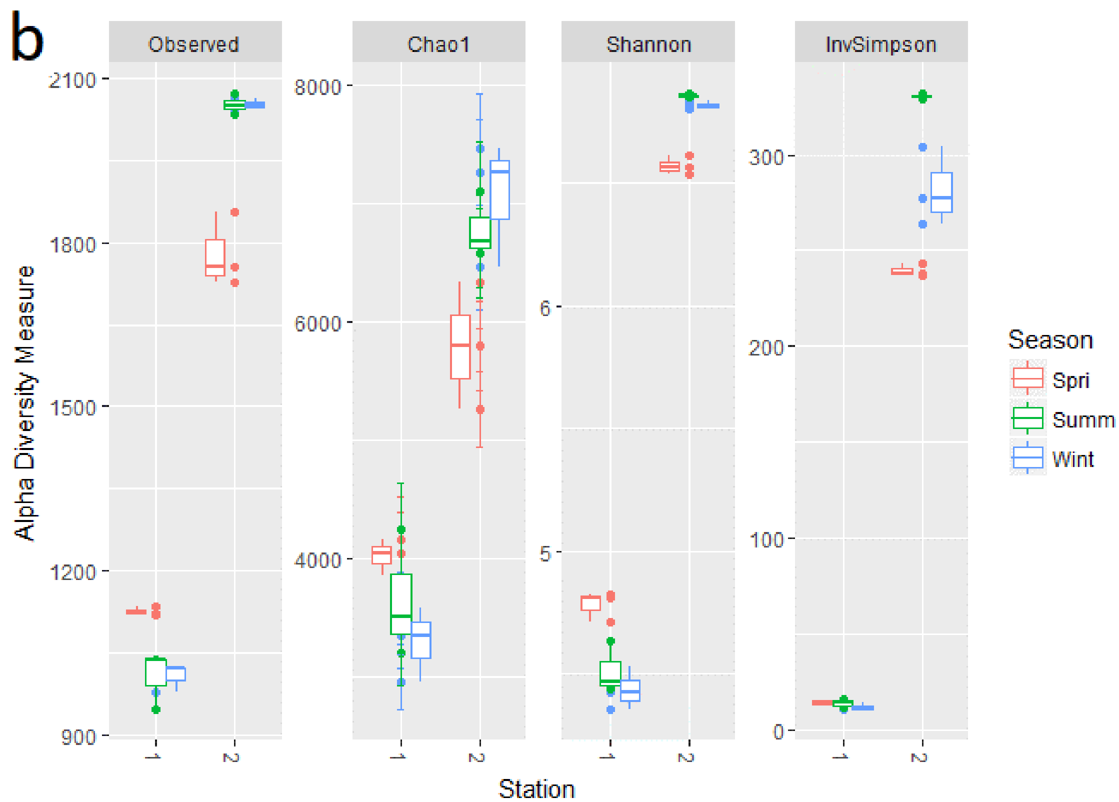


Fig. 4.4. Alpha diversity indices of nematode (a) and sediment microbiomes (b) based on the rarefied (4600 sequences per sample) dataset. Samples are labelled with a letter followed by a number (a). Letters M, P, and T refer to the nematode species *Metachromadora remanei, Praeacanthonchus punctatus* and *Theristus acer*, respectively (a), while numbers (1, 2) refer to stations 1 and 2. Sampling was conducted in three subsequent seasons: Wint (Winter: December), Spri (Spring: April), Summ (Summer: June).

Those phyla were Proteobacteria, Bacteroidetes and Verrucomicrobia in *M. remanei*, with the relative frequency of Verrucomicrobia differentiating most sampling stations and seasons. In *P. punctatus* and *T. acer*, these were Cyanobacteria, Bacteroidetes and Firmicutes, with addition of Verrucomicrobia in *P. punctatus* and of Actinobacteria (in *T. acer*), with the relative frequency of each of these phyla varying between sampling dates. The taxonomic profile at class level is further visualised in Fig. 4.3c.

The proportion of OTUs shared between sediment and each nematode species varied from 17 % in *M. remanei* over 19 % in *P. punctatus* to 23 % in *T. acer*. Each nematode host species harboured ca. 15,000 OTUs, which was ca. one quarter of the OTU richness of sediment, and shared ca 3000 OTUs with sediment. The percentages of unique OTUs per nematode species were 43 % in *M. remanei*, 32 % in *P. punctatus* and 36 % in *T. acer*. More details about the number of shared OTUs among different hosts are shown in Fig. S4.4.

4.3.2.2 Nematode and sediment microbiomes

4.3.2.2.1 Diversity

The interaction among host, station and season significantly influenced all microbiome diversity indices (observed OTU numbers, Chao1, Shannon-Wiener diversity and InvSimpson index) calculated on the rarefied dataset (Table S4.5). An overview of all pairwise tests can be found in table S4.5b. In short, sediments hosted much higher values of all alpha diversity indices than all the nematode species at the two stations and across all three seasons (Fig. 4.4). Pairwise tests further revealed that sediment of the silty station (st2) had a much higher microbiome richness and evenness (high values of InvSimpson) than that of the sandy station (st1) in all three seasons.

In nematodes, spatial and temporal differences of microbiome diversity indices within a given nematode species were only observed on a few occasions in *P. punctatus* and *T. acer*, whereas *M. remanei* had similar diversity indices across stations and seasons. Specifically, a higher microbiome richness occurred in winter in *T. acer* (observed OTUs and Chao1) and in *P. punctatus* (Chao1) at the silty compared to the sandy station, whereas a lower Shannon-Wiener diversity in *P. punctatus* was observed at the silty station, also in winter (Fig. 4.4a). Differences among seasons were only observed in *T. acer* at the silty station, with a higher number of observed OTUs, as well as a higher Shannon-Wiener diversity and InvSimpon index in winter compared to spring (Table S4.5b).

Differences of microbiome diversity indices among nematode species were mainly due to differences between the microbiomes of *M. remanei* and those of the other two species. *M. remanei* had a higher microbiome richness (observed OTU numbers) than *P. punctatus* at the sandy station in all three seasons, and a lower Shannon-Wiener diversity at the silty station in spring. *M. remanei* also had a higher microbiome richness than *T. acer* in summer (Chao1) and in winter (observed OTU) at the sandy

station, and a lower Shannon-Wiener diversity and InvSimpson index in summer at the sandy station and in winter at the silty station. Differences in diversity among the microbiomes of *P. punctatus* and *T. acer* were only observed in winter at the silty station, with higher numbers of observed OTUs and a higher Shannon-Wiener diversity in *T. acer* (Table S4.5b).

4.3.2.2.2 Microbiome composition

The interaction among host, station and season significantly influenced microbiome composition (table S4.7a, Pseudo-F = 1.4, p = 0.001). An overview of the results of the pairwise tests can be found in table S4.7b.

Sediment microbiomes differed significantly from each nematode microbiome across all three seasons (Table S4.5; Fig. 4.5a). When looking at only sediment microbiomes, these differed significantly between both stations but not between seasons (Fig. 4.5f, Table S4.5b).

Nematode microbiomes differed among the three species (Table S4.5b) and mainly differentiated *M. remanei* from *T. acer* and *P. punctatus* (Fig. 4.5b): the *M. remanei* microbiome differed from *T. acer* in all three seasons at both stations and from most *P. punctatus* (Fig. 4.5b). Within species, the microbiome of *P. punctatus* differed between stations in all three seasons (Fig. 4.5d); the microbiome of *M. remanei* differed between stations in summer and in winter (Fig. 4.5c), whereas that of *T. acer* only differed between stations in summer (Fig. 4.5e).

Seasonal variation was observed in the microbiomes of all three nematode species, mainly separating spring from summer and winter in *M. remanei* at st1, and winter from spring and summer in *M. remanei* at st2 (Fig. 4.5c) and in *P. punctatus* at st1. Microbiomes of *P. punctatus* differed among all three seasons at st2 (Fig. 4.5d). In *T. acer*, seasonal variation was only apparent at st1, differentiating the summer microbiome from those in spring and winter (Fig. 4.5e).

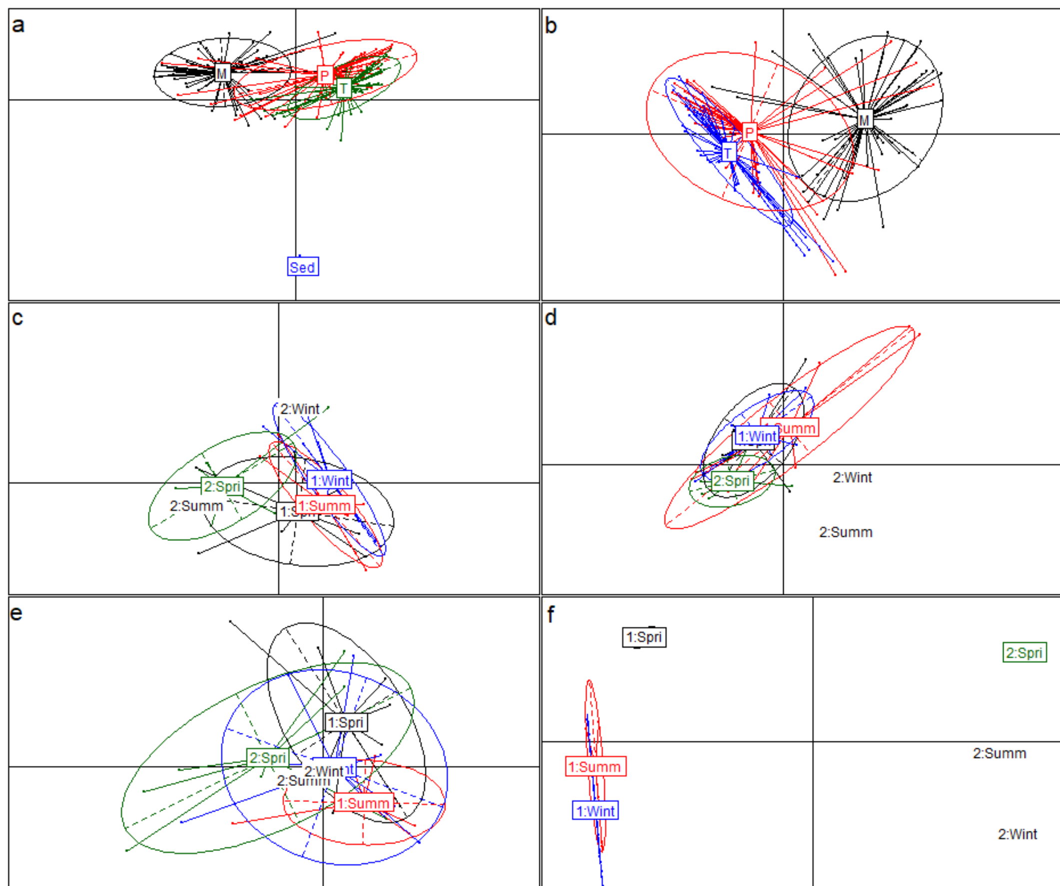


Fig. 4.5. Variation in microbiome composition different dataset: complete (a), nematodes (b), *Metachromadora* (c), *Praeacanthonchus* (d), *Theristus* (e) and sediment (f), visualized in principal coordinates analysis plot using Generalized UniFrac distances based on rarefied data (depth: 4600). Letters: M, P, T, Sed indicated microbiome host *M. remanei*, *P. punctatus*, *T. acer* and sediment, respectively; Numbers: 1 and 2 indicated sampling station 1 and 2, respectively; Spri, Summ, Wint indicated sampling time falling in season: spring, summer and winter, respectively.

4.3.3 Core microbiomes

An overview of the relative frequencies of core microbiome phyla per host and per 'HostStationSeason' can be found in Fig. S4.6, Table S4.7. No more than two OTUs (New.ReferenceOTU803, New.Cleanup.Reference 369849) were found in all nematode samples. The core microbiome (i.e. the assemblage of OTUs present in at least 80 % of all samples of all hosts across stations and seasons) was composed of 32 OTUs belonging to six phyla of bacteria, with Proteobacteria being most prominent. Proteobacteria (21 OTUs) was also the most dominant phylum in each nematode species, which differed from sediments where Bacteroidetes were the dominant phylum in the core microbiome. Proteobacteria was consistently the most dominant phylum per nematode HostStationSeason, except for some individuals of *M. remanei* and *P. punctatus* where Verrucomicrobia dominated. The taxonomic profile of the core microbiome at class level (Fig. S4.6c) varied between nematode hosts.

An overview of the distribution and relative abundances of core OTUs in all specimens of each host is given in the form of heatmaps (Fig. S4.8). In sediments, all 32 core OTUs were present in low abundance (Fig. S4.8d). In nematodes (Fig. S4.8a, S4.8b, S4.8c), three OTUs (New.ReferenceOTU803, New.Cleanup.Reference 369849 and NR6083) were abundant in most specimens of all three nematode species. Except for these three OTUs, NCR145209, NR10069, NR10514 and NR5973 were also abundant in most specimens of *M. remanei* (Fig. S4.8a). The abundances of other OTUs were strongly dependent on station and season.

The effects of the factors host, season and station and their interactions on the composition of the core microbiome (PERMANOVA, Fig. 4.6, Table S4.5) were highly similar to those obtained for complete microbiomes (see section Microbiome composition), with a perfect match between both in terms of pairwise differences for the three-way interaction effect. This similarity is less pronounced when comparing the PCoA ordinations of core and whole microbiomes (Fig. 4.5, Fig. 4.6), probably because a majority of core OTUs occurred in low abundance in sediments (Fig. S4.8).

An overview of the most differentially abundant taxa among the three nematode species (factor nematode host) as detected with Lefse analysis can be found in table S4.9. Verrucomicrobiales and Verrucomicrobiaceae differentiated *M. remanei* from the other two host species, while the most differentially abundant taxa in *P. punctatus* and *T. acer* were Proteobacteria and Flavobacteriales, respectively (Table S4.9a).

When focusing on individual host species, an overview of the most differentially abundant prokaryotic taxa that discriminated the microbiomes among stations and seasons in each nematode species can be found in table S4.9. In short, the most differentially abundant taxa were observed at all sampling occasions in *M. remanei* (Table S4.9b), and at most sampling occasions in *P. punctatus* (except for sampling at st1 in winter) (Table S4.9c) and *T. acer* (except for sampling at st2 in summer) (Table S4.9d). Ten taxa (*Delftia*, Methylobacteriaceae, *Methylobacterium*, Bacteria, Proteobacteria, Pseudomonadales, *Acinetobacter*, Gammaproteobacteria, Bacteroidetes and Moraxellaceae) were observed as the most differentially abundant ones in all three nematode species. Four of these (*Delftia* (NR3964), Methylobacteriaceae (NR3143), *Methylobacterium* (NR3143), Bacteria) were observed at the same station and same season in all three nematode species, with the first three observed at st1 in summer and Bacteria observed at st2 in spring. Other taxa differed between nematode species.

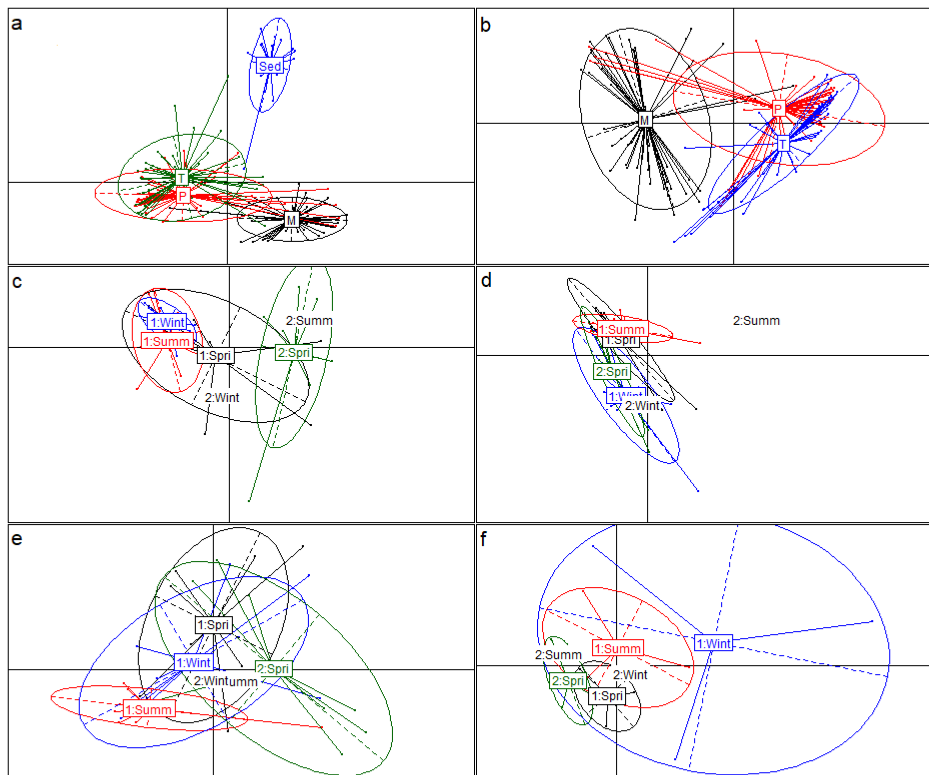


Fig.6 Variation in core microbiome (OTUs across at 80% of all the unrarefied samples) of different dataset: complete (a), nematodes (b), *Metachromadora* (c), *Praeacanthonchus* (d), *Theristus* (e) and sediment (f), visualized in principal coordinates analysis plot using Generalized UniFrac distances based on rarefied data (depth:4600). Letters: M, P, T, Sed indicated microbiome host *M. remanei*, *P. punctatus*, *T. acer* and sediment, respectively; Numbers: 1 and 2 indicated sampling station 1 and 2, respectively; Spri, Summ, Wint indicated sampling time falling in season: spring, summer and winter, respectively.

4.3.4 Relation of environmental variables with microbiomes

An overview of the correlation between environmental variables and microbiomes of separate hosts examined with DistLM analysis can be found in table 4.2. Marginal tests showed that all variables had a significant individual effect on sediment and *M. remanei* associated microbiomes, with single variables explaining 16-48 % of the variation in sediment microbiomes but only 4-8 % in *M. remanei* microbiomes. Most variables (14 out of 16) had an individual effect on *P. punctatus* associated microbiomes, with single variables capturing no more than 4-7 % of the variation in microbiome composition (Table 4.2). Fewer (8 out of 16) variables had an individual effect on *T. acer* associated microbiomes. Sequential tests revealed that a combination of median grain size (MGS), zeaxanthin and pheophytin a concentration best explained the variation (61 %) in sediment microbiomes. A combination of MGS, diadinoxanthin, chlorophyll a and pheophytin a best explained the variation (23 %) *in M. remanei* associated microbiomes, while a combination of zeaxanthin, lutein and pheophytin a best explained the variation (16 %) in *P. punctatus* associated microbiomes. MGS and zeaxanthin together explained no more than 7 % of the variation in *T. acer* associated microbiomes.

Table 4.2. Result of distance-based linear model (DistLM) analyses showing the influence of environmental parameters on each microbiome host: Nematoda, *Metachromadora remanei*, *Praeacanthonchus punctatus*, *Theristus acer* and Sediments. Marginal tests showed the individual effect and sequential tests showed the significantly explained variation (percentage: P.) by fitting variables within sets sequentially using step-wise selection, and conditional tests using 9999 permutations of residuals under a reduced model. Values in bold indicate P < 0.05.

DistLM tests		Nematoda		*Metachromadora*		*Praeacanthonchus*		*Theristus*		Sediments	
	Variable	P	P.	P	P.	P	P.	P	P.	P	P.
Marginal test	bcar	***0.003***	2%	***0.001***	6%	***0.002***	5%	0.064	3%	***0.001***	34%
	c1c2	***0.06***	1%	***0.01***	4%	***0.031***	4%	0.418	2%	***0.002***	28%
	chlb	***0.002***	2%	***0.001***	6%	***0.002***	5%	0.066	3%	***0.001***	32%
	chl*a*	***0.036***	1%	***0.003***	5%	***0.026***	4%	***0.039***	3%	***0.001***	37%
	diadino	***0.039***	1%	***0.001***	6%	0.545	2%	***0.037***	3%	***0.031***	16%
	diato	***0.072***	1%	***0.002***	5%	0.056	3%	***0.019***	4%	***0.001***	35%
	fuco	***0.011***	1%	***0.001***	6%	***0.014***	4%	***0.023***	4%	***0.002***	39%
	lutein	***0.001***	2%	***0.001***	7%	***0.003***	5%	0.1	3%	***0.001***	26%
	peridinin	***0.03***	1%	***0.001***	5%	***0.022***	4%	***0.024***	3%	***0.001***	37%
	pheo	***0.007***	2%	***0.001***	6%	***0.007***	5%	***0.049***	3%	***0.001***	36%
	pyro	***0.002***	2%	***0.001***	6%	***0.003***	5%	0.096	3%	***0.001***	28%
	zea	***0.001***	2%	***0.001***	6%	***0.001***	7%	0.08	3%	***0.001***	31%
	MGS	***0.002***	2%	***0.001***	8%	***0.003***	5%	***0.006***	4%	***0.001***	48%
	TOM	***0.001***	2%	***0.001***	8%	***0.001***	5%	0.107	3%	***0.002***	38%
	log(PAP+1)	***0.002***	2%	***0.001***	7%	***0.001***	5%	***0.048***	3%	***0.001***	42%
	chl*a*/TOM	***0.005***	2%	***0.001***	7%	***0.006***	4%	0.245	3%	***0.001***	27%
Sequential tests	+MGS	0.001	2%	0.001	8%			0.013	4%	0.001	48%
	+pheo	0.005	2%								
	+zea	0.021	1%								
	+diadino	0.013	1%	0.001	6%						
	+fuco	0.034	1%								
	+chl*a*			0.001	5%						
	+zea					0.002	7%	0.07	3%	0.001	7%
	+lutein					0.001	6%				
	+chl*a*/TOM										
	+pheo			0.002	4%	0.019	3%			0.001	6%

4.4 Discussion

4.4.1 Nematode microbiomes differ from the microbiomes of the sediments they inhabit

Microbiomes of nematodes clearly differed from those of the sediments from which they were collected, in line with results on the terrestrial nematode *Caenorhabditis elegans* (Dirksen et al., 2016) and on six limnoterrestrial tardigrade species (Vecchi et al., 2018). In our study, sediment microbiomes had a much higher diversity than those of nematodes. In addition, roughly 3000 OTUs, corresponding

to only ca. 5 % of the sediment microbiome diversity, were also found in nematodes, where they accounted for no more than ca. 20 % of the OTUs of nematode microbiomes.

This prominent presence of 'sediment bacteria' in the microbiomes of nematodes (ca. 20 % of nematode OTUs) can be a consequence of several, non-mutually exclusive causes. First, microbiomes in part reflect microbiota ingested as food (Derycke et al., 2016; Schulenburg and Félix, 2017); hence, the presence of sediment bacteria in nematode microbiomes is most likely a consequence of bacterivory. Bacteria are often considered a major food source of marine nematodes, yet with few exceptions, there is very little evidence on the importance – if any – of bacteria in the diet of specific marine nematode species (Moens and Vincx, 1997; Moens et al., 2004). There is also considerable debate as to whether marine nematodes feed selectively: depending on size and morphology of their mouth, nematodes have traditionally been assigned as 'selective' or 'non-selective' feeders (Wieser, 1953; Romeyn and Bouwman, 1983). Controlled lab experiments, by contrast, have demonstrated or suggested that most nematodes are capable of picking particular food sources very selectively from a range of options (e.g. Blanchard, 1991; Moens et al., 1999, 2014; De Mesel et al., 2003). Whether or not their feeding is selective under field conditions remains unknown.

Our data strongly support the idea of selective bacterivory: not only were but 5 % of the sediment OTUs ever encountered in our nematode species, there was also no link between the proportional abundance of specific bacterial taxa in sediments and in nematodes. For instance, several 'sediment bacteria' with high abundances (in terms of numbers of reads) in the microbiomes of *P. punctatus* and *T. acer* were only present in very low read numbers in sediments (Fig. S4.8). Vice versa, whilst Cyanobacteria were the most abundant prokaryotic taxon in st1, they were present in only very low abundances in nematode microbiomes. Moreover, although the numbers of shared OTUs between sediment and nematodes of *M. remanei*, *P. punctatus* and *T. acer* were very similar (2928, 2962 and 3072, respectively), only just less than half of these strains were shared between different nematode species (Fig. S4.4). As a consequence, whereas there was a substantial portion of sediment bacteria that was present in all nematode microbiomes, a larger portion of prokaryotes shared by nematodes and sediment was species-specific, again suggesting selective relationships between nematode species and sediment bacteria.

The presence of shared OTUs between nematodes and sediments may also reflect the occurrence of a specific gut microflora in nematodes. Derycke et al. (2016) studied the microbiomes of three very closely related species of bacterivorous marine nematodes. They concluded that roughly half of the nematode microbiome reflected their microbial food, whilst the other half likely comprised commensal and mutualistic bacteria. Insofar as these bacteria are 'free-living' in the lumen of the

nematode guts, they would likely also occur in nematode faeces and hence become inoculated into the sediments where the nematodes live. In harpacticoid copepods – small crustaceans which often are the second most abundant meiofauna-sized higher taxon in marine soft sediments after nematodes – the presence of an abundant and diverse microflora on and in copepod faecal pellets has been demonstrated (De Troch et al., 2010; Cnudde et al., 2013). Approximately half of these bacteria – both in terms of diversity and abundance – were packed inside the pellets; given the fact that these pellets are surrounded by a peritrophic membrane, such bacteria almost have to originate from the copepod guts and are hence either feeding-derived or gut microflora-derived (Cnudde et al., 2013). Nematode faeces have not been studied in this respect, but it is plausible that these also contain gut microflora that can (temporarily) survive or even remain active in the surrounding sediment.

Alternatively, some bacterial taxa may use nematode guts or outer body surfaces as a temporary or semi-permanent environmental niche, as was also observed in *C. elegans* (Dirksen et al., 2016). It has been suggested that bacteria can co-evolve with their invertebrate hosts and lose their virulence while retaining their ability to accumulate inside their hosts (Schulenburg and Félix, 2017; Shoemaker and Moisander, 2017). It is also well-known that a portion of the bacteria that are ingested by nematodes pass the nematode gut alive (Bird and Ryder, 1993; Ghafouri and McGhee, 2007); some may even benefit from nutrients obtained during their passage through the nematode gut (Schulenburg and Félix, 2017).

In addition, nematode microbiomes may differ from those of their immediate environment because of species-specific nematode-bacteria symbioses (Dirksen et al., 2016) (see next discussion section). Only two bacterial taxa (NR803: *Sphingomonas* and NCR369849: *Burkholderia bryophila*) were present in all nematode individuals and also in sediments. *Sphingomonas* has been documented as a diatom-associated bacteria (Amin et al., 2012). Other bacterial taxa that have been reported in association with diatoms and that were common in our nematode species were *Pseudoalteromonas* (NR9994) (Amin et al., 2012) and Comamonadaceae (NR10667, NR11505 and NR3964) (Decleyre et al., 2015). They were particularly abundant in *P. punctatus* and *T. acer*, but (much) less so in *M. remanei*. Given that microalgae are the prime carbon source for the three nematode species studied here (Wu et al., chapter 3 of this PhD), and more commonly for estuarine tidal flat nematodes (Moens et al., 2005a, 2014; Rzeznik Orignac et al., 2008), it is possible that these bacteria were co-ingested when feeding on diatoms. However, whilst the importance of microphytobenthos as a carbon source for tidal flat nematodes appears well-supported, there may be additional routes of uptake of microphytobenthos carbon other than through direct grazing (Moens et al., 2014; D'Hondt et al., 2018). Bacteria may utilize microphytobenthos expolymeric substances and/or remains of cell walls and as such act as a

trophic intermediate between diatom biofilms and nematodes. *Burkholderia bryophila* was found to associate with mosses and to have anti-fungal activity against phytopathogens (Vandamme et al., 2007), but to our knowledge had not previously been reported from marine habitats.

4.4.2 Nematode microbiomes are species-specific

Our data support the contention that nematode microbiomes are species-specific (Derycke et al., 2016; Dirksen et al., 2016), an observation which is common across invertebrate (Vecchi et al., 2018) as well as vertebrate (Fraune and Bosch, 2007) taxa. However, whereas Derycke et al. (2016) and Dirksen et al. (2016) found significant differences in microbiomes between congeneric species of bacterivores, the present study mainly found pronounced differences in the microbiomes (both in terms of taxonomic composition and diversity) between *M. remanei* on the one hand and *P. punctatus* and *T. acer* on the other. These nematode species belong to three different orders of Nematoda, yet the microbiomes of *P. punctatus* and *T. acer* did not differ consistently, as no significant differences were detected in winter at both stations, and in summer at st1. It is tempting to explain this pattern in relation to the feeding habits of these species, where *T. acer* and *P. punctatus* are mainly ingesters of entire food particles, whereas *M. remanei* feeds by selectively puncturing cells and sucking out the contents.

With respect to feeding strategy, *P. punctatus* and *T. acer* are commonly denoted as deposit feeders (Moens and Vincx, 1997), whereas *M. remanei* is an epigrowth feeder. Deposit feeders ingest food particles whole, whereas epigrowth feeders use a partly evertible tooth to puncture and suck out food particles, or scrape them off from substrates to which they are attached (Moens and Vincx, 1997; Moens et al., 2014). Members of both feeding guilds may, however, utilize essentially the same resources (here mostly diatoms; they are 'unicellular eukaryote feeders' *sensu* (Moens et al., 2004), but in different ways. In this respect, it is plausible that nematodes which ingest particles whole are more likely to co-ingest bacteria from the environment, either attached to their food particles or present in their immediate vicinity. This was confirmed by our observation that most bacteria shared by sediments and nematodes were more abundant in *T. acer* and *P. punctatus* than in *M. remanei*. Still, considerably higher relative abundances of some environmental bacteria (e.g. Myxococcales (NR5973) and VC21_Bac22 (NR10514)) were found in the microbiomes of *M. remanei* than in *P. punctatus* and *T. acer* (Fig. S4.8).

Admittedly, our focus on only three nematode species lacks replication of the factor 'feeding type', so we cannot draw firm conclusions on the effects of feeding strategy on nematode microbiomes. Indeed, a study that analysed the microbiomes of 281 nematode specimens belonging to 33 genera and comprising multiple members of all marine nematode feeding guilds, did not find any consistent

relationships in microbiomes between feeding guilds from local to global scales (Schuelke et al., 2018). Part of this discrepancy in results may be because Schuelke et al. (2018) compared a large(r) number of species from different environments, and found differences between species within feeding groups to be very substantial. On a more technical note, our study used Generalized UniFrac distances to detect differences, while Schuelke et al. (2018) used unweighted UniFrac distances; both measures are powerful to detect differences in rare and highly abundant lineages (Chen et al., 2012), but the latter is less powerful to detect changes in moderately abundant taxa than Generalized UniFrac distance (Chen et al., 2012). Hence, depending on whether differences in nematode microbiomes are mostly caused by moderately abundant prokaryotes or by rare and abundant taxa, GuniFrac distances may or may not prove more powerful in detecting differences.

In addition to differences in microbiome that can be related to species-specific and/or feeding-type specific factors, nematode microbiomes may also differ as a consequence of species-specific nematode-bacteria symbioses in the broadest sense of the word (Dirksen et al., 2016), ranging from pathogenic over commensal all the way to mutualistic relationships. *Metachromadora* is a potentially interesting genus in this respect, as it belongs to the marine family Desmodoridae and order Desmodorida, where some other species (Stilbonematinae) have been reported to be prominently associated with symbiotic, sulphur-oxidizing Gammaproteobacteria (Blome and Riemann, 1987; Polz et al., 1992; Dubilier et al., 2008; Zimmermann et al., 2016). On some nematode hosts, members of the respective clades within the Gammaproteobacteria are found externally, on the cuticle, but switches to endosymbiosis in other hosts have been observed (Zimmermann et al., 2016). In the nematode genus *Astomonema*, also within the order Desmodorida, endosymbiotic Chromatiaceae are present (Musat et al., 2007). We have not observed prominent presence of bacteria on the cuticles of *Metachromadora remanei*, nor did we find bacteria belonging to the clades that form ectosymbioses with Stilbonematinae nematodes in the microbiomes of our nematode species. Chromatiaceae, by contrast, were present in all nematode host species and in sediments, albeit in very low proportional abundances (amounting to < 1 % of bacterial sequence reads in the rarefied dataset), not suggestive of a prominent symbiotic relationship.

Next to these symbiotic Gammaproteobacteria, many Rickettsiales (belonging to the Alphaproteobacteria) have been reported from eukaryotic cells (Yu and Walker, 2006; Vecchi et al., 2018); Rickettsiales have an obligate intracellular life style and highly species-specific relationships with other ecdysozoans, such as tardigrades, have been reported (Vecchi et al., 2018). In our study, Rickettsiales (NR12623 and NR2190) were abundant in the microbiome of *M. remanei*, but they did not differentiate *M. remanei* from *P. punctatus* and *T. acer*, again not indicating signs of species-specific symbioses.

4.4.3 Nematode microbiomes are context-dependent

A remarkable result from a study encompassing a large number of nematode species from very different marine habitats (Schuelke et al., 2018), was the absence of consistent differences in nematode microbiome composition between different marine habitats. Nevertheless, in our 'local' study, the microbiomes of both sediments and nematodes were strongly context-dependent, differing both among stations (which represented two somewhat different intertidal habitats) and between seasons.

Distance-based Linear Modelling demonstrated that a large portion (61 %) of the variation in sediment microbiomes could be explained by the environmental variables measured here. These variables were mostly related to sediment granulometry and to the presence and composition of microphytobenthos. A combination of median grain size, zeaxanthin and pheophytin a had the highest explanatory power. Sediments that differ substantially in granulometry have contrasting biogeochemical properties and biofilms and hence also harbour distinct microbial communities (Herman et al., 2001; Currie et al., 2017). It is therefore not surprising that the fine sandy sediment of st1 harboured a different microbiome compared to the muddy st2. Pheophytin a in our dataset was correlated with total organic matter concentration, supporting its detrital (including decomposing benthic and planktonic microalgae) origin. Zeaxanthin is a pigment found predominantly in Cyanobacteria, which constituted the most abundant prokaryotic taxon in the sandy station 1 throughout the year, whereas they occurred in much lower relative abundance in st2. A high prominence of Cyanobacteria in sandier sediments is not uncommon (Hoffman, 1942; Watermann et al., 1999; Evrard et al., 2010); yet, they have not been found to provide an important food source to nematodes (Evrard et al., 2010). This is supported by a comparison of the microbiomes of sediments and nematodes: Cyanobacteria were virtually absent from the microbiome of *M. remanei* and were present in very low proportional abundances in the other two nematode species, even in st1 where Cyanobacteria were the most dominant prokaryotic group.

In contrast to sediment microbiomes, only a small part of the variation (7-23 %) in nematode-associated microbiomes could be explained in relation to the measured environmental variables. Nevertheless, much like for sediment microbiomes, median grain size and the concentrations of several pigments derived from microalgae and/or Cyanobacteria contributed significantly to the variation in nematode microbiomes. This likely reflects the close interrelationships between sediment grain size, benthic microbial (pro- and eukaryotic) community structure and biomass, and the benthic invertebrates feeding on those microbiota. The highest proportion (23 %) of microbiome variation explained by these factors was in *M. remanei*, suggesting that its microbiome is significantly related to variations in microphytobenthos biomass and/or composition. The smallest proportion of explained

variation (7 %) was in *T. acer*, suggesting that variations in microphytobenthos have little impact on its microbiome. In fact, *T. acer* exhibited the most limited variation in microbiome composition across stations and seasons of the three nematode species, suggesting that its microbiome is relatively little affected by environmental conditions. Insofar as (part of) its microbiome is food-derived, this definitely indicates that the label 'non-selective deposit feeder' (Wieser, 1953) does not fit the feeding ecology of this species. One would rather expect the diets of non-selective feeders to vary along with the variations in food availability, whereas selective feeders may be better capable of utilizing similar resources throughout the year; from that perspective, our results are counter to the idea that epigrowth feeders like *M. remanei* feed more selectively than deposit feeders like *T. acer* (Wieser, 1953). It is also noteworthy that – albeit within the limits of the small proportions of explained variation – the microbiomes of the different nematode species responded to changes in different pigments, suggesting that these species have (trophic or other) relationships with different groups of benthic primary producers and are thus affected by the temporal and spatial variation in MPB composition.

Some biomarker taxa clearly contributed to microbiome differences across stations and seasons in all three nematodes species. As an example, the high abundances of *Methylobacterium* and *Delftia* differentiated microbiomes of all three nematode species in summer from those in spring and winter at st1. Like *Delftia*, Enterobacteriaceae were also biomarker taxa of *M. remanei* and *T. acer* in summer. Both prokaryotic taxa have been linked with diatoms (D'Hondt et al., 2018) and seem to underline a more general shift in biomarker taxa for the microbiome of these two nematode species from more sediment-associated to more diatom-associated taxa in summer. If such shifts are related to nematode feeding, they may point at seasonal changes in nematode feeding ecology.

However, the predominant share of nematode microbiome variation that remained unexplained by the measured environmental drivers clearly demonstrates that nematode microbiome composition is additionally controlled by very different drivers than the sediment ones. It is therefore doubtful that bacterial community composition could predict the population states of these nematodes in their natural environment, as was proposed for the microbiomes of rotting fruits and the populations of *Caenorhabditis elegans* in terrestrial soils (Samuel et al., 2016). Then again, unlike *C. elegans*, the present species are not strong niche specialists nor mainly bacterivores. Nematode microbiome composition has been demonstrated to vary with multiple host-related factors, from host species to genotype and even developmental stage (Dirksen et al., 2016), and can further be 'controlled' by host immunological responses (Kamada et al., 2013) or other stimulatory, inhibitory and suppressive processes (Vecchi et al., 2018), as well as by different 'transfer routes', since bacterial symbionts can be transmitted from environment to host, but also among hosts (Bright and Bulgheresi, 2010).

4.5 Conclusions

This study is one of the first to characterize the microbiomes of marine nematodes in relation to the microbiomes of the nematodes' substrates. We demonstrate that only ca. 20 % of the microbiomes of three nematode species from an estuarine intertidal flat can be directly linked to the microbiomes of their environment, suggesting highly selective trophic and non-trophic relationships between hosts and prokaryotes. Our results join results on other nematodes and other taxa in demonstrating that nematode microbiomes are species-specific. Nevertheless, both the entire and the core nematode microbiomes vary in space and time, and only a small portion of that variation can be explained by variation in environmental factors that drive the composition of the sediment microbiome. The influence of the host on its microbiome is probably the largest knowledge gap and challenge for future research.

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Supplementary information of Chapter 4

Table S4.1. Primer sequences of PCR, with forward and reverse primer sequences indicated by number 1 and 2 respectively. Forward primer was barcoded, barcod sequences were indicated by "X".

	Illumina adapter	golay barcode	pad	linker	primer (515F)/806R
1	AATGATACGGCGACCACCGAGAT-CTACACGCT	XXXXXXX XXXXX	TATGGTAATT	GT	GTGYCAGCMGCCGCGGTAA
2	CAAGCAGAAGACGGCATACGAGAT		AGTCAGCCAG	CC	GGACTACNVGGGTWTCTAAT

S4.2. Website of taxonomic profile of microbiomes.

bar_charts.html

Table S4.3. Summary of total reads of sequences and observed numbers of OTUs in four microbiome hosts (*Metachromadora*, *Praeacanthonchus*, *Theristus* and sediment) at two stations (st1, st2) across three seasons (spring, winter and summer). Observed OTUs were based on rarefied dataset.

Variable	Season	Station	*Metachromadora*	*Theristus*	*Praeacanthonchus*	Sediment
Total reads	Spring	st1	78454 ± 36910	80431 ± 71394	38551 ± 42636	136256 ± 14452
		st2	95577 ± 52593	82090 ± 71085	20407 ± 14523	94945 ± 17226
	Summer	st1	111107 ± 39067	94983 ± 60804	276707 ± 429463	156965 ± 38125
		st2	170998± 6523	167809± 9678	228003± 1487	77382± 7061
	Winter	st1	125173 ± 75573	74757 ± 84341	56521 ± 47352	136390 ± 43396
		st2	149204 ± 135931	108523 ± 20994	193179 ± 120248	72662 ± 5111
Observed OTUs	Spring	st1	140 ± 45	125 ± 36	93 ± 26	1125 ± 8
		st2	129 ± 37	119 ± 32	109 ± 26	1779 ± 68
	Summer	st1	153 ± 32	128 ± 40	113 ± 20	1007 ± 54
		st2	138 ± 22	153 ± 42	135 ± 39	2052 ± 18
	Winter	st1	163 ± 44	109 ± 43	117 ± 38	1007 ± 27
		st2	200 ± 87	186 ± 14	119 ± 26	2053 ± 10

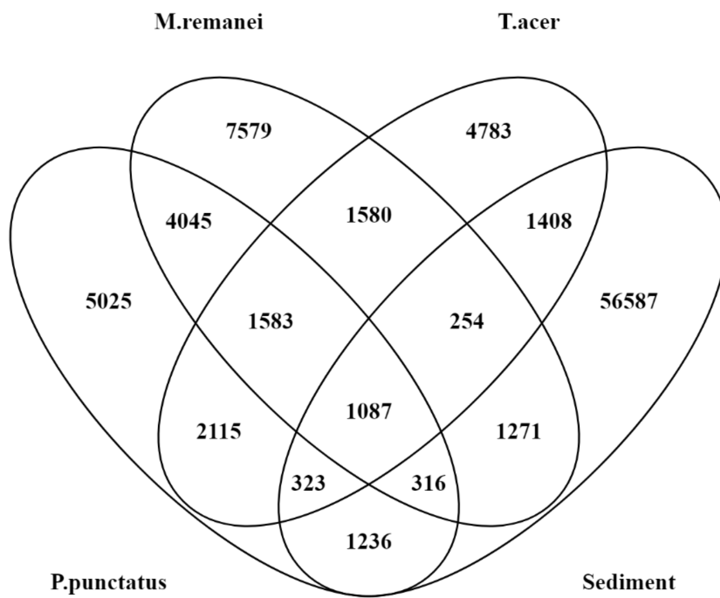


Fig S4.4. Relationship of microbiome composition among three nematode species and sediment, with a combination of all three seasons and two stations, indicated by the numbers of shared OTUs among microbiome hosts.

Table S4.5. Summary of PERMANOVA analysis, including a design of three factors: Host (Ho), Station (St) and Season (Se) conducted separately on all samples (a, b) of diversity indices (observed OTUs number, Chao 1, Shannon diversity index and InvSimpson), of microbiome composition and of core microbiome composition, respectively; with all main tests shown in table a and pairwise test presented in table b. Letters: M, P, T, Sed indicated microbiome host *M. remanei*, *P. punctatus*, *T. acer* and sediment, respectively; Numbers: 1 and 2 indicated sampling station 1 and 2, respectively; Spri, Summ, Wint indicated sampling time falling in season: spring, summer and winter, respectively. Significant values were indicated by bold numbers, p values obtained through Monte Carol test were indicated by bold and italic numbers.

a

dataset	Source	Host (Ho)	Station (St)	Season (Se)	Ho x St	Ho x Se	St x Se	Ho x St x Se
OTU number	df	3	1	2	3	6	2	6
	SS	3.00E+07	1.90E+06	4.10E+04	3.20E+06	1.70E+04	9.90E+04	1.30E+05
	MS	9.90E+06	1.90E+06	2.10E+04	1.10E+06	2.90E+03	5.00E+04	2.10E+04
	Pseudo-F	6.20E+03	1.20E+03	1.30E+01	6.70E+02	1.80E+00	3.10E+01	1.40E+01
	P(perm)	0.001	0.001	0.001	0.001	0.107	0.001	**0.001**
	P(PERMDISP)							**0.001**
Chao1	df	3	1	2	3	6	2	6
	SS	3.60E+08	1.90E+07	4.00E+05	3.20E+07	2.40E+05	1.90E+06	2.50E+06
	MS	1.20E+08	1.90E+07	2.00E+05	1.10E+07	3.90E+04	9.60E+05	4.10E+05

dataset	Source	Host (Ho)	Station (St)	Season (Se)	Ho x St	Ho x Se	St x Se	Ho x St x Se
	Pseudo-F	3.60E+03	5.70E+02	5.90E+00	3.20E+02	1.20E+00	2.90E+01	1.20E+01
	P(perm)	0.001	0.001	0.002	0.001	0.318	0.001	**0.001**
	P(PERMDISP)							**0.001**
Shannon	df	3	1	2	3	6	2	6
	SS	190.2	9.9	0.2	19.2	7.4	0.2	8
	MS	63.4	9.9	0.1	6.4	1.2	0.1	1.3
	Pseudo-F	121.9	19	0.2	12.3	2.4	0.2	2.6
	P(perm)	0.001	0.001	0.822	0.001	0.031	0.859	**0.024**
	P(PERMDISP)							**0.014**
InvSimpson	df	3	1	2	3	6	2	6
	SS	3.20E+05	1.50E+05	3.10E+03	2.90E+05	5.70E+03	2.70E+03	6.10E+03
	MS	1.10E+05	1.50E+05	1.60E+03	9.50E+04	9.40E+02	1.30E+03	1.00E+03
	Pseudo-F	4.80E+03	6.70E+03	6.90E+01	4.20E+03	4.20E+01	5.90E+01	4.50E+01
	P(perm)	0.001	0.001	0.001	0.001	0.001	0.001	**0.001**
	P(PERMDISP)							**0.001**
microbiome composition	df	3	1	2	3	6	2	6
	SS	10	1.3	1	2.7	2.3	0.6	2
	MS	3.3	1.3	0.5	0.9	0.4	0.3	0.3
	Pseudo-F	14	5.5	2	3.8	1.6	1.3	1.4
	P(perm)	0.001	0.001	0.001	0.001	0.001	0.036	**0.001**
	P(PERMDISP)							**0.001**
core microbiome composition	df	3	1	2	3	6	2	6
	SS	10.0	1.3	1.0	2.7	2.3	0.6	2.0
	MS	3.3	1.3	0.5	0.9	0.4	0.3	0.3
	Pseudo-F	14.0	5.5	2.0	3.8	1.6	1.3	1.4
	P(perm)	0.001	0.001	0.001	0.001	0.001	0.039	**0.001**
	P(PERMDISP)							**0.001**

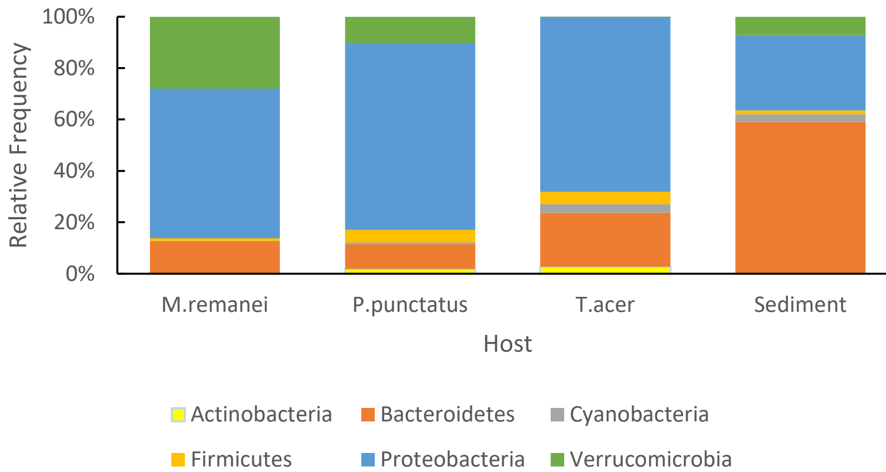
b

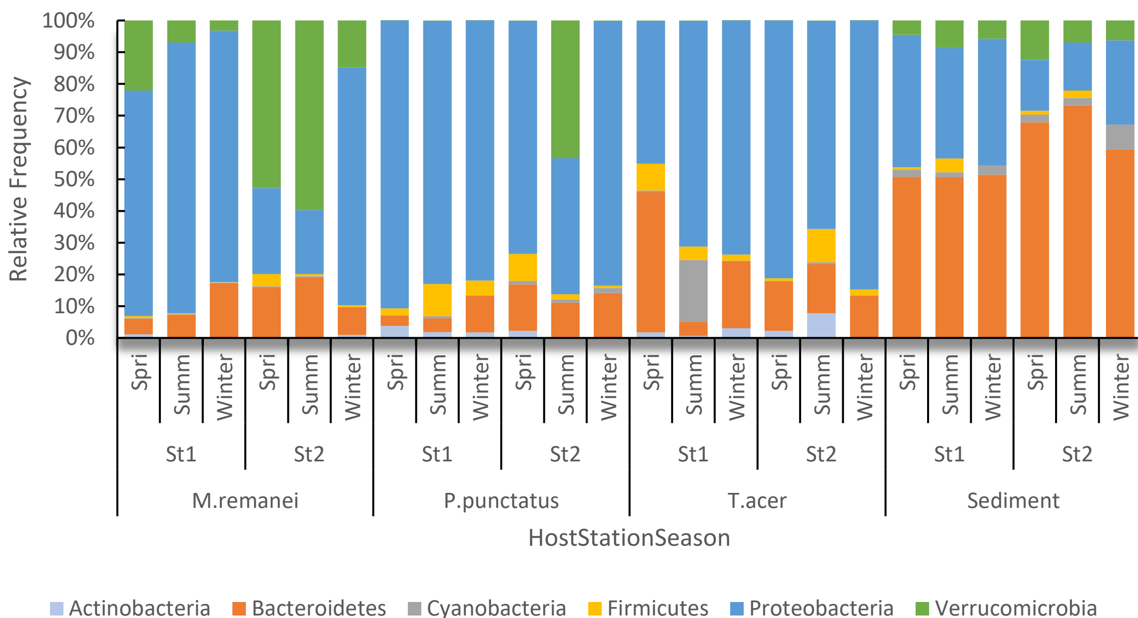
pairwise	test	Groups	Observed	Observed	Chao1	Chao1	Shannon	Shannon	InVsimpson	InVsimpson	microbiome	microbiome	core microbiome	core microbiome
Level	factor		t	P	t	P	t	P	t	P	t	P	t	P
M	Summ	1, 2	1.1	0.292	0.7	0.512	0.6	0.524	0.6	0.565	2.1	**0.001**	2.1	**0.001**
M	Wint	1, 2	1.1	0.314	1.2	0.239	0.8	0.481	1.1	0.277	1.5	**0.001**	1.5	**0.001**
M	Spri	1, 2	0.5	0.622	0.2	0.828	0.1	0.89	0.3	0.773	1.2	0.077	1.2	0.080
T	Summ	1, 2	1.2	0.238	0.8	0.42	0.1	0.923	0.9	0.374	1.3	**0.01**	1.3	**0.017**
T	Wint	1, 2	3.4	**0.01**	2.8	**0.023**	2	0.079	2.8	**0.025**	1	0.275	1.0	0.280
T	Spri	1, 2	0.3	0.763	0.1	0.935	1.1	0.294	0.3	0.73	1.2	0.081	1.2	0.064

pairwise	test	Groups	Observed	Observed	Chao1	Chao1	Shannon	Shannon	InVsimpson	InVsimpson	microbiome	microbiome	core microbiome	core microbiome
P	Summ	1, 2	1.4	0.187	1.7	0.11	0.5	0.595	1	0.373	1.6	**0.005**	1.6	**0.003**
P	Wint	1, 2	0.1	0.897	2.4	**0.045**	2.4	**0.048**	1.8	0.084	1.5	**0.02**	1.5	**0.018**
P	Spri	1, 2	1.3	0.214	0.5	0.646	2.2	0.051	2.2	**0.038**	1.3	**0.004**	1.3	**0.002**
Sed	Summ	1, 2	32	***0.001***	9.1	***0.003***	39	***0.001***	233.2	***0.001***	2.7	***0.012***	2.7	***0.009***
Sed	Wint	1, 2	63.7	***0.001***	10.7	***0.002***	45.8	***0.001***	22.6	***0.001***	2.6	***0.013***	2.6	***0.004***
Sed	Spri	1, 2	16.6	***0.001***	5.5	***0.009***	43.3	***0.001***	114.9	***0.001***	2.9	***0.008***	2.9	***0.008***
st1	Summ	M, T	1.3	0.203	2.3	**0.041**	3	**0.01**	3.2	**0.008**	2.4	**0.001**	2.4	**0.001**
st1	Summ	M, P	3	**0.007**	2.5	**0.023**	0.9	0.376	1.4	0.17	2.2	**0.001**	2.2	**0.001**
st1	Summ	M, Sed	33.6	**0.005**	18	**0.006**	7.9	**0.004**	6.8	**0.009**	2.9	**0.004**	2.9	**0.006**
st1	Summ	T, P	1	0.366	0	0.983	1.4	0.19	0.4	0.754	1.3	0.064	1.3	0.074
st1	Summ	T, Sed	29.8	**0.005**	19.4	**0.01**	3.5	**0.011**	2.6	**0.031**	2.2	**0.01**	2.2	**0.005**
st1	Summ	P, Sed	43.1	**0.005**	19.8	**0.004**	3.5	**0.028**	1.6	0.143	2.2	**0.007**	2.2	**0.008**
st1	Wint	M, T	2.5	**0.027**	2	0.071	0.8	0.47	1.7	0.116	2	**0.001**	2.0	**0.001**
st1	Wint	M, P	2.2	**0.044**	1.4	0.182	1.7	0.118	2.1	0.061	2.1	**0.001**	2.1	**0.001**
st1	Wint	M, Sed	30.5	**0.009**	23.8	**0.011**	12.8	**0.006**	6.6	**0.011**	2.5	**0.008**	2.5	**0.01**
st1	Wint	T, P	0.4	0.694	0.6	0.519	0.4	0.73	0.3	0.754	1.1	0.087	1.1	0.096
st1	Wint	T, Sed	33.1	**0.003**	25.3	**0.012**	4.1	**0.008**	2.6	**0.027**	2.2	**0.008**	2.2	**0.008**
st1	Wint	P, Sed	37	**0.009**	24.8	**0.003**	5.7	**0.007**	2.3	0.068	2.2	**0.008**	2.2	**0.006**
st1	Spri	M, T	0.7	0.481	0.1	0.958	1.3	0.228	1	0.311	1.8	**0.001**	1.8	**0.002**
st1	Spri	M, P	2.6	**0.012**	0.3	0.769	0.7	0.522	0.8	0.454	1.6	**0.005**	1.6	**0.005**
st1	Spri	M, Sed	36.5	**0.006**	43.7	**0.01**	7	**0.007**	6.8	**0.005**	2.4	**0.005**	2.4	**0.009**
st1	Spri	T, P	2	0.064	0.3	0.729	0.7	0.528	0.1	0.942	1.4	**0.002**	1.4	**0.003**
st1	Spri	T, Sed	45.9	**0.007**	31	**0.007**	7.9	**0.009**	7	**0.012**	2.4	**0.003**	2.4	**0.007**
st1	Spri	P, Sed	65.6	**0.007**	31.6	**0.003**	8	**0.01**	5	**0.005**	2.3	**0.009**	2.3	**0.008**
st2	Summ	M, T	0.9	0.423	1.1	0.282	1.8	0.09	2.5	**0.027**	2.2	**0.001**	2.2	**0.001**
st2	Summ	M, P	0.2	0.829	0.1	0.931	0.1	0.934	0.3	0.736	1.2	0.118	1.2	0.127
st2	Summ	M, Sed	136.5	**0.01**	62.8	**0.009**	15.1	**0.009**	240.9	**0.004**	3	**0.012**	3.0	**0.005**
st2	Summ	T, P	0.9	0.366	0.9	0.376	1.6	0.13	2.3	**0.027**	1.6	**0.001**	1.6	**0.001**
st2	Summ	T, Sed	73.4	**0.007**	67	**0.008**	5.7	**0.007**	69.2	**0.011**	2.1	**0.006**	2.1	**0.01**

pairwise	test	Groups	Observed	Observed	Chao1	Chao1	Shannon	Shannon	InvSimpson	InvSimpson	microbiome	microbiome	core microbiome	core microbiome
st2	Summ	P, Sed	80.6	**0.011**	60	**0.007**	9.4	**0.008**	202.4	**0.013**	2.2	**0.008**	2.2	**0.005**
st2	Wint	M, T	0.3	0.744	0.4	0.691	2.5	**0.029**	3	**0.014**	1.5	**0.005**	1.5	**0.008**
st2	Wint	M, P	1.8	0.084	0.4	0.617	1.8	0.13	1.1	0.294	1.6	**0.005**	1.6	**0.007**
st2	Wint	M, Sed	35.8	**0.007**	28.2	**0.004**	11.1	**0.006**	40	**0.007**	2.1	**0.007**	2.1	**0.003**
st2	Wint	T, P	4.5	***0.004***	0.1	***0.898***	3.3	***0.013***	2.9	***0.025***	1.4	***0.106***	1.4	***0.094***
st2	Wint	T, Sed	192.6	***0.001***	25.3	***0.001***	8.2	***0.004***	24.1	***0.001***	2.1	***0.017***	2.1	***0.012***
st2	Wint	P, Sed	119.1	***0.001***	25.9	***0.001***	9.8	***0.001***	27.7	***0.001***	2.1	***0.008***	2.1	***0.015***
st2	Spri	M, T	0.6	0.56	0.2	0.83	0	0.959	0.1	0.921	1.9	**0.001**	1.9	**0.001**
st2	Spri	M, P	1.3	0.218	0	0.971	2.7	**0.018**	2.5	**0.032**	2.3	**0.001**	2.3	**0.001**
st2	Spri	M, Sed	53.5	**0.005**	29.5	**0.007**	11	**0.011**	107.5	**0.008**	2.5	**0.007**	2.5	**0.011**
st2	Spri	T, P	0.7	0.525	0.2	0.823	2.1	0.058	2.3	**0.032**	1.6	**0.001**	1.6	**0.001**
st2	Spri	T, Sed	57.7	**0.008**	28.7	**0.01**	7.5	**0.006**	97.4	**0.01**	2.1	**0.007**	2.1	**0.003**
st2	Spri	P, Sed	63.1	**0.01**	26.2	**0.012**	12.3	**0.01**	63	**0.001**	2.7	**0.007**	2.7	**0.006**
M	st1	Summ, Wint	0.5	0.632	0.7	0.491	1.5	0.141	0.7	0.504	1.2	0.098	1.2	0.096
M	st1	Summ, Spri	0.7	0.534	1.8	0.097	0.8	0.399	0.7	0.484	1.3	**0.018**	1.3	**0.018**
M	st1	Wint, Spri	1	0.311	1.1	0.297	0.4	0.71	0.1	0.936	1.3	**0.013**	1.3	**0.016**
M	st2	Summ, Wint	2	0.106	1.2	0.223	1.2	0.227	1.1	0.267	1.9	**0.001**	1.9	**0.002**
M	st2	Summ, Spri	0.6	0.596	1	0.282	0.1	0.904	0.5	0.677	1	0.433	1.0	0.419
M	st2	Wint, Spri	2.1	0.08	1.7	0.132	1	0.345	0.6	0.568	1.5	**0.006**	1.5	**0.003**
T	st1	Summ, Wint	0.9	0.373	1.2	0.248	1.1	0.286	1.1	0.302	1.3	**0.01**	1.3	**0.011**
T	st1	Summ, Spri	0.2	0.843	0.1	0.953	1.3	0.227	2	0.078	1.4	**0.002**	1.4	**0.003**
T	st1	Wint, Spri	0.8	0.446	0.8	0.478	0.1	0.938	0.7	0.482	1.1	0.122	1.1	0.118
T	st2	Summ, Wint	1.5	0.172	2.2	0.05	0.9	0.368	1	0.349	1.1	0.177	1.1	0.169
T	st2	Summ, Spri	1.8	0.093	0.5	0.656	1.5	0.193	2	0.057	1.1	0.315	1.1	0.308
T	st2	Wint, Spri	3.9	**0.005**	1.7	0.147	2.5	**0.035**	3.3	**0.018**	1.1	0.189	1.1	0.196
P	st1	Summ, Wint	0.3	0.821	0.4	0.652	0.8	0.461	0.1	0.946	1.4	**0.013**	1.4	**0.009**
P	st1	Summ, Spri	1.8	0.097	0.4	0.753	0.1	0.904	0.7	0.565	1.3	0.08	1.3	0.091
P	st1	Wint, Spri	1.5	0.182	0.1	0.918	1	0.326	1	0.303	1.3	**0.003**	1.3	**0.003**
P	st2	Summ, Wint	0.7	0.499	1.1	0.27	0.8	0.497	0.7	0.497	1.5	**0.008**	1.5	**0.008**
P	st2	Summ, Spri	1.5	0.155	0.6	0.599	2.5	**0.016**	2.6	**0.014**	1.8	**0.001**	1.8	**0.002**
P	st2	Wint, Spri	0.6	0.582	1	0.354	3.3	**0.011**	2.3	**0.041**	1.5	**0.019**	1.5	**0.014**
Sed	st1	Summ, Wint	0	***0.994***	1	***0.345***	1	***0.387***	1.2	***0.311***	0.9	***0.503***	0.9	***0.517***
Sed	st1	Summ, Spri	3.8	***0.021***	1.2	***0.304***	3.9	***0.023***	0.4	***0.724***	1.4	***0.121***	1.4	***0.108***
Sed	st1	Wint, Spri	7.4	***0.002***	3.6	***0.029***	5.7	***0.004***	1.8	***0.125***	1.5	***0.116***	1.5	***0.095***
Sed	st2	Summ, Wint	0.1	***0.947***	0.8	***0.499***	3.3	***0.028***	4.1	***0.017***	1.5	***0.111***	1.5	***0.106***

pair wise	test	Groups	Observed	Observed	Chao1	Chao1	Shannon	Shannon	InVsimpson	InVsimpson	microbiome	microbiome	core microbiome	core microbiome
Sed	st2	Summ, Spri	6.8	***0.003***	2.8	***0.039***	12.7	***0.002***	46.4	***0.001***	1.7	***0.063***	1.7	***0.059***
Sed	st2	Wint, Spri	6.9	***0.006***	2.9	***0.031***	10	***0.001***	3.5	***0.019***	1.6	***0.087***	1.6	***0.069***





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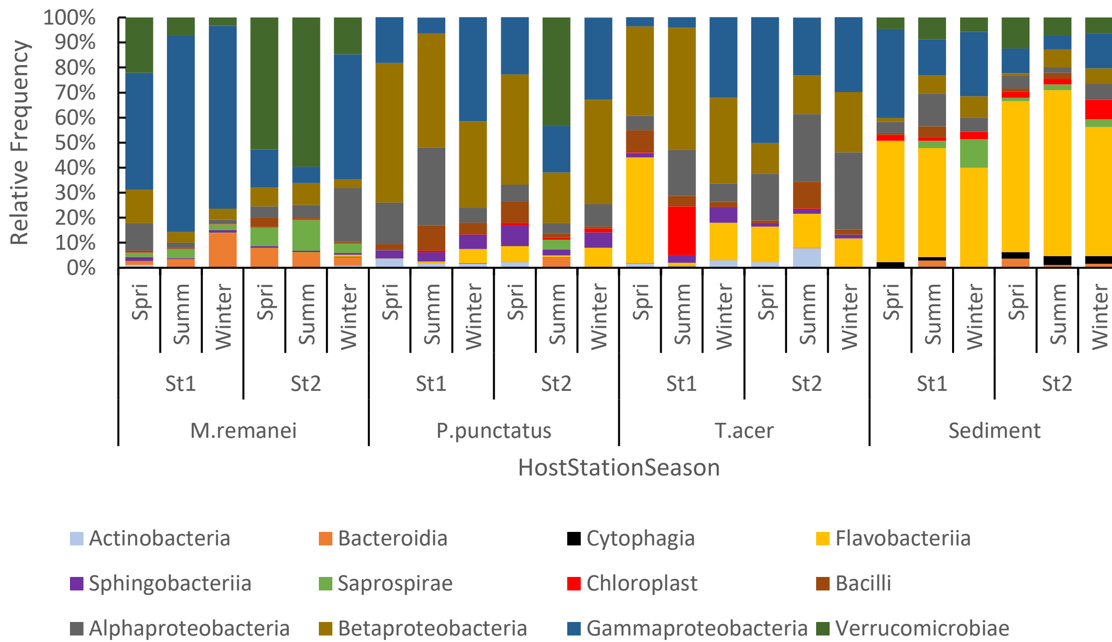


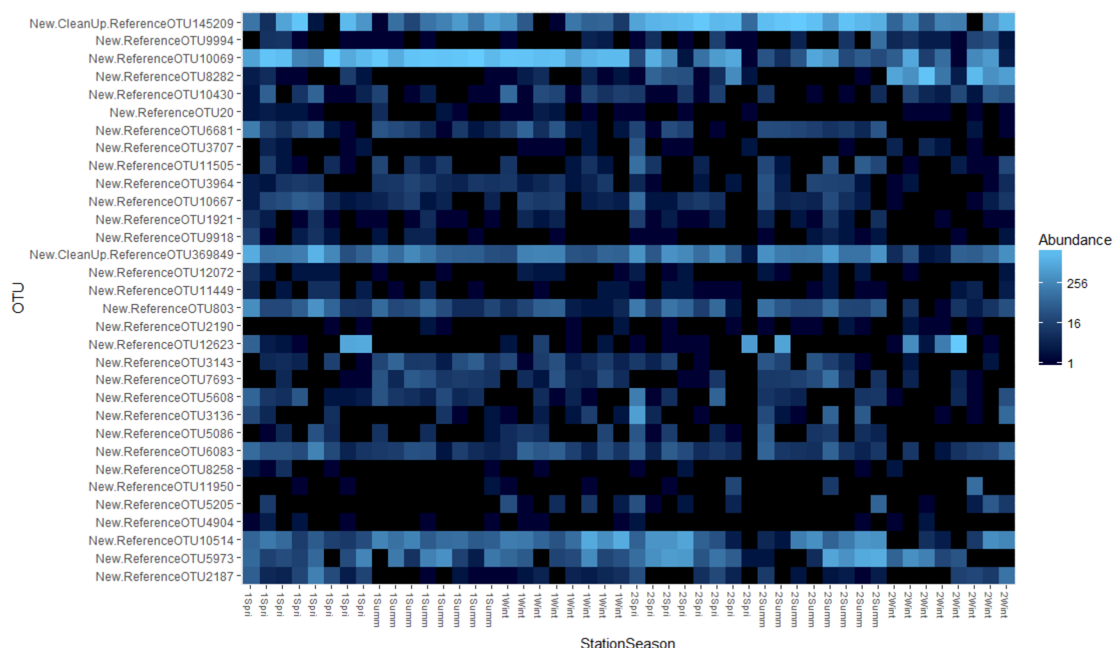
Fig S4.6. Taxonomic profile of core microbiome at the Phylum level per microbiome host (a), per HostStationSeason (b) and taxonomic at the class level per HostStationSeason (c).

Table S4.7. Taxonomy of core OTUs defined as OTUs presented at least 80% samples, with abbreviation of NR and NCR indicating New.ReferenceOTU and New.CleanUp.Reference, and of EV indicating environment associated taxa.

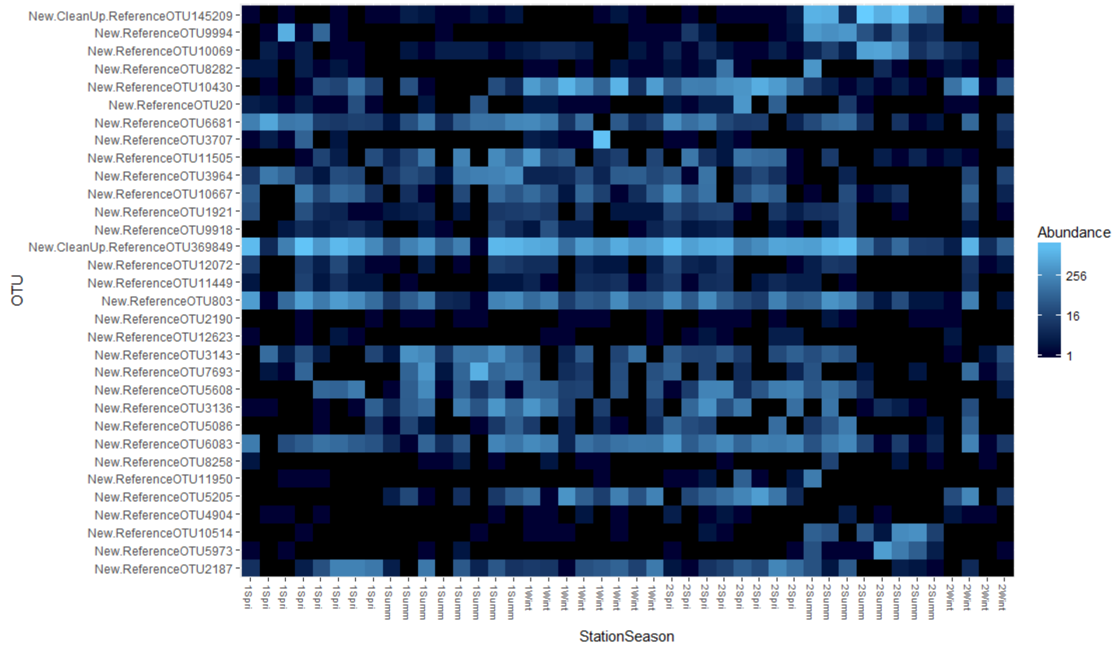
Core OTUs	Phylum	Class	Order	Family	Genus	Species	Associated taxa or function
NR803	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingomonas*		diatoms
NR9994	Proteobacteria	Gammaproteobacteria	Vibrionales	Pseudoalteromonadaceae	*Pseudoalteromonas*		diatoms
NR10514	Bacteroidetes	Bacteroidia	Bacteroidales	VC21			EV
NR10667	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae			EV
NR11505	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Tepidimonas*		EV
NR3964	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Delftia*		EV
NR5973	Bacteroidetes	Saprospirae	Saprospirales	Saprospiraceae			EV
NR6083	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	*Sphingobacteriaceae*			EV
NR11950	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae			EV
NR8258	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	*Polaribacter*		EV
NR5205	Bacteroidetes	Flavobacteriia	Flavobacteriales	Weeksellaceae	*Cloacibacterium*		EV
NR2190	Proteobacteria	Alphaproteobacteria	Rickettsiales	Anaplasmataceae	*Neorickettsia*		endosymbionts of eukaryotic cells
NR12623	Proteobacteria	Alphaproteobacteria	Rickettsiales				endosymbionts of eukaryotic cells
NR3703	Proteobacteria	Gammaproteobacteria	Thiotrichales	Piscirickettsiaceae			all are aerobics and water bacteria
NCR369849	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	*Burkholderia*	*bryophila*	Anti-fugi

Core OTUs	Phylum	Class	Order	Family	Genus	Species	Associated taxa or function
NR11449	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	*Burkholderia*	*bryophila*	Anti-fugi
NR12072	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	*Burkholderia*	*bryophila*	anti-fugi
NR9918	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	*Burkholderia*	*bryophila*	anti-fugi
NR1921	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	*Burkholderia*		Anti-fugi
NR10069	Proteobacteria	Gammaproteobacteria	Thiohalorhabdales				found in coral microbiome
NR8282	Proteobacteria	Gammaproteobacteria	Thiohalorhabdales				found in coral microbiome
NR20	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae			Intestitine or food
NR6681	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae			Intestitine or food
NR4904	Bacteroidetes	Cytophagia	Cytophagales	[Amoebophilaceae]	*Candidatus*		low RF
NR10430	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	*Acinetobacter*		mineralization, enzyme
NR7963	Proteobacteria	Gammaproteobacteria					most Gmmaproteobacteria wildly distributed in enivronemtal and also in animals
NR5608	Firmicutes	Bacilli	Bacillales	Staphylococcaceae	*Staphylococcus*		mucous membrane related taxa
NR3143	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	*Methylobacterium*		Plant
NR5086	Cyanobacteria	Chloroplast	Streptophyta				plant and green algae
NR3136	Firmicutes	Bacilli	Bacillales	Bacillaceae	*Anoxybacillus*	*kestanbolensis*	Putative parasite in gut
NR2187	Actinobacteria	Actinobacteria	Actinomycetales	Propionibacteriaceae	*Propionibacterium*	*acnes*	skin associated bactrium
NCR145209	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae			unknown

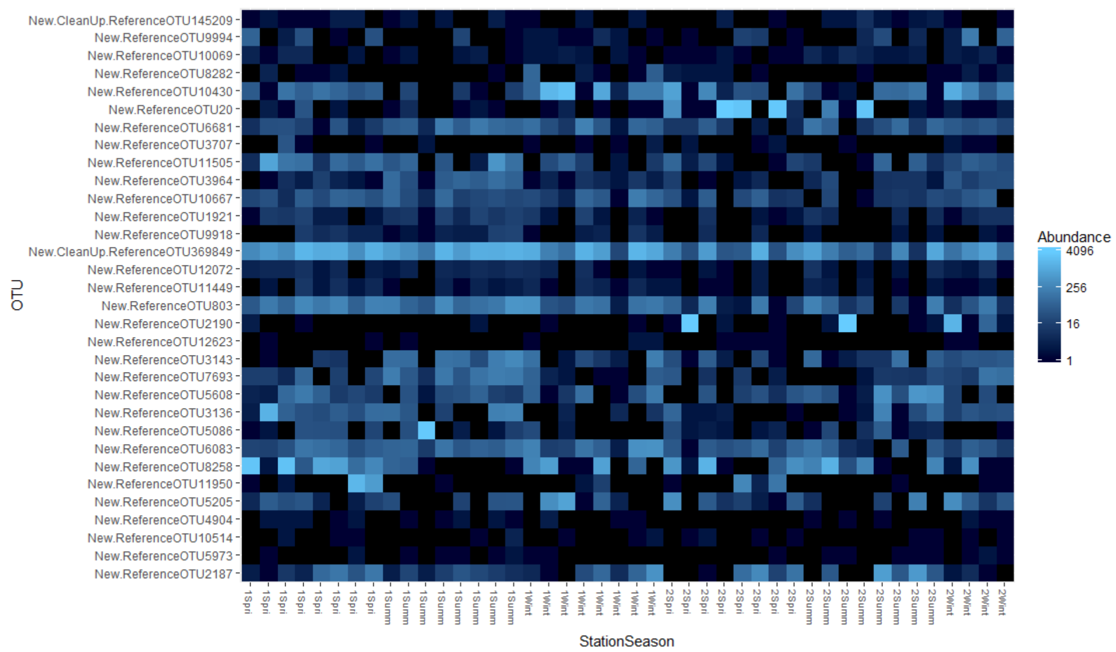
a-*M.remanei*



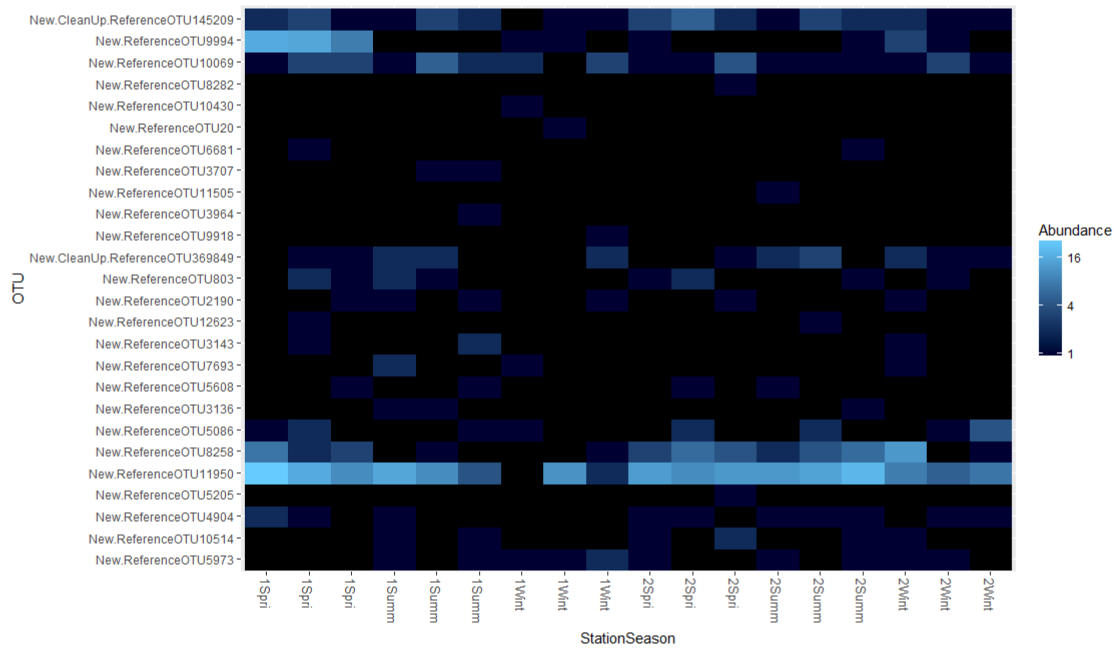
b-*P.punctatus*



c-*T.acer*



d-Sediment



e

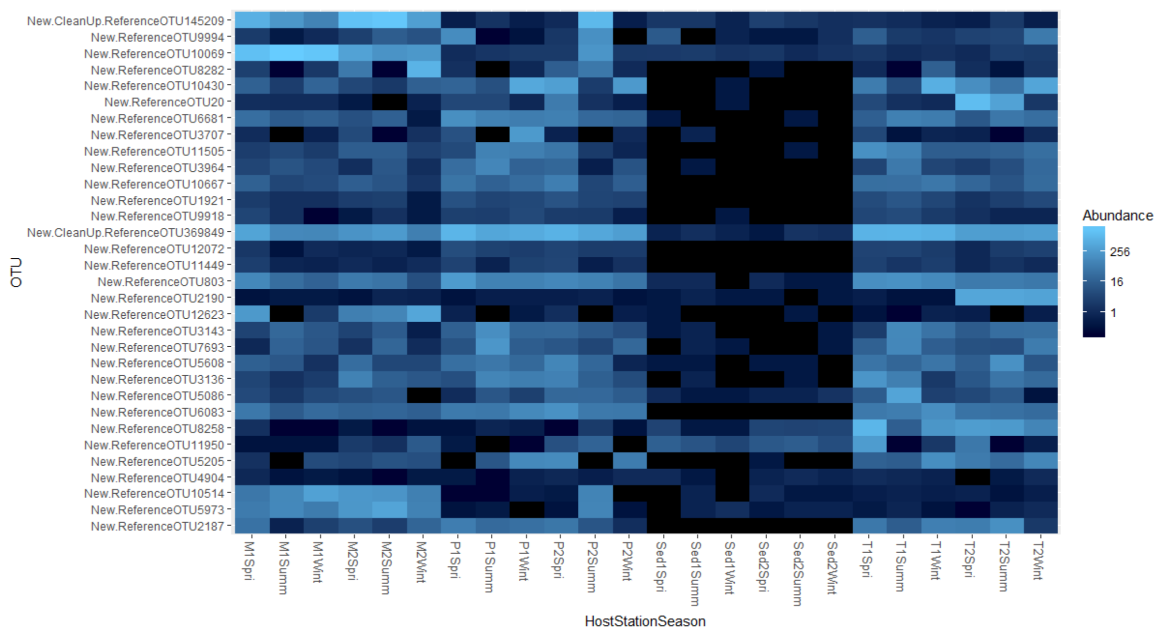


Fig S4.8. Heatmap plots depicting core microbiomes (defined as OTUs presented at least 80% samples on rarefied dataset) on StationSeason in each microbiome host (a, b, c, d) and on averaged HostStationSeason in nematodes and sediments (e). a, b, c and d indicated core microbiomes distribution in different microbiomes hosts: *Metachromadora remanei* (a), *Praeacanthonchus punctatus* (b), *Theristus acer* (c) and sediment (d), respectively. Stations were indicated by a number 1 for station 1 and 2 for station 2; Seasons were indicated by Spri for spring, Summ for summer and Wint for winter. Hosts (e) were indicated by M, P, T and Sed for *Metachromadora remanei*, *Praeacanthonchus punctatus*, *Theristus acer* and sediment, respectively.

Table S4.9. The most differentially abundant taxa of core microbiomes in three nematodes species identified by lefse analysis (http://mbac.gmu.edu:8080/), with factor being host (a) in dataset of three nematodes and stationseason (b: *M. remanei*, c: *P. punctatus* and d: *T. acer*) in dataset of each nematode species. Host (a), stationseason (e.g. 1Spri: station 1, spring) and taxa were indicated by different colors. Only the taxa meeting a significant LDA (Linear discriminant analysis) threshold value of > 2 are shown.

a

Taxa	Host
Verrucomicrobiaceae	*M. remanei*
Verrucomicrobiales	*M. remanei*
Flavobacteriales	*T. acer*

b

Taxa	StationSeason
Actinomycetales	1Spri
Burkholderiaceae	1Spri
Burkholderiales	1Spri
Propionibacteriaceae	1Spri
Propionibacterium	1Spri
Sphingomonadaceae	1Spri
Sphingomonadales	1Spri
Sphingomonas	1Spri
Delftia	1Summ
Methylobacteriaceae	1Summ
Methylobacterium	1Summ
Rhizobiales	1Summ
Staphylococcaceae	1Summ
Staphylococcus	1Summ
Acineobacter	1Wint
Bacteroidales	1Wint
Enterobacteriaceae	1Wint
Enterobacteriales	1Wint
Moraxellaceae	1Wint
Pseudomonadales	1Wint
Sphingobacteriaceae	1Wint
Sphingobacteriales	1Wint
VC_Bac22	1Wint
Verrucomicrobiaceae	2Summ
Verrucomicrobiales	2Summ
Rickettsiales	2Wint
Thiohalorhabdales	2Wint

c

Taxa	StationSeason
Sphingomonas	1Spri
Sphingomonadaceae	1Spri
Sphingomonadales	1Spri
Burkholderiales	1Spri
Pseudoalteromonas	1Spri
Pseudoalteromonadaceae	1Spri
Vibrionales	1Spri
Methylobacterium	1Summ
Methylobacteriaceae	1Summ
Rhizobiales	1Summ
Delftia	1Summ
Sphingobacteriaceae	2Spri
Sphingobacteriales	2Spri
Bacillales	2Spri
VC_Bac22	2Summ
Bacteroidales	2Summ
Myxococcales	2Summ
Verrucomicrobiaceae	2Summ
Verrucomicrobiales	2Summ
Flavobacteriales	2Wint
Acineobacter	2Wint
Moraxellaceae	2Wint
Pseudomonadales	2Wint

d

Taxa	StationSeason
Flavobacteriales	1Spri
Methylobacterium	1Summ
Methylobacteriaceae	1Summ
Rhizobiales	1Summ
Burkholderia	1Summ
Delftia	1Summ
Enterobacteriaceae	1Summ
Enterobacteriales	1Summ
Acineobacter	1Wint
Moraxellaceae	1Wint
Pseudomonadales	1Wint
Thiohalorhabdales	1Wint
Neorickettsia	2Spri
Anaplasmataceae	2Spri

Chapter 5 General Discussion

Chapter 5 General discussion

5.1 Introduction

Microphytobenthos biofilms are a major food source for a wide variety of tidal flat (invertebrate) fauna and heterotrophic protists (Decho 1990; Stal 2010). These biofilms play crucial roles in a number of ecosystem functions, such as sediment stabilization and water quality improvement (Paterson and Black 1999; Stal 2010). Nevertheless, much remains to be discovered about the complex interplay between microphytobenthos (MPB), prokaryotes and benthic invertebrates, such as the highly abundant nematodes, in microbial biofilms on tidal flats, and hence also about the potential roles of these benthic invertebrates in the above-mentioned ecosystem functions.

Improving our understanding of the functional roles of nematodes in tidal flat sediments requires that trophic interactions between nematodes and biofilm-forming organisms are documented and understood. That was also the overarching goal of this PhD: to **elucidate trophic relationships between nematodes and microphytobenthos and bacteria** on an intertidal flat.

Under that 'research umbrella', we developed a three-tiered approach to advance our understanding of the trophic relationships of tidal-flat nematodes with benthic microalgae and bacteria. Based on the idea that ecosystem processes and their variation in time and space cannot be properly understood without a good background of the drivers of *in situ* patterns (Underwood and Kromkamp, 1999), we first set out to **describe spatial variability in the nematode assemblages on a tidal flat in relation to – mainly food-related – environmental drivers** (**Chapter 2**). We first investigated the nematode distribution patterns in abundance, diversity and genus composition at meso- and microscales and in different depth layers in the field, and related these patterns with several potential drivers, covering not only MPB as indicated by several pigments, but also sediment granulometry, tidal level and alternative potential food sources such as zooplankton and their faecal pellets (Fig. 5.1).

We then **zoomed in on the *in situ* feeding ecology of a limited number of abundant nematode species** (9) from the same tidal flat area, covering different feeding types, and using natural stable isotope ratios of carbon and nitrogen along with fatty acid composition as food-web markers (**Chapter 3**). Both trophic-marker approaches have their inherent limitations (Boschker et al., 2005; Mutchler et al., 2004; Neubauer and Jensen 2015), yet they are at least partly complementary, and their combined use may therefore offer a better resolution of food-web flows than their single use. For instance, with the exception of chemoautotrophic bacteria (Fry et al., 1991), stable-isotope approaches do not allow to disentangle the trophic importance of bacteria, because bacteria tend to

have stable-isotope ratios that are virtually the same as those of their resources (Boschker et al., 2005; Mutchler et al., 2004). Certain fatty acids, by contrast, only occur in prokaryotes, hence their presence and abundance in consumers can be used as a first indicator of their trophic importance (Canuel and Martens, 1993).

However, MPB is a complex mix of microalgae and bacteria, and the biomarker approach of chapter 3 only allows an overall (semi)quantitative assessment of the trophic importance of bacteria, whilst not providing more detailed information of which bacteria could be consumed by which nematodes. Hence, aspects of resource partitioning, which is potentially important among bacterivorous nematodes (Moens et al. 1999a), cannot be derived from such an approach. Surfing on the recent upsurge of microbiome studies in a wide variety of animal hosts (Ainsworth, et al., 2015; Hentschel et al., 2012; Sturgeon et al., 2014; Vredenburg et al., 2011), including marine nematodes (Derycke et al. 2016; Schuelke et al. 2018), we studied the microbiomes and their variability in space and time, of three tidal-flat nematode species with presumed different feeding behaviours (**chapter 4**). These were also the three nematode species which in chapter 3 showed the largest direct dependence on MPB as a food source. Nematode-associated microbiomes (NAM) not only encompass remnants of bacteria ingested as food, but also the bacteria on and inside nematodes, including all kinds of symbioses, both positive (mutualistic) and negative (pathogenic) (Dirksen et al., 2016; Samuel et al., 2016; Zhang et al., 2017). Admittedly, therefore, our results cannot be straightforwardly interpreted in the context of trophic interactions, but given the different feeding behaviours of the nematode species used, this approach did allow us to investigate whether any species-specific differences could be at all related to differences in the feeding ecology of these nematode species. Moreover, microbiomes can affect the fitness of their 'host' organisms in multiple ways (Amin et al., 2012; Derycke et al., 2016; Gerdts et al., 2013; Sison-Mangus et al., 2014), and may therefore indirectly also affect their overall ecology and interactions with other species.

In the below discussion, I first highlight some of the principal findings about the drivers of nematode community structure as they emerged from a combination of community analysis, biomarker approaches and microbiome work (section 5.2). I then discuss in detail what we have learnt, mainly from chapters 3 and 4, about the importance – or lack thereof – of bacteria as a food source for tidal flat nematodes (section 5.3). I then elaborate an example (the case of *Metachromadora remanei*) illustrating that, although our biomarker and microbiome approaches have yielded considerable new insights in the roles of MPB and bacteria as food for nematodes, they have left several questions 'unsettled' (section 5.4). At the same time, mainly our biomarker approach has also revealed several important aspects of the trophic structure at the basis of benthic food webs, and these aspects are discussed in section 5.5. I end with some perspectives into the future of this kind or research, with an

emphasis on new methodological developments and a plea for research at the level of species and even individuals.

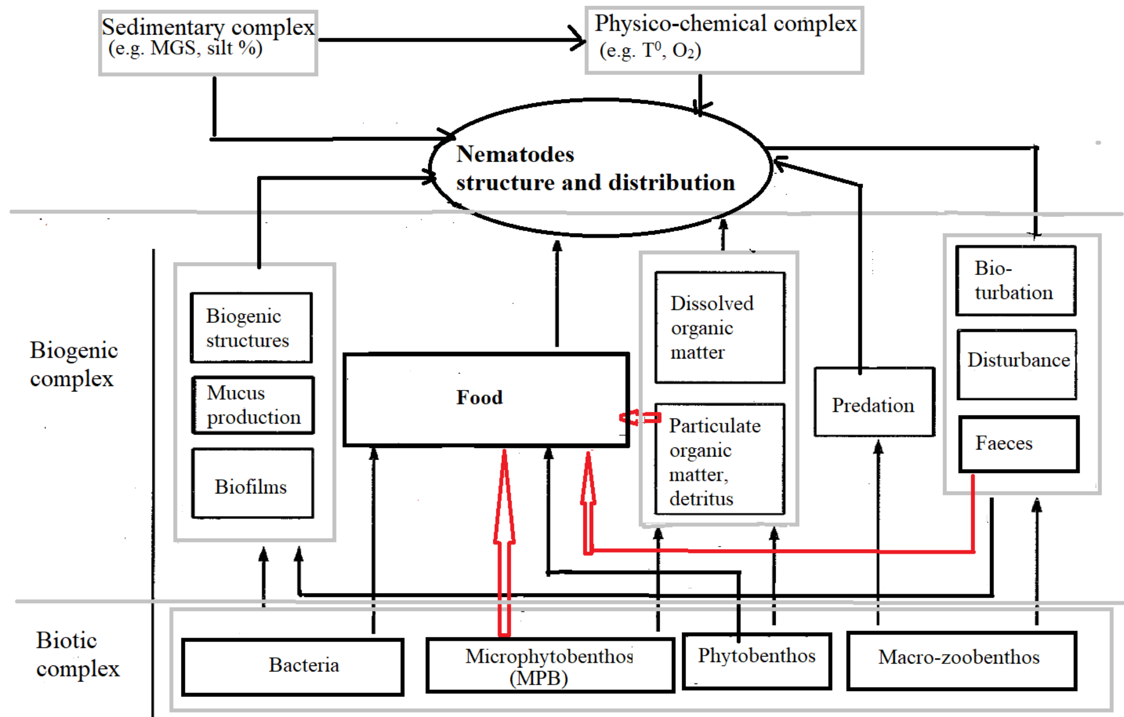


Fig. 5.1 A schematic factorial web of abiotic and biotic factors acting on and structuring nematode communities, with evidence provided by this study in red colour, modified from Giere, 2009.

5.2 Drivers of nematode community structure: what can we learn from a combination of a field survey, food-web biomarkers and nematode-associated microbiomes?

At the start of this PhD, the few available stable-isotope data at nematode genus level indicated that MPB provided the predominant carbon source to nematodes from different feeding types (Moens et al., 2005a; Rzeznik-Orignac et al., 2008). MPB biofilms at the Paulina tidal flat, and more broadly in the poly- and mesohaline reaches of the Scheldt Estuary, are typically strongly dominated in biomass by epipelic diatoms, particularly on muddy and fine-sandy sediments (Sabbe and Vyverman, 1991; Hamels et al., 1998). In conjunction to observations of nematodes feeding on diatoms (Moens and Vincx, 1997) and/or holding diatom frustules in their intestine (Nehring et al., 1993), this enhanced the idea that diatoms are the principal resource of intertidal nematodes (Moens et al., 2005a; Rzeznik-Orignac et al., 2008). The structuring role of diatom-derived phytopigments such as fucoxanthin and diatoxanthin to the variation in nematode community composition at the Paulina tidal flat (chapter 2), as well as the fact that one of only two bacterial species that occurred in all nematode microbiomes is known as a diatom-associate (chapter 4), further support the close trophic link between nematodes and diatoms.

While this thesis provides several lines of evidence in support of this idea, it also suggests that (meio)benthic food webs are more complex. First, while MPB carbon is clearly the main energy source fueling intertidal nematodes, the pathways from MPB to nematodes are manifold (see section 5.4; Figs. 5.2 and 5.3), ranging from direct grazing to predation on herbivores and on predators of herbivores (chapter 3, and this general discussion, section 5.5). Bacteria and/or fungi, which can utilize MPB and their exopolymeric substances, also provide a route from MPB carbon to nematodes (see section on the (un)importance of bacteria, i.e. 5.3).

Second, there are usually some caveats when looking at the natural stable-isotope abundances of MPB. These are traditionally determined after scraping off algal biofilm from the sediment surface (MacIntyre et al., 1996), in which case they are biased by other types of organic matter as well as by inorganic carbon; or after a migration-to-light assay, which yields almost exclusively the most motile diatom species (Fenchel and Straarup 1971; De Brouwer and Stal 2001).

Moreover, among those epipelic diatoms, different size fractions may have slightly different isotopic signatures, a factor which has only rarely been taken into account (but see Rzeznik-Orignac et al., 2008). As such, there are to our knowledge no earlier SI-based studies which have investigated feeding selectivity of nematodes within natural marine or estuarine MPB biofilms, and hence also no SI-based studies which have studied whether nematodes may preferentially utilize other microalgae over diatoms. In this study (chapter 3), the abundance of the diatom marker FA C16:1ω7 in *Metachromadora remanei* and to a lesser extent *Adoncholaimus fuscus* supports the idea that diatoms contribute importantly to their nutrition. However, at the same time, this diatom-derived FA contributed less than 10% of the FA in all other nematode species. By contrast, EPA and DHA often had considerably higher concentrations. High concentrations of C16:1ω7 may indicate a more selective feeding on diatoms, whereas high EPA and lowish C16:1ω7 may actually point at a less selective grazing on microalgal biofilms.

The sum of the PUFA EPA and DHA contributed between 29.9 (in *Oncholaimus*) and 51.7% (in *Daptonema*) of nematode FA. While neither FA is very source-specific, EPA tends to be very abundant in diatoms, while DHA is more prominent in dinoflagellates. Among the nine nematode species in our study, there appeared to be an inverse relationship in the relative contributions of these two biomarker FA, leading to EPA/DHA ratios that varied from well above 1 in *Praeacanthonchus, Adoncholaimus* (both > 2) and especially *Metachromadora* (= 3.5), to lower than 1 in *Daptonema, Odontophora* and *Enoploides*. This strongly suggests that some nematode species have a clear preference for diatoms over dinoflagellates, while others have an opposite preference. This is an important new result, since to our knowledge, the utilization of dinoflagellates by marine nematodes

hitherto had not been properly documented. Two harpacticoid copepod species from the Paulina tidal flat also exhibited important FA contributions of DHA, albeit only at specific stations and seasons (Cnudde et al., 2015), suggesting that this trophic link may be temporally and spatially variable. Similarly, in our study, for the three nematode species from which we obtained FA data from two different stations on the Paulina tidal flat, feeding on DHA appeared more prominent at the sandy station st1 than at the silty st16, in agreement with observations of temporal blooms of dinoflagellates at st1 but much less so at silty sediments (Moens, unpubl.). Still, while the FA data suggest an important trophic link with dinoflagellates for at least some abundant nematode species, chapter 2 found that only a very small portion of the observed variability in nematode community composition could be linked to concentrations of peridinin, a light-harvesting pigment characteristic of dinoflagellates. Hence, while our FA results provide an indication that dinoflagellates may substantially contribute to the nutrition of several abundant genera of tidal-flat nematodes, more research is needed to substantiate this claim.

In this context, it is important to highlight that the analysis of patterns and their drivers may be strongly dependent on the spatial and temporal scales and dimensions at which they are being observed (Levin 1992; Vieira and Fonseca, 2013). Mixing different scales of observation may hide important ecological information due to an interaction of different drivers at different spatial scales. In our study, for instance, we should be particularly careful when interpreting the often different drivers of nematode community composition in different sediment depth layers. The different phytopigments which explained variation in the different depth layers may point at the involvement of different drivers (such as different components of MPB), but also to the involvement of the same drivers (e.g. when different phytopigments are actually derived from the same organisms).

In addition to the potential significance of dinoflagellates as a resource for tidal-flat nematodes, chapters 2 and 3 provide first evidence that zooplankton faecal pellets (as reflected by pyropheophytin concentrations) as well as dead microzooplankton, as indicated by the concentration of arachidonic acid, may significantly contribute to the nutrition of at least some intertidal nematode species. The former were among the most important drivers of nematode community composition, whereas the latter FA mainly occurred in *Oncholaimus*. It is important in this respect that in addition to the 'local' zooplankton, substantial quantities of marine zooplankton enter the estuary at each high tide; a large fraction of these zooplankters die in the estuary (Soetaert and Herman, 1994), and to our knowledge, our data are the first to suggest that nematodes from intertidal sediments may feed on this carbon source.

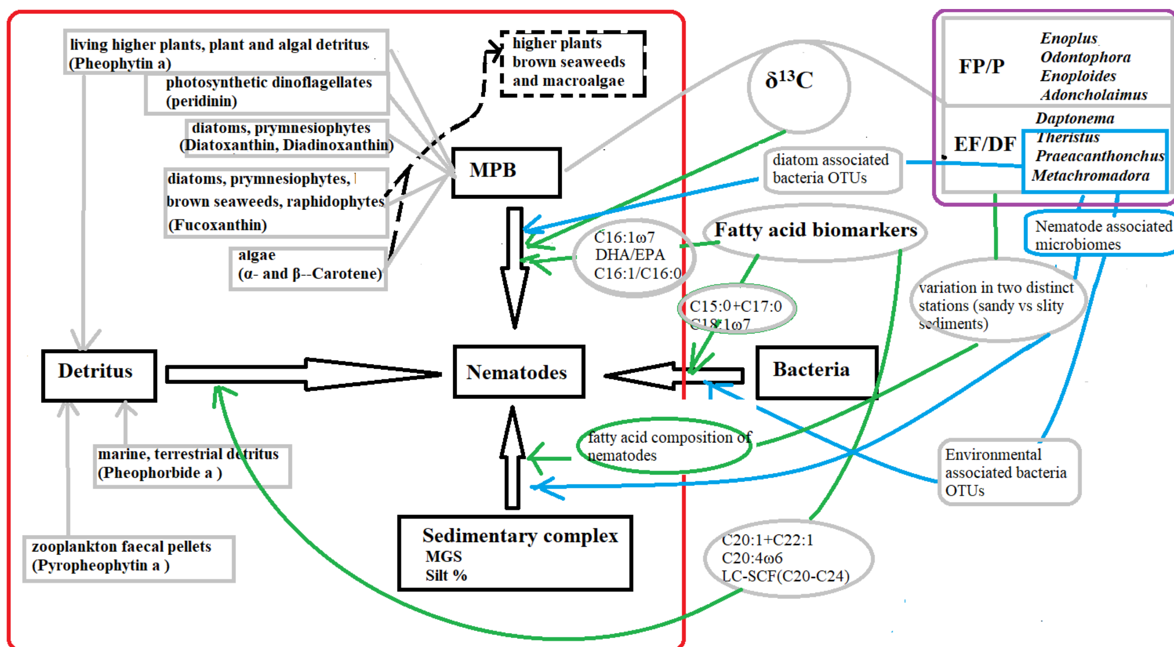


Fig. 5.2 Drivers of nematode community detected by Chapter 2 (in red box) and related evidences provided by chapter 3 (green arrow) and chapter 4 (blue arrow in purple box). Microphytobenthos was indicated by MPB, and nematode feeding types: facultative predator (FP), predator (P), epigrowth feeder (EF), deposit feeder (DF).

5.3 The (un)importance of bacteria as a resource for tidal flat nematodes? Lessons learnt from chapters 3 and 4

Prokaryotes are the most important decomposers of organic matter in tidal flat sediments (Hicks et al., 2018). Hence, in order to better understand the functioning of soft-bottom sediments such as intertidal flats, and the intricate trophic relationships between microalgae, bacteria and their grazers in MPB biofilms, the fate of benthic bacterial production remains an important topic. Given their high abundances and production rates, and their favourable nutrient stoichiometry (typically lower C/N ratios than those of their benthic invertebrate consumers (Abrams and Mitchell, 1980), bacteria might constitute an important food source for nematodes and other benthic meiofauna. Nevertheless, evidence of the trophic role of bacteria for meiofauna remains both fragmentory and equivocal.

In situ pulse-chase experiments on estuarine tidal flats (Middelburg et al., 2000; Herman et al., 2001; Van Oevelen et al., 2006a) as well as modelling exercises based on these experiments (Van Oevelen et al., 2006b), have suggested that bacterial production is mostly a dead-end in the benthic food chain, because the fate of bacterial biomass is largely (viral-induced?) mortality rather than consumption by grazers. Van Oevelen et al. (2006a, b), for instance, estimated that nematodes can only consume ca 3% of bacterial carbon production, far less than in earlier estimates based on radioactive tracer experiments (Montagna, 1995). It is not, because grazing is not a major fate of bacterial production, that bacterial production cannot substantially contribute to the nutrition of some benthic organisms.

Still, bacteria contributed only 6% to the carbon requirements of nematodes in the experiment by Van Oevelen et al. (2006a, b). These results contrast with considerably higher estimates of the contribution of bacteria in Pascal et al. (2008, 2009), and in older studies which used radioactive labelling techniques in sediment slurries to estimate meiofaunal grazing rates (reviewed in Montagna, 1995). However, all the above estimates are community-based, hence it is plausible that at least some nematode species would rely more heavily on prokaryotic biomass.

Since stable isotope (SI) ratios of bacteria and their resources tend to be highly similar (Boschker et al., 2000), natural SI ratios cannot be used to discriminate the roles of bacteria and other food sources of meiofauna. This is one example where fatty acid (FA) biomarkers can complement information from SI (Neubauer and Jensen 2015). Based on the sum of the general bacterial marker FA C15:0 and C17:0 and the potential bacterial marker C18:1ω7, we nevertheless conclude that bacteria indeed contribute a considerably smaller part to the energy requirements of nematodes than do microalgae (here with contributions estimated based on the FA EPA, DHA and C16:1ω7). Microalgal FA, then, contributed between 38 and 57% of the total FA in the nine nematode species studied in chapter 3, whereas prokaryotic FA only contributed between 5 and 12 %, and between 1 and 3 % when we omit C18:1ω7. Considering their completely different approaches, the FA proportions and the tracer-based assimilation estimates by Van Oevelen et al. (2006a, b) agree remarkably well. Recently, van der Heijden (2018) combined FA data with SI data and linear inverse food web modelling on coastal tidal flats in France and Germany. Although in contrast to our results, he found similar proportions of microalgae-derived and prokaryotic FA in nematodes, the modelled fluxes of carbon from microalgae to nematodes were on average ca 8 times higher than the fluxes from bacteria to nematodes. The combined results of FA, tracer-based experiments and modelling exercises therefore clearly suggest that MPB is preferred as a food source over bacteria, and does not – or at least not in a quantitatively dominant way – pass to nematodes through a bacterial intermediate.

This is supported by an experiment in a Louisiana salt marsh, where nutrient enrichment was found to enhance grazing by nematodes (and a variety of other benthic invertebrates) on MPB but not on bacteria (Pascal et al., 2013). On the other hand, while bacteria are not the 'preferred' food of meiofauna, they may still be utilized at significant rates when more preferred resources such as MPB are scarce. As an example, rates of bacterivory by benthic nematodes increased as microalgal abundance decreased in an intertidal mudflat, suggesting that bacteria constitute an alternative resource that is consumed when more preferred resources become scant (Pascal et al., 2008). In this context, it is important to note that our field samplings for chapters 2 and 3 were performed in June and under excellent weather conditions, which implies a high microphytobenthic productivity at the time and site of these samplings, and hence plenty of microalgal food available to benthic consumers.

As such, caution is due when extrapolating our results to other time moments, other locations and, especially, to other marine habitats. Ingels et al. (2010) recorded the preferred utilization of bacterial over phytodetrital carbon in Antarctic deep-sea sediments. Since their experiment utilized batch cultures of a single phytoplankton species and a bacterial mixture of probably limited diversity, it is unclear whether their results can be opened up to a more general preference for bacteria over algae. In contrast to Ingels et al. (2010), Guilini et al. (2010), while labeling different functional groups of bacteria in an Arctic deep-sea sediment with SI and tracing these into nematodes, concluded that the very limited transfer of carbon from bacteria to nematodes was not even due to direct bacterivory, but to the consumption of other substrates to which the tracer could adsorb. So for polar deep-sea sediments, the same controversy that long governed views on tidal flat bacterivory seems to apply.

Closer to bare intertidal mudflats, the resource utilization of meiofauna in intertidal and shallow subtidal seagrass beds has been studied on a number of occasions during the last decade (Leduc et al., 2009; Lebreton et al., 2012; Vafeiadou et al., 2014; Mascart et al., 2018). The first two studies both concluded that MPB was the most important carbon source to nematodes in seagrass sediments, seagrass detritus coming second with variable yet often substantial contributions. In contrast to these two studies, Vafeiadou et al. (2014) analysed SI of nematodes at genus rather than at the whole-community level, and found a reasonably clear isotopic discrimination between MPB and seagrass detritus. In other words, because of the substantial overlap in natural SI ratios between MPB and seagrass detritus in Leduc et al. (2009) and Lebreton et al. (2012), any conclusion on the relative importance of these two carbon sources for nematodes remained inconclusive. In the study by Vafeiadou et al. (2014), despite the seagrass vegetation being sparse, a substantial number of nematode genera had SI ratios that reflected a predominant contribution of seagrass detritus, up to 70 – 85 % in *Metachromadora* and *Daptonema*. In addition, other genera that are also common in the Paulina tidal flat, such as *Theristus* and *Ptycholaimellus*, also had major contributions of seagrass-derived carbon. Furthermore, next to seagrass detritus and MPB, suspended particulate organic matter (SPOM) contributed substantially (up to 37%) to the carbon utilization of some genera, including *Spirinia* and *Sabatieria*. We consider it unlikely that seagrass detritus would be directly consumed by nematodes, and therefore expect that most of the seagrass carbon entered the nematodes through consumption of the principal decomposers of seagrass detritus, i.e. bacteria and fungi. Similarly, depending on the quality of the SPOM, this resource may be used directly or also mainly indirectly (through grazing on associated bacteria) by nematodes (Moens et al., 1999a; Vafeiadou et al., 2014). Although bacteria lack some essential molecules for nematode growth (e.g. sterols and PUFA (Bolla, 1979; Vanfleteren, 1980; Cho and Mo, 1999), they have a favourable elemental stoichiometry (Abrams and Mitchell, 1980), and their exo-enzymatic activity may release

dissolved organic molecules that could be utilized by benthic invertebrates (Ederington et al., 1995; Cnudde et al., 2015). Bacteria and perhaps fungi may thus provide a pathway through which low-quality detritus may enter the traditional metazoan food web by microbial reworking of organic matter (e.g. Iken et al., 2001; Danovaro, 1996; Azam 1998; Cnudde et al., 2015), in accordance with the 'old' idea that especially in subsurface sediment strata, meiofauna would principally rely on older, more refractory detritus and the micro-organisms that are associated with it (Rudnick, 1989). However, alternative routes of detritus utilization by meiofauna are possible, including the consumption of faecal pellets of detritivorous macrofauna (Mascart et al., 2018).

Interestingly, Mascart et al. (2018), who investigated resource utilization of four species of harpacticoid copepods in seagrass detritus accumulations, also found that seagrass detritus and heterotrophic biomass were among the major carbon sources for these copepods, and concluded that copepods could constitute a significant trophic link between seagrass detritus (+ biomass of detritus decomposers) and higher trophic levels. The results of Vafeiadou et al. (2014), in combination with our own results on the high PUFA-levels of nematodes, suggest that this may also hold for at least some abundant nematode genera. However, these same genera at the Paulina tidal flat relied primarily on MPB carbon. The combination of these data strongly suggests that we should be extremely cautious not to generalize findings from one habitat type to others. Moreover, we should be equally careful not to make simple generalizations about the feeding ecology and principal resources of specific nematode genera, as these may differ in different environments (see also section 5.4.2) as well as within the same environment over time, as demonstrated for harpacticoid copepods (Mascart et al., 2018). *A fortiori*, this also implies that the use of nematode feeding types, which make generalized inferences on feeding ecology for very heterogeneous groupings of nematodes, further looses relevance (Vafeiadou et al., 2014).

While above, our main contention is that bacteria are of limited nutritional importance to tidal-flat nematodes, the results of chapter 4 of this PhD show an entirely different picture: marine nematodes hold microbiomes composed of large numbers of cells and strains of prokaryotes. Since none of the three species we studied was expected to feed predominantly on bacteria, our results support those of Schuelke et al. (2018) that probably all marine nematodes have a well-developed microbiome. Although these microbiomes may have various functions and consequences for their hosts (Samuel et al., 2016; Schulenburg and Félix, 2017; Zhang et al., 2017) and certainly do not only reflect ingested food (Derycke et al., 2016), they do highlight some potentially important conclusions. First, when we consider that microbiomes are part of, or contribute to the delineation of, a host's niche, the microbiome results at least partly support FA/SI in that nematode niches differ among species. Indeed, the microbiome of *Metachromadora* differed significantly from that of *Praeacanthonchus* and

Theristus, the latter two not always having such a clearcut difference. Second, the fact that only a small portion (up to 20%) of the sediment bacteria was ever picked up from a nematode microbiome indicates that – insofar as nematode microbiomes do reflect nematode feeding on bacteria – nematode bacterivory is selective: nematodes, then, ingest only a small portion of the bacterial strains present in their environment, and this fraction likely differs between nematode species.

An additional conclusion is that the network of 'indirect interactions' between nematodes and bacteria is even larger and much less 'one-way' than expected. Several studies had already indicated that nematodes can affect the species composition of microbial assemblages, bacterivory offering only one of several possible mechanisms (De Mesel, 2004; D'Hondt et al., 2018), in addition to other effects such as microbioturbation (Cullen, 1973; Bonaglia et al., 2014), vectoring and others (see chapter 1 for more info on this topic). These interactions all allow nematodes to affect populations and communities of bacteria. Chapter 4 of this thesis , however, demonstrates that microbiomes are not restricted to bacterial-feeding nematodes and/or to species with known bacterial symbioses (Ababa et al., 2009; Ott et al., 2004; Zhang et al., 2017), but are also common among species where bacterivory is at best a secondary trophic strategy. We have not yet arrived at the point where we can properly assess the multiple consequences of the microbiome and its exact composition on the performance and dynamics of host nematodes, but in analogy to other host-microbiome interactions (e.g. Greenblum et al., 2012; Huttenhower et al., 2012), we consider it plausible that microbiomes form an important part of the nematode niche and contribute in multiple ways to the nematodes' fitness and population dynamics. Hence, the current development of microbiome research in multiple fields, including ecology, can probably lead to a focus away from the mere determination of assimilation rates during bacterivory, and the realization that bacteria can be important for nematodes in multiple other, non-trophic ways.

Technically, however, identifying the importance of bacteria to the ecology of nematodes remains a challenge. We are but at the point of describing host microbiomes, and beginning to gain some understanding of how these microbiomes can affect their hosts (Samuel et al., 2016; Schulenburg and Félix, 2017; Zhang et al., 2017). In doing so, it is hitherto impossible to make a clear distinction between the bacteria that have been ingested by nematodes as food, and the microbiome *sensu stricto* (Derycke et al., 2016), even though dedicated experiments may offer first pointers here (Derycke et al., 2016; De Meester et al., unpubl.). At the same time, the abundances of specific prokaryotic marker fatty acids have to be cautiously interpreted: they may at least in part derive from the microbiome *sensu stricto* and not from nematode feeding on bacteria. As such, the above-mentioned percentages of prokaryotic marker FA should be considered as overestimates of the prokaryotic contribution to the nematodes' nutrition.

The fact that both microbiomes and FA profiles of the nematodes studied here demonstrated significant differences among nematode species, highlights the importance of studying nematodes and their ecology at the species level to be able to provide insights on feeding strategies (our FA data, for instance, reveal that *M. remanei* feeds more selectively on diatoms as indicated by high levels of C16:1ω7, while *P. punctatus* feeds less selectively on microalgae as indicated by a more pronounced prominence of EPA) as well as life histories.

In summary, the importance of bacteria and bacterivory for nematodes in coastal sediments remains insufficiently understood.

5.4 Trophic positioning and roles of marine nematodes: a tale of more than just primary and secondary consumers

In the 'classical' literature on benthic ecology, the meiofauna has long been considered a black box, consuming carbon and energy from primary producers (such as MPB) and primary decomposers (mainly bacteria), but not substantially transferring it to higher trophic levels (McIntyre and Murison, 1973). With time, two separate evolutions have modified this view (Fig. 5.3).

First, there was an increasing awareness that predation among meiofauna was not unusual but fairly common. Predatory relationships were observed between different major meiofaunal taxa, examples being the predation of turbellarians on free-living nematodes (Kreuzinger-Janik et al., 2018), or the observation of nematodes inside the guts of harpacticoid copepods (Kennedy, 1994). Moreover, with the feeding type classification of Wieser (1953) gaining increasing recognition, it became broadly accepted that predatory nematodes can be common or even abundant representatives of the marine benthic nematofauna. Our $\delta^{15}N$ data support the idea that several abundant nematodes from our study sites are at least to a significant extent carnivorous, in line with literature on the genera concerned (*Enoploides*: Moens et al., 1999a; Moens et al., 2000; Gallucci et al., 2005; *Enoplus*: Hellwig-Armonies et al., 1991; *Oncholaimus*: Heip et al., 1978; *Adoncholaimus*; Moens et al., 1999; Moens et al., 2000). They also indicate that some supposedly 'deposit-feeding' species may complement their diets through predation (here mainly *Daptonema*, but see also *Praeacanthonchus* in Moens et al. 2014) on unknown prey. In this context, it can be mentioned that Xyalidae (the family to which *Daptonema* belongs) with small nematode prey in their guts (sometimes juveniles of their own species (Moens and Vincx 1997)) have been regularly observed in our lab (e.g. Vanhove unpubl., Bezerra unpubl.). Unfortunately, neither of the two FA-markers which have been suggested as indicators of carnivory (PUFA/SFA, C20:1ω9) showed relative concentration patterns consistent with our $\delta^{15}N$ data and with existing observations on the predatory behaviour of the species concerned. Similar results were observed in copepods (e.g. Cnudde et al., 2015). It thus seems that the mentioned FA are not adequate,

or at least not generally applicable, biomarkers of carnivory. Many predatory nematodes have body sizes well above average nematode body sizes, and it has been suggested that large-bodied nematodes are a more likely prey to epi- and hyperbenthic macrofauna such as gobiid fish (Hamerlynck and Vanreusel, 1993).

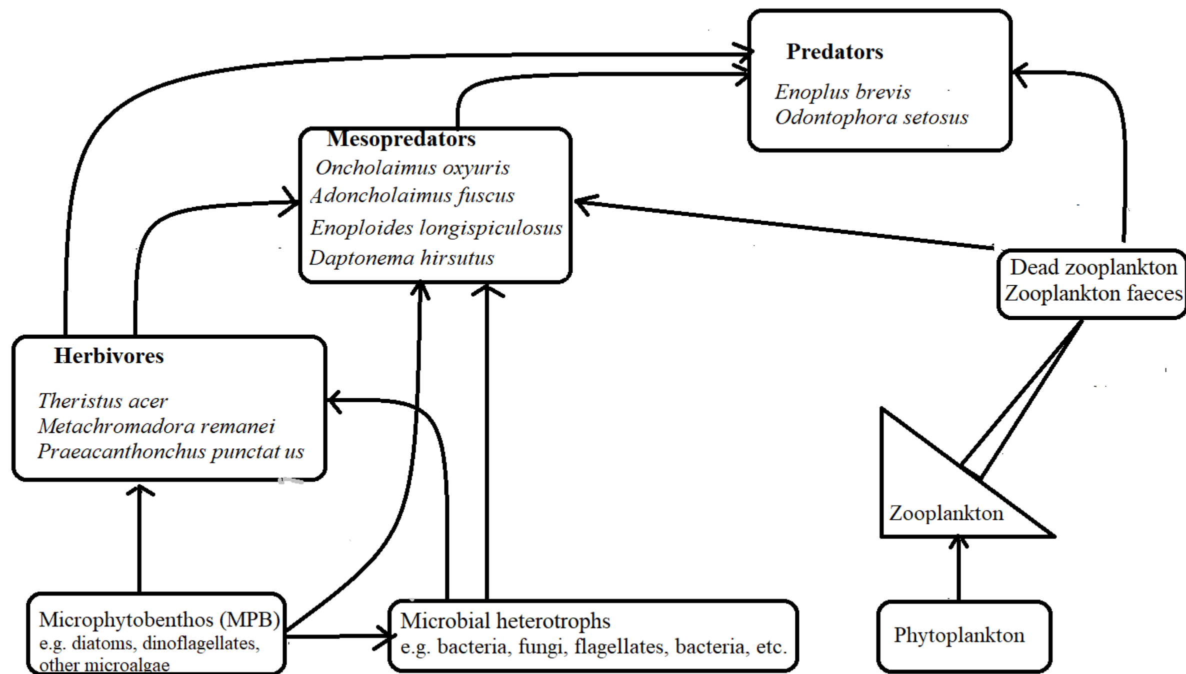


Fig. 5.3 Food sources and nematodes trophic levels revealed by fatty acid composition.

This last observation links to the second evolution away from the classical black-box view on meiofauna: an increasing number of studies have demonstrated the importance of meiofauna as prey for higher trophic levels (Coull, 1999; Danovaro et al., 2007; Gee, 1989; Beier et al., 2004; McCall and Fleeger, 1995). Although the focus in these papers has often been on harpacticoid copepods as prey organisms for epi- and hyperbenthic predators (e.g. McCall and Fleeger, 1995), supported by studies on predator gut content (Flinkman et al., 1994; Uye, 2011) and by the common idea that harpacticoid copepods live more epibenthically and make regular excursions into the water column (Thistle and Sedlacek, 2004), some authors have stressed that the lesser prominence of the relatively soft-bodied nematodes inside predator guts is a consequence of their more rapid digestion compared to, for instance, copepods (Scholz et al., 1991). Studies on freshwater habitats have demonstrated that predation by, among others, juvenile fish can be an important structuring factor for nematode assemblages (Weber and Traunspurger, 2016; Majdi et al., 2018).

Hence it is now commonly accepted that meiofauna are a potentially important link of benthic primary producer and primary consumer biomass to higher trophic levels (Kuipers et al., 1981; Coull, 1999;

Leduc, 2009); however, this link is far better established for harpacticoid copepods than for nematodes. Nevertheless, nematodes too may make short excursions from the sediment into the overlying water (Jensen, 1987; Lorenzen et al., 1987; Thomas and Lana, 2011; De Meester et al., 2018), and several species are restricted to the upper mms of the sediment (Coull 1988), making them inherently vulnerable to predation by mysids, shrimp and fish that forage at or near the sea floor. Moreover, copepods in general, and more specifically also harpacticoid copepods, are considered a high-quality food source for predators because of their relatively high levels of polyunsaturated fatty acids (Bell et al., 2003; Caramujo et al., 2008). However, these PUFA levels appear to vary a lot both among species and environmental contexts, also at the location of our own work (Paulina tidal flat), ranging from < 5 % of total FA in *Pseudostenhelia wellsi* to well over 50 % for *Delavalia palustris* (Cnudde et al., 2015). In seagrass leaf deposits, Mascart et al. (2018) found a PUFA range of 9.7 to 34.8 % in four harpacticoid copepod species sampled across different seasons. Compared to these, admittedly fragmentary, literature data, the PUFA proportions in our nine nematode species from the Paulina tidal flat both showed a relatively small range (from 39.5 % in *Metachromadora* to 60.8 % in *Daptonema* and *Theristus*) and comparatively high values. Our results also largely exceed HUFA levels in two other marine nematodes (18 – 19.5 %), the bacterivorous macroalgal inhabitant *Litoditis marina* (Leduc and Probert, 2009) and the sandy beach omnivore *Oncholaimus moanae* (Leduc, 2009). It is possible that different food sources and/or food availability could explain these differences in PUFA levels, or that these depend on environmental context. Combined with the generally much higher abundances of nematodes in intertidal sediments, this high nutritional quality suggests that nematodes can be at least as prominent a trophic link to higher trophic levels as harpacticoid copepods.

Still, the new view of ‘trophic structure’ and trophic levels in marine nematodes remains quite restrictive, now recognizing the importance of secondary consumers (predators of mainly other meiofauna) in addition to primary consumers (of primary producer or decomposer biomass). Our stable-isotope (SI) data from chapter 3 suggest that this is still too simplistic a representation.

First, depending on the trophic fractionation factor used, the nine nematode species from chapter 3 spanned almost three complete trophic levels. This can be explained in several non-mutually exclusive ways: first, some predacious nematodes may eat other predacious nematodes and meiofauna, which would yield a higher TL. Secondly, some nematode genera, especially large-bodied nematodes belonging to the facultative predators *sensu* Moens and Vincx (1997), have good swimming capacities and can move substantial distances towards carcasses of dead fish and other invertebrates (Lorenzen et al., 1987; Abolafia et al., 2015; Brüggemann, 2012), where they probably feed at least in part through a scavenging life style. This was already suggested by Jensen (1987), although more commonly

in a context of scavenging on dead benthic invertebrates. Obviously, when such nematodes obtain carbon and nitrogen from, for instance, a dead fish, this would affect the estimation of their TL. Nevertheless, we do not really believe that this phenomenon was very important in our model nematode species. First, we have SI data from several species from multiple moments in time, and these do not demonstrate substantial fluctuations in TL, which we would expect to be the case if scavenging on dead vertebrates were an opportunistic strategy to complement diet. Second, such vertebrate carcasses would in many cases have much more depleted $\delta^{13}C$ values than our nematodes, because they originate from the pelagic food web (exceptions being epi- and hyperbenthic species), and these were not observed here, except to a very limited extent in *Oncholaimus oxyuris*. We can thus carefully conclude that the high TL of species such as *Enoplus brevis* and *Odontophora setosum* really reflects a longer chain length of the 'meiobenthic part' of benthic food webs.

At the same time, the absolute food chain length is partly constrained by the prominent presence of omnivory. *Enoplus communis*, for instance, the species with the highest TL in our study, is known to be a generalist consumer, feeding on a very broad range of resources, from cyanobacteria to various meiofauna (Hellwig-Armonies et al., 1991). Actually, with the exception of *Metachromadora remanei*, *Praeacanthonchus punctatus* and *Theristus acer*, which all fed mainly on MPB (including bacteria to a limited extent, see section 5.3), most nematode species in our study did not have integer trophic levels, demonstrating that they are omnivores in the true ecological sense of the word, i.e. organisms that consume prey from more than one trophic level. That omnivory appears to be rule rather than exception in the (facultative) predators of our work, strongly suggests that the distinction between facultative and strict predators is artificial and could be abandoned. Indeed, the genus *Enoploides* was one of the model species upon which the feeding type of strict predators was based (Moens and Vincx, 1997; Moens et al., 2000), but it has meanwhile been shown to also feed on ciliates (Hamels et al., 2001), deposited phytoplankton (Franco et al., 2008) and diatoms (Moens et al., 2014), quite probably in an opportunistic manner, utilizing those high-quality resources that are most available. This matches the idea that most free-living nematodes are not very specialist feeders, but rather respond flexibly to available resources (Moens et al., 2004).

Despite such flexible feeding strategies of individual nematode species, our FA and SI data strongly support the idea that niche differentiation based on resource divergence is a major structuring factor of tidal-flat nematode assemblages. Particularly when combined with the patchy distribution of resources in space and time, and with differential dispersal rates (De Meester et al., 2015; De Meester et al., 2018; Thomas and Lana, 2011), this niche differentiation may account for the coexistence of large numbers of species at a local scale.

5.5 Dedicated experiments do not always provide straightforward answers: The example of *Metachromadora remanei*

The genus *Metachromadora* is very common in estuarine tidal flats, and in Europe is often mainly represented by *M. remanei* and *M. vivipara* (Steyaert et al., 2007; Rzeznik-Orignac et al., 2004), both of which are also common in the Paulina intertidal area (Wu, unpubl.). This genus illustrates rather well that advances we make in our knowledge on the ecology of marine nematodes sometimes paint a more complex picture than anticipated.

In terms of its occurrence in intertidal flats, *Metachromadora*, like many other epistrate-feeding nematodes, has often been found to attain its highest abundances in the surficial layer of the sediment (Steyaert et al., 2007). The same was true for the present study. Nevertheless, in a microcosm experiment where several nematode species were exposed to hypoxic and/or anoxic conditions, *M. vivipara* was the only species which not only survived all treatments but even increased in abundance under oxygen deprivation (Steyaert et al., 2007). Its ovoviviparous reproduction strategy was even considered an adaptation to life in anoxic and/or sulphidic habitats. At the same time, in the field, *M. vivipara* – like *M. remanei* – was most abundant in the surface layer. This is but one example where dedicated lab experiments do not always explain patterns observed in the field.

With respect to feeding ecology, the genus *Metachromadora* has long been considered a predator/omnivore *sensu* Wieser (1953) because of its very muscular pharynx and presence of a prominent tooth. It was not until natural stable isotope data were obtained from this genus, that it became clear that it feeds as an epistrate feeder and obtains most or all of its carbon on tidal flats from microphytobenthos (Moens et al., 2005). Indeed, in a year-round survey (with bimonthly samplings) at the Paulina tidal flat, *Metachromadora* consistently had (one of) the lowest $\delta^{15}N$ values of all abundant nematode genera present here, consistent with the idea of herbivory (Bezerra and Moens, unpubl.). In the present study, its feeding on diatom-dominated benthic biofilms was corroborated by the highest levels of C16:1ω7 (see chapter 3).

By contrast, *Metachromadora* had the lowest PUFA and HUFA levels of the nine nematode species investigated here, as well as the highest proportion of prokaryotic FA C15:0 and C17:0, even though the latter two FA still only comprised slightly over 3% of the total FA in *Metachromadora*. This raises the question as to whether *Metachromadora* may perhaps obtain some part of its nutrition from bacteria rather than from grazing on microalgae. This would be in line with results from an intertidal location sparsely vegetated with *Zostera marina*, where isotope mixing models suggested that *Metachromadora* obtained up to 85% of its carbon from seagrass detritus, most likely through the consumption of bacteria and/or fungi growing on the seagrass detritus (Vafeiadou et al., 2014). It may

also provide an indication that MPB grazers do not only depend on microalgae for their nutrition, but rely to some extent on the complex mix of microalgae, exopolymer secretions and bacteria that form MPB biofilms (Herlory et al., 2004; Hanlon et al., 2006; Agogué et al., 2014). In this context, it is interesting that up to a quarter of the variation in the microbiome composition of *Metachromadora* could be related to a mix of pigment concentrations, many of which indicative of the abundance of certain microalgal groups (chapter 4). Elucidating the exact roles of nematodes and other small invertebrates in the dynamics of these benthic biofilms is one of the principal challenges of tidal flat ecological research (Moens and Beninger, 2018).

5.6 Future perspectives

5.6.1. Technological opportunities to improve the 'performance' of nematode community ecology

The typical traditional approach to assess nematode communities was through the use of a light microscope to identify a portion of the nematodes present based on their morphological characteristics. A large body of literature has been using, and continues to use, this approach, and admittedly, there is currently probably no better or more efficient method. However, this approach is highly time- and energy-demanding and requires considerable taxonomic expertise, which is often not or insufficiently available (Taberlet et al., 2012). Because a typical meiofauna sample of intertidal sediments contains hundreds, if not more, of nematodes, it is usually not feasible to identify them all, and different 'schools' follow different approaches with respect to the question whether to identify a fixed number of specimens per sample, or rather a fixed proportion of the specimens in a sample (Barbour and Gerritsen, 1996; Giere, 2009). Both approaches have their advantages and drawbacks.

Because of the time-consuming nature of the identification work, it is not uncommon to see the nematode/meiofauna results of interdisciplinary projects lag one or two years behind the results of microbiologists and macrobenthologists. Moreover, the classical identification approach not only has limits in terms of numbers of samples that can be processed and specimens per sample that can be identified; it is now clear that the existence of cryptic species, i.e. species that cannot be differentiated unambiguously based on morphological characters alone, is widespread in marine nematodes (Bhadury et al., 2006; Derycke et al., 2005, 2008a, 2010). Such cryptic diversity cannot be uncovered using traditional approaches alone. Hence, DNA-based approaches such as meta-barcoding, are gaining increasing attention in the study of the diversity and community composition of nematodes (e.g. Creer et al., 2010; Fonseca et al., 2010; Fonseca et al., 2017).

Meta-barcoding can be an economically attractive alternative to classical approaches; it can produce millions of sequences of bulk samples at once after a relatively simple and rapid sample processing

procedure, and thus alleviate both fundamental limitations of traditional morphological methods mentioned above (time-consuming and unable to detect cryptic species) by offering rapid and reliable identifiers (operational taxonomic units, OTUs) that are independent of taxonomic expertise. Metabarcoding combines DNA taxonomy with high-throughput DNA sequencing (Ji et al., 2013). The former offers powerful and reliable identification due to its consideration of invisible morphological characteristics, while the latter allows the analysis of bulk samples or at least of bulk extracts (for instance a sample of nematodes collected from a sieve after repeated sample decantation), and hence of hundreds of nematodes, at once. It has already proven its potential for ecological research on communities of small-size organisms that are difficult to identify, such as meiofauna in marine sediments (Carugati et al., 2015; Chariton et al., 2015; Fonseca et al., 2014).

However, metabarcoding has not arrived at the point yet where it can render classical microscopical work redundant. This mainly has three reasons. First, our ability to put species names on OTUs depends on the quality of the reference database. There are currently several thousands of nematode species for which sequences of the 18S ribosomal RNA gene are available (Quast et al., 2012). Hence, in most evolutionary lineages of the phylum, a sequence can be assigned at least to a family, often to a genus, and regularly to a species. However, not all sequences in GenBank stem from reliably identified nematodes. More importantly, the 18S rRNA gene in nematodes has a poor identification resolution at the species level (Powers, 2004; Hebert et al., 2003); hence, species-level diversity in general, and cryptic diversity in particular, is unlikely to be adequately assessed based on 18S sequences. For any other target gene that would allow a higher identification resolution, such as the mitochondrial cytochrome oxidase subunit I gene (CO I) (Hebert et al., 2003; Derycke et al., 2005), databases contain sequences from at most a few hundreds of nematode species and do not offer a complete coverage of all major evolutionary lineages (Mitreva et al., 2011). This problem can only be resolved through a concerted effort of ‘classical’ and DNA taxonomists, aiming to substantially increase the extent and the quality/reliability of the reference database. Therefore, a combination of classical and DNA-based approaches is still direly needed (Rzeznik-Orignac et al., 2017).

A second major problem with metabarcoding is that, while it should theoretically be able to detect all species present in a sample, neither of the two most commonly used marker genes, 18S and CO I, is easily amplified from all nematodes species. Amplification success depends, among others, on the primers used and on the specific partition of the target gene (Derycke et al., 2010), and there tends to be a significant minority of species whose sequences are not amplified by the primer sets which we commonly use in our lab.

Finally, probably the main issue with metabarcoding of multicellular organisms like nematodes, is that there are currently no sufficiently reliable ways of quantifying the relative abundances of species in a sample (Elbrecht and Leese, 2015), because gene copy numbers differ between species, between specimens of a species, and even between cells of an individual (Schrider and Hahn, 2010; Katju and Bergthorsson, 2013). Hence, even if we overcome the above problems and manage to detect all species present in a sample, what we end up with is a richness estimate and a species/OTU list, but not a reliable quantitative assessment of community composition. During the course of this PhD, I metabarcoded nematode communities from part of the sampling locations of chapter 2 and determined environmental drivers of the metabarcoding-based community composition to compare these with what we found based on the classical approach of chapter 2. Unfortunately, time did not permit me to include these results in this PhD, but I can nevertheless say that the results are encouraging for the future use of metabarcoding. If this would prove to be more generally true, it would definitely increase our ability to deal with much larger numbers of samples, and hence to produce more powerful statistical analyses of the relationships between community composition and diversity on the one hand, and potential environmental drivers on the other.

5.6.2. Expanding the microbiome approach to elucidate nematode diets

Both stable-isotope and fatty-acid based approaches to elucidate the feeding ecology of nematodes have strong limitations; even if they complement each other, this set of techniques is largely unable to assess resource selectivity that would involve (Cashman et al., 2016), for instance, a nematode's preference of certain diatom or bacterial species over others. In our quest for much more detailed diet information, we applied metagenomics to analyse nematode microbiomes, under the assumption that these would in part reflect the trophic relationships between nematodes and bacteria.

However, we also tried essentially the same approach, but targeting the 18S rRNA gene, to elucidate the eukaryotic prey organisms of a range of nematode species from the Paulina tidal flat. Such an approach is possible, although we know of only one successful application to nematodes (Schuelke et al., 2018). Schuelke et al. (2018) concluded that nematode-associated microbiomes do not correlate with host phylogeny, geographic region or feeding morphology in marine sediments, but this seems at odds with species-specific microbiome differences between even very closely related species (Derycke et al., 2016) as well as between species belonging to different feeding types (chapter 4 of this study).

Our attempt to deep-sequence the 18S 'biome' of nematodes failed because of an unfortunate miscommunication about the correct primer sequences. However, while we expect that the vast majority of 18S sequences which we will pick up from any nematode will reflect the 18S of the

nematode itself, we nevertheless expect that this technique should allow us to document in unprecedented detail the recent diet of individual nematodes. The main potential limitation is in the word 'recent' in the previous sentence, because the non-host sequences we expect to find will reflect gut content, and hence represent only a very short snapshot in time of a nematode's diet (see, e.g., Moens et al., 1999a).

5.6.3. Getting more out of our microbiome analyses

While in humans and several other model organisms (Hentschel et al., 2012; Sturgeon et al., 2014; Turnbaugh, et al., 2007), microbiome research has progressed well beyond the stage of characterising the prokaryotes that are present, in nematodes this has been less the case. Marker-gene deep-sequencing has therefore largely advanced our understanding of microbial communities associated with hosts (e.g. Costello et al., 2012; Huttenhower et al., 2012), and this is also where the present PhD work has contributed, but it doesn't provide evidence of a microbiome's functional aspects (Langille et al., 2013), for example related to cellular processes (e.g. cell communication, cell growth and death, cell motility), to the processing of environmental information (membrane transport, signal transduction), to metabolism (amino acid metabolism, biosynthesis of other secondary metabolities, carbohydrate metabolism, energy metabolism, enzyme families, lipid metabolism,…), immunological responses etc.. This thesis did not include such in-depth information, as that would require a closed-reference OTU picking strategy, while chapter 4 of this thesis used an open-reference OTU picking strategy. For details of pros and cons of these two strategies of picking OTUs, see the following website (http://qiime.org/tutorials/otu_picking.html).

PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) (Langille et al., 2013) is a software designed to identify metagenome functional traits linked to OTUs detected from marker gene sequencing. It has been widely applied in human-associated microbiomes (e.g.), however, it has not so far been applied to nematode microbiomes. PICRUSt may be one future tool to link composition of the nematode-associated microbiomes to potential functioning, and hence to better understand where, when and how microbiomes may affect the fitness of nematodes. I personally believe that this may raise completely new ideas and open new avenues of research on how nematodes respond and adapt to their environment, deal with stress (environmental as well as biotic) and optimize their fitness in a variable and stressful environment.

5.6.4. A plea for more species-level and individual-based research

Nematode ecosystem functioning is often inferred from their assumed feeding groups based on their morphological characteristics, especially their mouth structure (Wieser, 1953; Yeates et al., 1993), and sometimes from a combination of morphology and life-history based traits (Schratzberger et al., 2007). For every trait considered, however, a limited number of trait groups are defined and hence, many

nematode species are lumped together in each trait group. Increasingly, studies are showing that these trait groups do not adequately represent the functional diversity of nematodes (e.g. Vafeiadou et al., 2014; Monteiro et al., 2018; chapter 3 of the present study).

Because of the biomass requirements for most biomarker studies, and because of the above-mentioned taxonomic challenges, it is tempting to lump species together in 'community samples' or at best according to feeding types when performing stable isotope or fatty acid studies. The results of chapter 3 underline that this is essentially useless, because diversity in trophic strategies within and between feeding groups is similarly large. Hence, we make a strong plea for studies which focus at the level of individual species. Although lumping species within a genus or family may sometimes be necessary, one should not forget that functional roles of nematodes may differ even among congeneric species (De Mesel et al., 2004; Vafeiadou et al., 2014; De Meester et al., 2016).

In addition, an often neglected feature in ecology is inter-individual variation and its importance for the dynamics of populations and communities (a.o. Bolnick et al., 2007; Ashton et al., 2010; Violle et al., 2012). Indeed, when we assign species to a particular trait group, we implicitly assign the same mean trait value to all individuals belonging to that species. This may lead to a severe underestimation of a species' ability to tolerate, and/or adapt to, stressful environmental conditions. It may also lead to an underestimation of the niche breadth of a species, and therefore of the degree of niche overlap and hence competition between species (Ashton et al., 2010; Violle et al., 2012). Hence, if we want to fully understand how species interactions may contribute to coexistence in multispecies communities, we may have to consider interindividual variation within species as well. Complex though this may seem, technology is rapidly developing the means to achieve this. Whereas until recently, it was impossible to obtain reliable diet information on individual nematodes, microbiome and eukaryotic biome analyses can now be performed on single specimens (see above), and techniques like NanoSIMS (Nanoscale secondary ion (emission) mass spectrometry) (Herrmann et al., 2007), although complex and tedious, equally allow detailed SI analyses on single individuals and even substructures/tissues/cells of organisms. Although the degree of expertise required for, and the costs associated with these analyses still hamper a more routine use, they will undoubtedly become cheaper in the future, and be complemented by yet newer technological advancements. The challenge will be to 'advertise' meiofauna/nematodes as sufficiently interesting model organisms to attract sufficient funding so that we can readily incorporate these novel technological evolutions into our research.

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References list

Abrams, B.I., and Mitchell, M.J. (1980) Role of nematode-bacterial interactions in heterotrophic systems with emphasis on sewage sludge decomposition. *Oikos* **34**: 404-410.

Abolafia, J., Ruiz-Cuenca, A.N., Fernandes, B.M., Cohen, S.C., and Cardenas, M.Q. (2015) Description of free-living marine nematodes found in the intestine of fishes from the Brazilian coast. *Zootaxa* **3948**: 549-572.

Agogué, H., Mallet, C., Orvain, F., De Crignis, M., Mornet, F., and Dupuy, C. (2014) Bacterial dynamics in a microphytobenthic biofilm: a tidal mesocosm approach. *Journal of Sea Research* **92**: 36-45.

Ainsworth, T.D., Krause, L., Bridge, T., Torda, G., Raina, J.-B., Zakrzewski, M. et al. (2015) The coral core microbiome identifies rare bacterial taxa as ubiquitous endosymbionts. *The ISME journal* **9**: 2261.

Alkemade, R., Wielemaker, A., De Jong, S., and Sandee, A. (1992) Diplolaimella dievengatensis in stimulating the mineralization of Spartina anglica detritus. *Marine Ecology Progress Series* **90**: 149-155.

Aller, R.C., and Aller, J.Y. (1992) Meiofauna and solute transport in marine muds. *Limnology and Oceanography* **37**: 1018-1033.

Alperin, M.J., and Hoehler, T.M. (2009) Anaerobic methane oxidation by archaea/sulfate-reducing bacteria aggregates: 2. Isotopic constraints. *American Journal of Science* **309**: 958-984.

Alves, M., Pereira, A., Matos, P., Henriques, J., Vicente, C., Aikawa, T. et al. (2016) Bacterial community associated to the pine wilt disease insect vectors *Monochamus galloprovincialis* and *Monochamus alternatus*. *Scientific Reports* **6**: 23908.

Amin, S.A., Parker, M.S., and Armbrust, E.V. (2012) Interactions between diatoms and bacteria. *Microbiology and Molecular Biology Reviews* **76**: 667-684.

Anderson, M.J. (2005) Permutational multivariate analysis of variance. *Department of Statistics, University of Auckland, Auckland* **26**: 32-46.

Anderson, M.J., and Robinson, J. (2003) Generalized discriminant analysis based on distances. *Australian & New Zealand Journal of Statistics* **45**: 301-318.

Anderson, M.J., Gorley, R.N., and Clarke, K.R. (2008) PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods. PRIMER-E Ltd, Plymouth.

Appeltans, W., Ahyong, S.T., Anderson, G., Angel, M.V., Artois, T., Bailly, N. et al. (2012) The magnitude of global marine species diversity. *Current Biology* **22**: 2189-2202.

Armonies, W., and Reise, K. (2000) Faunal diversity across a sandy shore. *Marine Ecology Progress Series* **196**: 49-57.

Ashton, I.W., Miller, A.E., Bowman, W.D., and Suding, K.N. (2010) Niche complementarity due to plasticity in resource use: plant partitioning of chemical N forms. *Ecology* **91**: 3252-3260.

Austen, M.C., Widdicombe, S., and Villano-Pitacco, N. (1998) Effects of biological disturbance on diversity and structure of meiobenthic nematode communities. *Marine Ecology Progress Series* **174**: 233-246.

Azam, F. (1998) Microbial control of oceanic carbon flux: the plot thickens. *Science* **280**: 694-696.

Azovsky, A., Saburova, M., Chertoprood, E., and Polikarpov, I. (2005) Selective feeding of littoral harpacticoids on diatom algae: hungry gourmands? *Marine Biology* **148**: 327-337.

Baquiran, J.-P., Thater, B., Sedky, S., De Ley, P., Crowley, D., and Orwin, P.M. (2013) Culture-independent investigation of the microbiome associated with the nematode *Acrobeloides maximus*. *PLoS One* **8**: e67425.

Barbour, M.T., and Gerritsen, J. (1996) Subsampling of benthic samples: a defense of the fixed-count method. *Journal of the North American Benthological Society* **15**: 386-391.

Beier, S., Bolley, M., and Traunspurger, W. (2004) Predator–prey interactions between *Dugesia gonocephala* and free‐living nematodes. *Freshwater Biology* **49**: 77-86.

Bell, J.G., Tocher, D.R., Henderson, R.J., Dick, J.R., and Crampton, V.O. (2003) Altered fatty acid compositions in Atlantic salmon (*Salmo salar*) fed diets containing linseed and rapeseed oils can be partially restored by a subsequent fish oil finishing diet. *The Journal of Nutrition* **133**: 2793-2801.

Bella, D.A., Ramm, A.E., and Peterson, P.E. (1972) Effects of tidal flats on estuarine water quality. *Journal Water Pollution Control Federation* **44**: 541-556.

Bezerra, T., Decraemer, W., Eisendle-Flöckner U, Holovachov O, Leduc D, Miljutin D et al. (2018) NeMys: World Database of Free-Living Marine Nematodes.

Bhadury, P., Austen, M.C., Bilton, D.T., Lambshead, P.J.D., Rogers, A.D., and Smerdon, G.R. (2006) Development and evaluation of a DNA-barcoding approach for the rapid identification of nematodes. *Marine Ecology Progress Series* **320**: 1-9.

Bioinformatics, B. (2011) FastQC: a quality control tool for high throughput sequence data. *Cambridge, Babraham Institute*.

Bird, A.F., and Ryder, M.H. (1993) Feeding of the nematode acrobeloides-nanus on bacteria. *Journal of Nematology* **25**: 493-499.

Bird, C.E., Franklin, E.C., Smith, C.M., and Toonen, R.J. (2013) Between tide and wave marks: a unifying model of physical zonation on littoral shores. *Peerj* **1**: e154.

Blanc, C., Sy, M., Djigal, D., Brauman, A., Normand, P., and Villenave, C. (2006) Nutrition on bacteria by bacterial-feeding nematodes and consequences on the structure of soil bacterial community. *European Journal of Soil Biology* **42**: S70-S78.

Blanchard, G. (1990) Overlapping microscale dispersion patterns of meiofauna and microphytobenthos. *Marine Ecology Progress Series* **68**: 101-111.

Blanchard, G.F. (1991) Measurement of meiofauna grazing rates on microphytobenthos: is primary production a limiting factor? *Journal of Experimental Marine Biology and Ecology* **147**: 37-46.

Blome, D., and Riemann, F. (1987) A sediment agglutination on females of the free-living marine nematode *Desmodora schulzi*. *Helgolander Meeresuntersuchungen* **41**: 113-119.

Blome, D., Schleier, U., and von Bernem, K.H. (1999) Analysis of the small-scale spatial patterns of free-living marine nematodes from tidal flats in the East Frisian Wadden Sea. *Marine Biology* **133**: 717-726.

Boldina, I., Beninger, P.G., and Le Coz, M. (2014) Effect of long-term mechanical perturbation on intertidal soft-bottom meiofaunal community spatial structure. *Journal of Sea Research* **85**: 85-91.

Bolla, R. (1979) Developmental nutrition of nematodes: The biochemical role of sterols, heme compounds, and lysosomal enzymes. *Journal of Nematology* **11**: 250.

Bolnick, D.I., Svanbäck, R., Araújo, M.S., and Persson, L. (2007) Comparative support for the niche variation hypothesis that more generalized populations also are more heterogeneous. *Proceedings of the National Academy of Sciences* **104**: 10075-10079.

Bonaglia, S., Nascimento, F.A., Bartoli, M., Klawonn, I., and Brüchert, V. (2014) Meiofauna increases bacterial denitrification in marine sediments. *Nature Communications* **5**: 5133.

Boon, A., and Duineveld, G. (1997) Phytopigments and fatty acids as molecular markers for the quality of near-bottom particulate organic matter in the North Sea. *Oceanographic Literature Review* **1**: 24.

Boschker, H., and Middelburg, J. (2002) Stable isotopes and biomarkers in microbial ecology. *FEMS Microbiology Ecology* **40**: 85-95.

Boschker, H., Kromkamp, J., and Middelburg, J. (2005) Biomarker and carbon isotopic constraints on bacterial and algal community structure and functioning in a turbid, tidal estuary. *Limnology and Oceanography* **50**: 70-80.

Boucher, G. (1990) Pattern of nematode species diversity in temperate and tropical subtidal sediments. *Marine Ecology* **11**: 133-146.

Braeckman, U., Provoost, P., Moens, T., Soetaert, K., Middelburg, J.J., Vincx, M., and Vanaverbeke, J. (2011b) Biological vs. physical mixing effects on benthic food web dynamics. *PLoS One* **6**: e18078.

Braeckman, U., Provoost, P., Sabbe, K., Soetaert, K., Middelburg, J.J., Vincx, M., and Vanaverbeke, J. (2015) Temporal dynamics in a shallow coastal benthic food web: Insights from fatty acid biomarkers and their stable isotopes. *Marine Environmental Research* **108**: 55-68.

Braeckman, U., Van Colen, C., Soetaert, K., Vincx, M., and Vanaverbeke, J. (2011a) Contrasting macrobenthic activities differentially affect nematode density and diversity in a shallow subtidal marine sediment. *Marine Ecology Progress Series* **422**: 179-191.

Bright, M., and Bulgheresi, S. (2010) A complex journey: transmission of microbial symbionts. *Nature Reviews Microbiology* **8**: 218-230.

Brüggemann, J. (2012) Nematodes as live food in larviculture: a review. *Journal of the World Aquaculture Society* **43**: 739-763.

Cabreiro, F., and Gems, D. (2013) Worms need microbes too: microbiota, health and aging in *Caenorhabditis elegans*. *EMBO Molecular Medicine* **5**: 1300-1310.

Cao, Y., Williams, D.D., and Larsen, D.P. (2002) Comparison of ecological communities: the problem of sample representativeness. *Ecological Monographs* **72**: 41-56.

Canuel, E.A., and Martens, C.S. (1993) Seasonal variations in the sources and alteration of organic matter associated with recently-deposited sediments. *Organic Geochemistry* **20**: 563-577.

Caramujo, M.J., Boschker, H.T., and Admiraal, W. (2008) Fatty acid profiles of algae mark the development and composition of harpacticoid copepods. *Freshwater Biology* **53**: 77-90.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K. et al. (2010) QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* **7**: 335-336.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N. et al. (2012) Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *The ISME Journal* **6**: 1621-1624.

Carman, K.R., and Fry, B. (2002) Small-sample methods for $\delta^{13}C$ and $\delta^{15}N$ analysis of the diets of marsh meiofaunal species using natural-abundance and tracer-addition isotope techniques. *Marine Ecology Progress Series* **240**: 85-92.

Carpentier, A., Como, S., Dupuy, C., Lefrancois, C., and Feunteun, E. (2014) Feeding ecology of *Liza spp*. in a tidal flat: Evidence of the importance of primary production (biofilm) and associated meiofauna. *Journal of Sea Research* **92**: 86-91.

Carugati, L., Corinaldesi, C., Dell'Anno, A., and Danovaro, R. (2015) Metagenetic tools for the census of marine meiofaunal biodiversity: An overview. *Marine Genomics* **24(1)**: 11-22.

Cashman, M.J., Pilotto, F., Harvey, G.L., Wharton, G., and Pusch, M.T. (2016) Combined stable‐isotope and fatty‐acid analyses demonstrate that large wood increases the autochthonous trophic base of a macroinvertebrate assemblage. *Freshwater Biology* **61**: 549-564.

Chariton, A.A., Stephenson, S., Morgan, M.J., Steven, A.D., Colloff, M.J., Court, L.N., and Hardy, C.M. (2015) Metabarcoding of benthic eukaryote communities predicts the ecological condition of estuaries. *Environmental Pollution* **203**: 165-174.

Chen, J., Bittinger, K., Charlson, E.S., Hoffmann, C., Lewis, J., Wu, G.D. et al. (2012) Associating microbiome composition with environmental covariates using generalized UniFrac distances. *Bioinformatics* **28**: 2106-2113.

Cho, I., and Blaser, M.J. (2012) The human microbiome: at the interface of health and disease. *Nature Reviews Genetics* **13**: 260-270.

Cho, K.W., and Mo, S.J. (1999) Screening and characterization of eicosapentaenoic acid-producing marine bacteria. *Biotechnology Letters* **21**: 215-218.

Clarke KR, Gorley RN. 2006. PRIMER v6 User manual/tutorial. PRIMER-E Ltd, Plymouth.

Claustre, H., Marty, J.-C., Cassiani, L., and Dagaut, J. (1988) Fatty acid dynamics in phytoplankton and microzooplankton communities during a spring bloom in the coastal Ligurian Sea: ecological implications. *Marine Microbial Food Webs* **3**: 51-66.

Cnudde, C. (2013) Trophic ecology of intertidal harpacticoid copepods, with emphasis on their interactions with bacteria. Dissertitation, University of Ghent.

Cnudde, C., Clavano, C.J.S., Moens, T., Willems, A., and De Troch, M. (2013) Structural and functional patterns of active bacterial communities during aging of harpacticoid copepod fecal pellets. *Aquatic Microbial Ecology* **71**: 25-42.

Cnudde, C., Moens, T., Werbrouck, E., Lepoint, G., Van Gansbeke, D., and De Troch, M. (2015) Trophodynamics of estuarine intertidal harpacticoid copepods based on stable isotope composition and fatty acid profiles. *Marine Ecology Progress Series* **524**: 225-239.

Commito, J.A., and Tita, G. (2002) Differential dispersal rates in an intertidal meiofauna assemblage. *Journal of Experimental Marine Biology and Ecology* **268**: 237-256.

Compton, T.J., Holthuijsen, S., Koolhaas, A., Dekinga, A., ten Horn, J., Smith, J. et al. (2013) Distinctly variable mudscapes: Distribution gradients of intertidal macrofauna across the Dutch Wadden Sea. *Journal of Sea Research* **82**: 103-116.

Cook, P.L., Van Oevelen, D., Soetaert, K., and Middelburg, J.J. (2009) Carbon and nitrogen cycling on intertidal mudflats of a temperate Australian estuary. IV. Inverse model analysis and synthesis. *Marine Ecology Progress Series* **394**: 35-48.

Costello, E.K., Stagaman, K., Dethlefsen, L., Bohannan, B.J., and Relman, D.A. (2012) The application of ecological theory toward an understanding of the human microbiome. *Science* **336**: 1255-1262.

Coull, B.C. (1988) Ecology of marine meiofauna. *In* Higgins R.P. and Thiel H (eds). *Introduction to the study of meiofauna.* Smithsonian institution scholarly press, Washington, p18-38.

Coull, B.C. (1990) Are members of the meiofauna food for higher trophic levels? *Transactions of the American Microscopical Society* **109**: 233-246.

Coull, B.C. (1999) Role of meiofauna in estuarine soft-bottom habitats. *Australian Journal of Ecology* **24**: 327-343.

Creer, S., Fonseca, V., Porazinska, D., Giblin-Davis, R., Sung, W., Power, D. et al. (2010) Ultrasequencing of the meiofaunal biosphere: practice, pitfalls and promises. *Molecular Ecology* **19**: 4-20.

Cripps, G., and Atkinson, A. (2000) Fatty acid composition as an indicator of carnivory in Antarctic krill, Euphausia superba. *Canadian Journal of Fisheries and Aquatic Sciences* **57**: 31-37.

Cullen, D.J. (1973) Bioturbation of superficial marine sediments by interstitial meiobenthos. *Nature* **242**: 323-324.

Currie, A.R., Tait, K., Parry, H., de Francisco-Mora, B., Hicks, N., Osborn, A.M. et al. (2017) Marine microbial gene abundance and community composition in response to ocean acidification and elevated temperature in two contrasting coastal marine sediments. *Frontiers in Microbiology* **8**: 1599.

Dalsgaard, J., John, M.S., Kattner, G., Müller-Navarra, D., and Hagen, W. (2003) Fatty acid trophic markers in the pelagic marine environment. *Advances in Marine Biology* **46**: 225-340.

Danovaro, R. (1996) Detritus-bacteria-meiofauna interactions in a seagrass bed (*Posidonia oceanica*) of the NW Mediterranean. *Marine Biology* **127**: 1-13.

Danovaro, R., Scopa, M., Gambi, C., and Fraschetti, S. (2007) Trophic importance of subtidal metazoan meiofauna: evidence from *in situ* exclusion experiments on soft and rocky substrates. *Marine Biology* **152**: 339-350.

Day JW, Hall CAS, Kemp WM,Yanez-Arancibia A. 1989. Estuarine Ecology. John Wiley & Sons, New York.

De Grisse, A. (1965) A labour-saving method for fixing and transferring eelworms to anhydrous glycerin. *LandbHogesch OpzoekStns—Leerstoel Dierk Gent*.

De Brouwer, J., and Stal, L. (2001) Short-term dynamics in microphytobenthos distribution and associated extracellular carbohydrates in surface sediments of an intertidal mudflat. *Marine Ecology Progress Series* **218**: 33-44.

De Jorge, V., and Van Beusekom, J. (1995) Wind ‐ and tide ‐ induced resuspension of sediment and microphytobenthos from tidal flats in the Ems estuary. *Limnology and Oceanography* **40**: 776-778.

De Meester, N., Derycke, S., and Moens, T. (2012) Differences in time until dispersal between cryptic species of a marine nematode species complex. *PLoS One* **7**: e42674.

De Meester, N., Derycke, S., Rigaux, A., and Moens, T. (2015) Temperature and salinity induce differential responses in life histories of cryptic nematode species. *Journal of Experimental Marine Biology and Ecology* **472**: 54-62.

De Meester, N., Gingold, R., Rigaux, A., Derycke, S., and Moens, T. (2016) Cryptic diversity and ecosystem functioning: a complex tale of differential effects on decomposition. *Oecologia* **182**: 559-571.

De Meester N, Van Daele T, Van Malderen J, Monteiro L, Van Colen C, Derycke S, Moens T (2018) Active and species-specific dispersal behaviour in a marine nematode cryptic species complex. *Marine Ecology Progress Series* **600**:71-83.

De Mesel, I. (2004) Nematodes associated with phytodetritus in salt marshes. Dissertitation, University of Ghent.

De Mesel, I., Derycke, S., Swings, J., Vincx, M., and Moens, T. (2003) Influence of bacterivorous nematodes on the decomposition of cordgrass. *Journal of Experimental Marine Biology and Ecology* **296**: 227-242.

De Mesel, I., Derycke, S., Swings, J., Vincx, M., and Moens, T. (2006) Role of nematodes in decomposition processes: Does within-trophic group diversity matter? *Marine Ecology Progress Series* **321**: 157-166.

De Troch, M., Boeckx, P., Cnudde, C., Van Gansbeke, D., Vanreusel, A., Vincx, M., and Caramujo, M.J. (2012) Bioconversion of fatty acids at the basis of marine food webs: insights from a compound-specific stable isotope analysis. *Marine Ecology Progress Series* **465**: 53-67.

De Troch, M., Chepurnov, V., Gheerardyn, H., Vanreusel, A., and Olafsson, E. (2006) Is diatom size selection by harpacticoid copepods related to grazer body size? *Journal of Experimental Marine Biology and Ecology* **332**: 1-11.

De Troch, M., Cnudde, C., Willems, A., Moens, T., and Vanreusel, A. (2010) Bacterial colonization on fecal pellets of harpacticoid copepods and on their diatom food. *Microbial Ecology* **60**: 581-591.

Decho, A.W. (1990) Microbial exopolymer secretions in ocean environments: their role(s) in food webs and marine processes. *Oceanography and Marine Biology* **28**: 9-16.

Decleyre, H., Heylen, K., Sabbe, K., Tytgat, B., Deforce, D., Van Nieuwerburgh, F. et al. (2015) A doubling of microphytobenthos biomass coincides with a tenfold increase in denitrifier and total bacterial abundances in intertidal sediments of a temperate estuary. *PLoS One* **10**: e0126583.

Derycke, S., Backeljau, T., and Moens, T. (2013) Dispersal and gene flow in free-living marine nematodes. *Frontiers in Zoology* **10**: 1.

Derycke, S., De Meester, N., Rigaux, A., Creer, S., Bik, H., Thomas, W., and Moens, T. (2016) Coexisting cryptic species of the *Litoditis marina* complex (Nematoda) show differential resource use and have distinct microbiomes with high intraspecific variability. *Molecular Ecology* **25**: 2093-2110.

Derycke, S., Fonseca, G., Vierstraete, A., Vanfleteren, J., Vincx, M., and Moens, T. (2008a) Disentangling taxonomy within the *Rhabditis* (*Pellioditis*) *marina* (Nematoda, Rhabditidae) species complex using molecular and morhological tools. *Zoological Journal of the Linnean Society* **152**: 1-15.

Derycke, S., Hendrickx, F., Backeljau, T., D'Hondt, S., Camphijn, L., Vincx, M., and Moens, T. (2007) Effects of sublethal abiotic stressors on population growth and genetic diversity of *Pellioditis marina* (Nematoda) from the Westerschelde estuary. *Aquatic Toxicology* **82**: 110-119.

Derycke, S., Remerie, T., Backeljau, T., Vierstraete, A., Vanfleteren, J., Vincx, M., and Moens, T. (2008b) Phylogeography of the *Rhabditis* (*Pellioditis*) *marina* species complex: evidence for long-distance dispersal, and for range expansions and restricted gene flow in the northeast Atlantic. *Molecular Ecology* **17**: 3306-3322.

Derycke, S., Remerie, T., Vierstraete, A., Backeljau, T., Vanfleteren, J., Vincx, M., and Moens, T. (2005) Mitochondrial DNA variation and cryptic speciation within the free-living marine nematode *Pellioditis marina*. *Marine Ecology Progress Series* **300**: 91-103.

Derycke, S., Vanaverbeke, J., Rigaux, A., Backeljau, T., and Moens, T. (2010) Exploring the Use of Cytochrome Oxidase c Subunit 1 (COI) for DNA Barcoding of Free-Living Marine Nematodes. *PLoS One* **5**: e13716.

DeSantis, T., Hugenholtz, P., Keller, K., Brodie, E., Larsen, N., Piceno, Y. et al. (2006) NAST: a multiple sequence alignment server for comparative analysis of 16S rRNA genes. *Nucleic Acids Research* **34**: W394-W399.

D'Hondt, A.-S., Stock, W., Blommaert, L., Moens, T., and Sabbe, K. (2018) Nematodes stimulate biomass production in a multispecies diatom biofilm. *Marine Environmental Research* **140**: 78-89.

Dirksen, P., Marsh, S.A., Braker, I., Heitland, N., Wagner, S., Nakad, R. et al. (2016) The native microbiome of the nematode *Caenorhabditis elegans*: gateway to a new host-microbiome model. *BMC Biology* **14**: 38.

Douglas, A., Douraghi-Zadeh, K., Eglinton, G., Maxwell, J., and Ramsay, J. (1970) Fatty acids in sediments including the Green River shale (Eocene) and Scottish torbanite (Carboniferous). In: Dep. Chem., Univ. Glasgow, Glasgow, Scot.

Doulgeraki, S., Lampadariou, N., and Sinis, A. (2006) Meiofaunal community structure in three Mediterranean coastal lagoons (North Aegean Sea). *Journal of the Marine Biological Association of the United Kingdom* **86**: 209-220.

Dubilier, N., Bergin, C., and Lott, C. (2008) Symbiotic diversity in marine animals: the art of harnessing chemosynthesis. *Nature Reviews Microbiology* **6**: 725-740.

Ederington, M.C., McManus, G.B., and Harvey, H.R. (1995) Trophic transfer of fatty acids, sterols, and a triterpenoid alcohol between bacteria, a ciliate, and the copepod Acartia tonsa. *Limnology and Oceanography* **40**: 860-867.

Elbrecht, V., and Leese, F. (2015) Can DNA-based ecosystem assessments quantify species abundance? Testing primer bias and biomass-sequence relationships with an innovative metabarcoding protocol. *PeerJ PrePrints* **3**: e1258.

Elhady, A., Adss, S., Hallmann, J., and Heuer, H. (2018) Rhizosphere microbiomes modulated by pre-crops assisted plants in defense against plant-parasitic nematodes. *Frontiers in Microbiology* **9**: 1133.

Elhadyl, A., Gine, A., Topalovic, O., Jacquiod, S., Sorensen, S.J., Sorribas, F.J., and Heuer, H. (2017) Microbiomes associated with infective stages of root-knot and lesion nematodes in soil. *PLoS One* **12(5)**: e0177145.

Elliott, M., and McLusky, D.S. (2002) The need for definitions in understanding estuaries. *Estuarine, Coastal and Shelf Science* **55**: 815-827.

Epstein, S. (1997) Microbial food webs in marine sediments. I. Trophic interactions and grazing rates in two tidal flat communities. *Microbial Ecology* **34**: 188-198.

Estifanos, T.K., Traunspurger, W., and Peters, L. (2013) Selective feeding in nematodes: a stable isotope analysis of bacteria and algae as food sources for free-living nematodes. *Nematology* **15**: 1-13.

Evans, G. (1965) Intertidal flat sediments and their environments of deposition in the Wash. *Quarterly Journal of the Geological Society* **121**: 209-240.

Evrard, V., Cook, P.L., Veuger, B., Huettel, M., and Middelburg, J.J. (2008) Tracing carbon and nitrogen incorporation and pathways in the microbial community of a photic subtidal sand. *Aquatic Microbial Ecology* **53**: 257-269.

Evrard, V., Huettel, M., Cook, P.L., Soetaert, K., Heip, C.H., and Middelburg, J.J. (2012) Importance of phytodetritus and microphytobenthos for heterotrophs in a shallow subtidal sandy sediment. *Marine Ecology Progress Series* **455**: 13-31.

Evrard, V., Soetaert, K., Heip, C.H., Huettel, M., Xenopoulos, M.A., and Middelburg, J.J. (2010) Carbon and nitrogen flows through the benthic food web of a photic subtidal sandy sediment. *Marine Ecology Progress Series* **416**: 1-16.

Fenchel, T., and Straarup, B.J. (1971) Vertical distribution of photosynthetic pigments and the penetration of light in marine sediments. *Oikos* **22**: 172-182.

Ferreira, R.C., Nascimento, A.B., Santos, P.J.P., Botter-Carvalho, M.L., and Pinto, T.K. (2015) Responses of estuarine nematodes to an increase in nutrient supply: An *in situ* continuous addition experiment. *Marine Pollution Bulletin* **90**: 115-120.

Findlay, S.E. (1981) Small-scale spatial distribution ofmeiofauna on a mud-and sandflat. *Estuarine, Coastal and Shelf Science* **12**: 471-484.

Flemming, B.W. (2011) Geology, morphology, and sedimntology of estuaries and coasts. In E. Wolanski, D.S. McLusky (Eds.), *Treatise on estuarine and coastal science*, Vol 3, *Academic Press, Waltham*.

Flinkman, J., Vuorinen, I., and Christiansen, M. (1994) Calanoid copepod eggs survive passage through fish digestive tracts. *ICES Journal of Marine Science* **51**: 127-129.

Floyd, R., Abebe, E., Papert, A., and Blaxter, M. (2002) Molecular barcodes for soil nematode identification. *Molecular Ecology* **11**: 839-850.

Fonseca, G., and Soltwedel, T. (2007) Deep-sea meiobenthic communities underneath the marginal ice zone off Eastern Greenland. *Polar Biology* **30**: 607-618.

Fonseca, V., Sinniger, F., Gaspar, J., Quince, C., Creer, S., Power, D.M., Peck L. S. and Clark M.S. (2017) Revealing higher than expected meiofaunal diversity in Antarctic sediments: a metabarcoding approach. *Scientific Reports* **7**: 6094.

Fonseca, V.G., Carvalho, G.R., Sung, W., Johnson, H.F., Power, D.M., Neill, S.P., Packer, M, Blaxter, M.L., Lambshead, P.J.D. and Thomas, W.K. (2010) Second-generation environmental sequencing unmasks marine metazoan biodiversity. *Nature communications* **1**: 98.

Fonseca, G., Hutchings, P., Vieira, D.C., and Gallucci, F. (2011) Meiobenthic community underneath the carcass of a stingray: a snapshot after natural death. *Aquatic Biology* **13**: 27-33.

Franco, M.A., Soetaert, K., Costa, M.J., Vincx, M., and Vanaverbeke, J. (2008a) Uptake of phytodetritus by meiobenthos using C^{13} labelled diatoms and Phaeocystis in two contrasting sediments from the North Sea. *Journal of Experimental Marine Biology and Ecology* **362**: 1-8.

Franco, M.A., Soetaert, K., Van Oevelen, D., Van Gansbeke, D., Costa, M.J., Vincx, M., and Vanaverbeke, J. (2008b) Density, vertical distribution and trophic responses of metazoan meiobenthos to phytoplankton deposition in contrasting sediment types. *Marine Ecology Progress Series* **358**: 51-62.

Fraune, S., and Bosch, T.C.G. (2007) Long-term maintenance of species-specific bacterial microbiota in the basal metazoan Hydra. *Proceedings of the National Academy of Sciences of the United States of America* **104**: 13146-13151.

Fry, B. (2007) Stable isotope ecology. Springer Science & Business Media, Berlin.

Fry, B., Jannasch, H.W., Molyneaux, S.J., Wirsen, C.O., Muramoto, J.A., and King, S. (1991) Stable isotope studies of the carbon, nitrogen and sulfur cycles in the Black Sea and the Cariaco Trench. *Deep Sea Research, Part A Oceanographic Research Papers* **38**: S1003-S1019.

Gallucci, F., Moens, T., Vanreusel, A., and Fonseca, G. (2008) Active colonisation of disturbed sediments by deep-sea nematodes: evidence for the patch mosaic model. *Marine Ecology Progress Series* **367**: 173-183.

Gallucci, F., Steyaert, M., and Moens, T. (2005) Can field distributions of marine predacious nematodes be explained by sediment constraints on their foraging success? *Marine Ecology Progress Series* **304**: 167-178.

Gee, J. (1989) An ecological and economic review of meiofauna as food for fish. *Zoological journal of the Linnean Society* **96**: 243-261.

Gerbersdorf, S.U., Bittner, R., Lubarsky, H., Manz, W., and Paterson, D.M. (2009) Microbial assemblages as ecosystem engineers of sediment stability. *Journal of Soils and Sediments* **9**: 640-652.

Gerdts, G., Brandt, P., Kreisel, K., Boersma, M., Schoo, K., and Wichels, A. (2013) The microbiome of North Sea copepods. *Helgoland Marine Research* **67**: 757.

Ghafouri, S., and McGhee, J.D. (2007) Bacterial residence time in the intestine of *Caenorhabditis elegans*. *Nematology* **9**: 87-91.

Gheskiere, T., Hoste, E., Vanaverbeke, J., Vincx, M., and Degraer, S. (2004) Horizontal zonation patterns and feeding structure of marine nematode assemblages on a macrotidal, ultra-dissipative sandy beach (De Panne, Belgium). *Journal of Sea Research* **52**: 211-226.

Gheskiere, T., Vincx, M., Urban-Malinga, B., Rossano, C., Scapini, F., and Degraer, S. (2005) Nematodes from wave-dominated sandy beaches: diversity, zonation patterns and testing of the isocommunities concept. *Estuarine Coastal and Shelf Science* **62**: 365-375.

Giere, O. (2009) Meiobenthology: the microscopic motile fauna of aquatic sediments. Springer Science & Business Media, Berlin.

Gingold, R., Ibarra-Obando, S.E., and Rocha-Olivares, A. (2011) Spatial aggregation patterns of free-living marine nematodes in contrasting sandy beach micro-habitats. *Journal of the Marine Biological Association of the United Kingdom* **91**: 615-622.

Gingold, R., Mundo-Ocampo, M., Holovachov, O., and Rocha-Olivares, A. (2010) The role of habitat heterogeneity in structuring the community of intertidal free-living marine nematodes. *Marine Biology* **157**: 1741-1753.

Gomes, T.P., and Rosa, J.S. (2009) Composition and spatio-temporal variability of meiofauna community on a sandy beach in the Amazon region (Ajuruteua, Para, Brazil). *Iheringia Serie Zoologia* **99**: 210-216.

Goto, N., Kawamura, T., Mitamura, O., and Terai, H. (1999) Importance of extracellular organic carbon production in the total primary production by tidal-flat diatoms in comparison to phytoplankton. *Marine Ecology Progress Series* **190**: 289-295.

Greenblum, S., Turnbaugh, P.J., and Borenstein, E. (2012) Metagenomic systems biology of the human gut microbiome reveals topological shifts associated with obesity and inflammatory bowel disease. *Proceedings of the National Academy of Sciences* **109**: 594-599.

Guckert, J.B., Antworth, C.P., Nichols, P.D., and White, D.C. (1985) Phospholipid, ester-linked fatty acid profiles as reproducible assays for changes in prokaryotic community structure of estuarine sediments. *FEMS Microbiology Letters* **31**: 147-158.

Guilini, K., Veit-Koehler, G., De Troch, M., Van Gansbeke, D., and Vanreusel, A. (2013) Latitudinal and temporal variability in the community structure and fatty acid composition of deep-sea nematodes in the Southern Ocean. *Progress in Oceanography* **110**: 80-92.

Guilini, K., Van Oevelen, D., Soetaert, K., Middelburg, J.J., and Vanreusel, A. (2010) Nutritional importance of benthic bacteria for deep-sea nematodes from the Arctic ice margin: Results of an isotope tracer experiment. *Limnology and Oceanography* **55**: 1977-1989.

Hamels, I., Moens, T., Mutylaert, K., and Vyverman, W. (2001) Trophic interactions between ciliates and nematodes from an intertidal flat. *Aquatic Microbial Ecology* **26**: 61-72.

Hamels, I., Sabbe, K., Muylaert, K., Barranguet, C., Lucas, C., Herman, P., and Vyverman, W. (1998) Organisation of microbenthic communities in intertidal estuarine flats, a case study from the

Molenplaat (Westerschelde estuary, The Netherlands). *European Journal of Protistology* **34**: 308-320.

Hamerlynck, O., and Vanreusel, A. (1993) *Mesacanthion diplechma* (Nematoda: Thoracostomopsidae), a link to higher trophic levels? *Journal of the Marine Biological Association of the United Kingdom* **73**: 453-456.

Hanlon, A.R., Bellinger, B., Haynes, K., Xiao, G., Hofmann, T.A., Gretz, M.R. et al. (2006) Dynamics of extracellular polymeric substance (EPS) production and loss in an estuarine, diatom‐dominated, microalgal biofilm over a tidal emersio—immersion period. *Limnology and Oceanography* **51**: 79-93.

Hebert, P.D., Ratnasingham, S., and de Waard, J.R. (2003) Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society of London. Series B: Biological Sciences* S96-S99.

Heip, C., Goosen, N., Herman, P., Kromkamp, J., Middelburg, J., and Soetaert, K. (1995) Production and consumption of biological particles in temperate tidal estuaries. *Oceanography and Marine Biology: an annual review* **33**: 1-149.

Heip, C., Vincx, M., and Vranken, G. (1985) The ecology of marine nematodes. *Oceanography and Marine Biology* **23**: 399-489.

Hellings, L., Dehairs, F., Tackx, M., Keppens, E., and Baeyens, W. (1999) Origin and fate of organic carbon in the freshwater part of the Scheldt Estuary as traced by stable carbon isotope composition. *Biogeochemistry* **47**: 167-186.

Hellwig-Armonies, M., Armonies, W., and Lorenzen, S. (1991) The diet of *Enoplus brevis* (Nematoda) in a supralittoral salt marsh of the North Sea. *Helgoländer Meeresuntersuchungen* **45**: 357.

Henrichs, S., and Doyle, A. (1986) Decomposition of ^{14}C-labelled organic substances in marine sediments. *Limnology Oceanography* **31**: 765-778.

Herlory, O., Guarini, J.-M., Richard, P., and Blanchard, G. (2004) Microstructure of microphytobenthic biofilm and its spatio-temporal dynamics in an intertidal mudflat (Aiguillon Bay, France). *Marine Ecology Progress Series* **282**: 33-44.

Herman, P., Middelburg, J., Van de Koppel, J., and Heip, C. (1999) Ecology of estuarine macrobenthos. *Advances in Ecological Research* **29**: 195-240.

Herman, P.M., Middelburg, J.J., and Heip, C.H. (2001) Benthic community structure and sediment processes on an intertidal flat: results from the ECOFLAT project. *Continental Shelf Research* **21**: 2055-2071.

Herrmann, A.M., Clode, P.L., Fletcher, I.R., Nunan, N., Stockdale, E.A., O'Donnell, A.G., and Murphy, D.V. (2007) A novel method for the study of the biophysical interface in soils using nano‐

scale secondary ion mass spectrometry. *Rapid Communications in Mass Spectrometry* **21**: 29-34.

Hentschel, U., Piel, J., Degnan, S.M., and Taylor, M.W. (2012) Genomic insights into the marine sponge microbiome. *Nature Reviews Microbiology* **10**: 641.

Hicks, N., Liu, X., Gregory, R., Kenny, J., Lucaci, A., Lenzi, L. et al. (2018) Temperature driven changes in benthic bacterial diversity Influences biogeochemical cycling in coastal sediments. *Frontiers in Microbiology* **9**: 1730.

Higgins, R.P., and Thiel, H. (1988) Introduction to the study of meiofauna. Smithsonian Institution Press, Washington DC.

Hochard, S., Pinazo, C., Grenz, C., Evans, J.L.B., and Pringault, O. (2010) Impact of microphytobenthos on the sediment biogeochemical cycles: A modeling approach. *Ecological Modelling* **221**: 1687-1701.

Hodda, M. (1990) Variation in estuarine littoral nematode populations over three spatial scales. *Estuarine, Coastal and Shelf Science* **30**: 325-340.

Hoffman, C. (1942) Beitrage zur Vegetation des Farbstreifen-Sandwattes. *Kieler Meeresforsch Sonderheft* **4**: 85-108.

Hooper, D.U., Chapin Iii, F., Ewel, J., Hector, A., Inchausti, P., Lavorel, S. et al. (2005) Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecological Monographs* **75**: 3-35.

Howarth, R.W. (1984) The ecological significance of sulfur in the energy dynamics of salt marsh and coastal marine sediments. *Biogeochemistry* **1**: 5-27.

Hua, E., Zhang, Z.N., Zhou, H., Mu, F.H., Li, J., Zhang, T. et al. (2016) Meiofauna Distribution in Intertidal Sandy Beaches Along China Shoreline (18 degrees-40 degrees N). *Journal of Ocean University of China* **15**: 19-27.

Hubas, C., Sachidhanandam, C., Rybarczyk, H., Lubarsky, H.V., Rigaux, A., Moens, T., and Paterson, D.M. (2010) Bacterivorous nematodes stimulate microbial growth and exopolymer production in marine sediment microcosms. *Marine Ecology Progress Series* **419**: 85-94.

Huttenhower, C., Gevers, D., Knight, R., Abubucker, S., Badger, J.H., Chinwalla, A.T. et al. (2012) Structure, function and diversity of the healthy human microbiome. *Nature* **486**: 207-214.

Iken, K., Brey, T., Wand, U., Voigt, J., and Junghans, P. (2001) Food web structure of the benthic community at the Porcupine Abyssal Plain (NE Atlantic): a stable isotope analysis. *Progress in Oceanography* **50**: 383-405.

Ingels, J., Van den Driessche, P., De Mesel, I., Vanhove, S., Moens, T., and Vanreusel, A. (2010) Preferred use of bacteria over phytoplankton by deep-sea nematodes in polar regions. *Marine Ecology Progress Series* **406**: 121-133.

Jackson, A.L., Inger, R., Parnell, A.C., and Bearhop, S. (2011) Comparing isotopic niche widths among and within communities: SIBER–Stable Isotope Bayesian Ellipses in R. *Journal of Animal Ecology* **80**: 595-602.

Jensen, P. (1987) Feeding ecology of free-living aquatic nematodes. *Marine Ecology Progress Series* **35**: 187-196.

Ji, Y., Ashton, L., Pedley, S.M., Edwards, D.P., Tang, Y., Nakamura, A., Kitching, R., Dolman, P. M., Woodcock, P., Edwards, F. A. (2013) Reliable, verifiable and efficient monitoring of biodiversity via metabarcoding. *Ecology Letters* **16**: 1245-1257.

Joint, I., Gee, J., and Warwick, R. (1982) Determination of fine-scale vertical distribution of microbes and meiofauna in an intertidal sediment. *Marine Biology* **72**: 157-164.

Jordan, S. (2012) Estuaries*:* Classification, Ecology, and Human Impacts. Nova Science Publishers, New York.

Kaiser, M.J., Attrill, M.J., Jennings, S., Thomas, D.N., and Barnes, D.K. (2011) Marine ecology: processes, systems, and impacts. Oxford University Press, Oxford.

Kamada, N., Seo, S.U., Chen, G.Y., and Nunez, G. (2013) Role of the gut microbiota in immunity and inflammatory disease. *Nature Reviews Immunology* **13**: 321-335.

Katju, V., and Bergthorsson, U. (2013) Copy-number changes in evolution: rates, fitness effects and adaptive significance. *Frontiers in Genetics* **4**: 273.

Kelly, J.R., and Scheibling, R.E. (2012) Fatty acids as dietary tracers in benthic food webs. *Marine Ecology Progress Series* **446**: 1-22.

Kennedy, A.D. (1994) Carbon partitioning with meiobenthic nematode communities in the Exe estuary, UK. *Marine Ecology Progress Series* **105**: 71-78.

Kreuzinger-Janik, B., Kruscha, S., Majdi, N., and Traunspurger, W. (2018) Flatworms like it round: nematode consumption by *Planaria torva* (Müller 1774) and *Polycelis tenuis* (Ijima 1884). *Hydrobiologia* **819**: 231-242.

Kromkamp JC, de Brouwer JFC, Blanchard GF, Forster RM, Creach V (2006) Functioning of microphytobenthos in estuaries: Proceedings of the Colloquium, Amsterdam, 21-23 August 2003. Royal Netherlands Academy of Arts and Sciences, Amsterdam.

Kuipers, B., and Dapper, R. (1984) Nursery function of Wadden Sea tidal flats for the brown shrimp Crangon crangon. *Marine Ecology Progress Series Oldendorf* **17**: 171-181.

Kuipers, B., De Wilde, P., and Creutzberg, F. (1981) Energy flow in a tidal flat ecosystem. *Marine Ecology Progress Series* **5**: 215-221.

Lambshead, P.J.D., and Boucher, G. (2003) Marine nematode deep-sea biodiversity hyperdiverse or hype? *Journal of Biogeography* **30**: 475-485.

Langille, M.G.I., Zaneveld, J., Caporaso, J.G., McDonald, D., Knights, D., a Reyes, J., Clemente, J.C., Burkepile, D.E., Vega Thurber, R.L., Knight, R., Beiko, R.G. and Huttenhower, C. (2013) Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nature Biotechnology* **8:** 1-10.

Layman, C.A., Arrington, D.A., Montaña, C.G., and Post, D.M. (2007) Can stable isotope ratios provide for community-wide measures of trophic structure? *Ecology* **88**: 42-48.

Le Hir, P., Roberts, W., Cazaillet, O., Christie, M., Bassoullet, P., and Bacher, C. (2000) Characterization of intertidal flat hydrodynamics. *Continental Shelf Research* **20**: 1433-1459.

Lebreton, B., Richard, P., Galois, R., Radenac, G., Brahmia, A., Colli, G. et al. (2012) Food sources used by sediment meiofauna in an intertidal *Zostera noltii* seagrass bed: a seasonal stable isotope study. *Marine Biology* **159**: 1537-1550.

Leduc, D. (2009) Description of *Oncholaimus moanae* sp nov (Nematoda: Oncholaimidae), with notes on feeding ecology based on isotopic and fatty acid composition. *Journal of the Marine Biological Association of the United Kingdom* **89**: 337-344.

Leduc, D., and Probert, P.K. (2009) The effect of bacterivorous nematodes on detritus incorporation by macrofaunal detritivores: A study using stable isotope and fatty acid analyses. *Journal of Experimental Marine Biology and Ecology* **371**: 130-139.

Leduc, D., Brown, J.C., Bury, S.J., and Lörz, A.-N. (2015) High intraspecific variability in the diet of a deep-sea nematode: Stable isotope and fatty acid analyses of *Deontostoma tridentum* on Chatham Rise, Southwest Pacific. *Deep Sea Research Part I: Oceanographic Research Papers* **97**: 10-18.

Lee, M. R. and M. Riveros, 2012. Latitudinal trends in the species richness of free-living marine nematode assemblages from exposed sandy beaches along the coast of Chile (18-42°S). *Marine Ecology-an Evolutionary Perspective* **33(3)**:317-325.

Levin, S.A. (1992) The problem of pattern and scale in ecology: the Robert H. MacArthur award lecture. *Ecology* **73**: 1943-1967.

Li, J., Vincx, M., and Herman, P.M.J. (1997) Carbon flows through meiobenthic nematodes in the Westerschelde Estuary. *Fundamental and Applied Nematology* **20**: 487-494.

Loreau, M., Naeem, S., and Inchausti, P. (2002) Biodiversity and ecosystem functioning: synthesis and perspectives. Oxford University Press, Oxford.

Lorenzen, S., Prein, M., and Valentin, C. (1987) Mass aggregations of the free-living marine nematode *Pontonema vulgare* (Oncholaimidae) in organically polluted fjords. *Marine Ecology Progress Series*: 27-34.

Lozupone, C., Lladser, M.E., Knights, D., Stombaugh, J., and Knight, R. (2011) UniFrac: an effective distance metric for microbial community comparison. *The ISME journal* **5**: 169.

MacIntyre, H.L., Geider, R.J., and Miller, D.C. (1996) Microphytobenthos: the ecological role of the "secret garden" of unvegetated, shallow-water marine habitats. I. Distribution, abundance and primary production. *Estuaries* **19**: 186-201.

Majdi, N., Weber, S., and Traunspurger, W. (2018) The early catfish catches the worm: predation of *Corydoras aeneus* (Siluriformes, Callichthyidae) on freshwater nematodes. In *Annales de Limnologie-International Journal of Limnology*: EDP Sciences, p. 29.

Maria, T.F., De Troch, M., Vanaverbeke, J., Esteves, A.M., and Vanreusel, A. (2011) Use of benthic vs planktonic organic matter by sandy-beach organisms: A food tracing experiment with C^{13} labelled diatoms. *Journal of Experimental Marine Biology and Ecology* **407**: 309-314.

Maria, T.F., Vanaverbeke, J., Gingold, R., Esteves, A.M., and Vanreusel, A. (2013) Tidal exposure or microhabitats: what determines sandy-beach nematode zonation? a case study of a macrotidal ridge-and-runnel sandy beach in Belgium. *Marine Ecology-an Evolutionary Perspective* **34**: 207-217.

Martin, M. (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet Journal* **17**: 10-12.

Mascart, T., De Troch, M., Remy, F., Michel, L.N., and Lepoint, G. (2018) Seasonal dependence on seagrass detritus and trophic niche partitioning in four copepod eco-morphotypes. *Food Webs* **16**: e00086.

Masood, A., Stark, K.D., and Salem, N. (2005) A simplified and efficient method for the analysis of fatty acid methyl esters suitable for large clinical studies. *Journal of Lipid Research* **46**: 2299-2305.

McCutchan, J.H., Lewis, W.M., Kendall, C., and McGrath, C.C. (2003) Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos* **102**: 378-390.

McCall, J., and Fleeger, J. (1995) Predation by juvenile fish on hyperbenthic meiofauna: a review with data on post-larval *Leiostomus xanthurus*. *Oceanographic Literature Review* **10**: 879.

McIntyre, A., and Murison, D. (1973) The meiofauna of a flatfish nursery ground. *Journal of the Marine Biological Association of the United Kingdom* **53**: 93-118.

McLachlan, A., and Jaramillo, E. (1996) Zonation on sandy beaches. *Oceanographic Literature Review* **12**: 1247.

McLachlan, A., DeRuyck, A., and Hacking, N. (1996) Community structure on sandy beaches: Patterns of richness and zonation in relation to tide range and latitude. *Revista Chilena De Historia Natural* **69**: 451-467.

McLusky, D.S. (1999) Estuarine benthic ecology: A European perspective. *Australian Journal of Ecology* **24**: 302-311.

McMurdie, P.J., and Holmes, S. (2013) phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One* **8**: e61217.

Meire, P., Ysebaert, T., Damme, S.V., Bergh, E.V.d., Maris, T., and Struyf, E. (2005) The Scheldt estuary: a description of a changing ecosystem. *Hydrobiologia* **540**: 1-11.

Mensens, C., De Laender, F., Janssen, C.R., Rivera, F.C., Sabbe, K., and De Troch, M. (2018) Selective and context-dependent effects of chemical stress across trophic levels at the basis of marine food webs. *Ecological Applications* **28**: 1342-1353.

Meyers, S., Hopper, B., and Cefalu, R. (1970) Ecological investigations of the marine nematode Metoncholaimus scissus. *Marine Biology* **6**: 43-47.

Middelburg, J.J., Barranguet, C., Boschker, H.T.S., Herman, P.M.J., Moens, T., and Heip, C.H.R. (2000) The fate of intertidal microphytobenthos carbon: An *in situ* C^{13}-labeling study. *Limnology and Oceanography* **45**: 1224-1234.

Middelburg, J.J., Soetaert, K., Herman, P.M., and Heip, C.H. (1996) Denitrification in marine sediments: A model study. *Global Biogeochemical Cycles* **10**: 661-673.

Minagawa, M., and Wada, E. (1984) Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between δ^{15}N and animal age. *Geochimica et Cosmochimica Acta* **48**: 1135-1140.

Miththapala, S. (2013) Tidal Flats. In *Coastal Ecosystems Series*. IUCN:Sri Lanka, Colombo.

Mitreva, M., Jasmer, D.P., Zarlenga, D.S., Wang, Z., Abubucker, S., Martin, J. et al. (2011) The draft genome of the parasitic nematode *Trichinella spiralis*. *Nature Genetics* **43**: 228.

Moens T, and Beninger P. (2018) Meiofauna – an inconspicuous but important player in mudflat ecology. In: Beninger P (Ed.), *Mudflat ecology*. Berlin, Springer, in press.

Moens, T., and Vincx, M. (1997) Observations on the feeding ecology of estuarine nematodes. *Journal of the Marine Biological Association of the United Kingdom* **77**: 211-227.

Moens, T., and Vincx, M. (2000) Temperature, salinity and food thresholds in two brackish-water bacterivorous nematode species: assessing niches from food absorption and respiration experiments. *Journal of Experimental Marine Biology and Ecology* **243**: 137-154.

Moens, T., Bouillon, S., and Gallucci, F. (2005a) Dual stable isotope abundances unravel trophic position of estuarine nematodes. *Journal of the Marine Biological Association of the United Kingdom* **85**: 1401-1407.

Moens, T., Braeckman U, Derycke S, Fonseca G, Gallucci F, Gingold R et al. (2013) Ecology of free-living marine nematodes. In *Schmidt-Rhaesa A (ed) Handbook of Zoology.* De Gruyter, Berlin.

Moens, T., dos Santos, G.A.P., Thompson, F., Swings, J., Fonseca-Genevois, V., Vincx, M., and De Mesel, I. (2005b) Do nematode mucus secretions affect bacterial growth? *Aquatic Microbial Ecology* **40**: 77-83.

Moens, T., Herman, P., Verbeeck, L., Steyaert, M., and Vincx, M. (2000) Predation rates and prey selectivity in two predacious estuarine nematode species. *Marine Ecology Progress Series* **205**: 185-193.

Moens, T., Luyten, C., Middelburg, J.J., Herman, P.M.J., and Vincx, M. (2002) Tracing organic matter sources of estuarine tidal flat nematodes with stable carbon isotopes. *Marine Ecology Progress Series* **234**: 127-137.

Moens, T., Vafeiadou, A.-M., De Geyter, E., Vanormelingen, P., Sabbe, K., and De Troch, M. (2014) Diatom feeding across trophic guilds in tidal flat nematodes, and the importance of diatom cell size. *Journal of Sea Research* **92**: 125-133.

Moens, T., Van Gansbeke, D., and Vincx, M. (1999) Linking estuarine nematodes to their suspected food. A case study from the Westerschelde Estuary (south-west Netherlands). *Journal of the Marine Biological Association of the United Kingdom* **79**: 1017-1027.

Moens, T., Vanhove, S., De Mesel, I., Kelemen, B., Janssens, T., Dewicke, A., and Vanreusel, A. (2007) Carbon sources of Antarctic nematodes as revealed by natural carbon isotope ratios and a pulse-chase experiment. *Polar Biology* **31**: 1-13.

Moens, T., Verbeeck, L., and Vincx, M. (1999a) Feeding biology of a predatory and a facultatively predatory nematode (*Enoploides longispiculosus* and *Adoncholaimus fuscus*). *Marine Biology* **134**: 585-593.

Moens, T., Verbeeck, L., de Maeyer, A., Swings, J., and Vincx, M. (1999b) Selective attraction of marine bacterivorous nematodes to their bacterial food. *Marine Ecology Progress Series* **176**: 165-178.

Moens, T., Yeates, G.W., and De Ley, P. (2004) Use of carbon and energy sources by nematodes. In: Cook, R.C., and Hunt, D.J. (eds). Proceeding of the Fourth International Congress of Nematology. p529-545.

Mokievsky, V., and Azovsky, A. (2002) Re-evaluation of species diversity patterns of free-living marine nematodes. *Marine Ecology Progress Series* **238**: 101-108.

Montagna, P.A., Coull, B.C., Herring, T.L., and Dudley, B.W. (1983) The relationship between abundances of meiofauna and their suspected microbial food (diatoms and bacteria). *Estuarine, Coastal and Shelf Science* **17**: 381-394.

Monteiro, L.C., Van Butsel, J., De Meester, N., Traunspurger, W., Derycke, S., and Moens, T. (2018) Differential heavy-metal sensitivity in two cryptic species of the marine nematode *Litoditis marina* as revealed by developmental and behavioural assays. *Journal of Experimental Marine Biology and Ecology* **502**: 203-210.

Musat, N., Giere, O., Gieseke, A., Thiermann, F., Amann, R., and Dubilier, N. (2007) Molecular and morphological characterization of the association between bacterial endosymbionts and the marine nematode Astomonema sp. from the Bahamas. *Environmental Microbiology* **9**: 1345-1353.

Mutchler, T., Sullivan, M.J., and Fry, B. (2004) Potential of 14N isotope enrichment to resolve ambiguities in coastal trophic relationships. *Marine Ecology Progress Series* **266**: 27-33.

Nascimento, F.J., Näslund, J., and Elmgren, R. (2012) Meiofauna enhances organic matter mineralization in soft sediment ecosystems. *Limnology and Oceanography* **57**: 338-346.

Nehring, S. (1991) Der Röhrenbau: Eine neuentdeckte, erfolgreiche Lebensweise bei den Nematoden. *Mikrokosmos* **80**: 134-138.

Nehring, S., Jensen, P., and Lorenzen, S. (1990) Tube-dwelling nematodes: tube construction and possible ecological effects on sediment-water interfaces. *Marine Ecology, Progress Series (Halstenbek)* **64**: 123-128.

Netto, S.A., and Meneghel, A. (2014) Pulse of marine subsidies: the role of surf diatom Asterionellopsis glacialis accumulations in structuring the meiofauna of sandy beaches. *Marine Biodiversity* **44**: 445-457.

Neubauer, P., and Jensen, O.P. (2015) Bayesian estimation of predator diet composition from fatty acids and stable isotopes. *PeerJ* **3**: e920.

Nicholas, W.L., and Hodda, M. (1999) The free-living nematodes of a temperate, high energy, sandy beach: faunal composition and variation over space and time. *Hydrobiologia* **394**: 113-127.

Ott, J., Novak, R., Schiemer, F., . Hentschel, U., Nebelsick, M., and Polz, M. (1991) Tackling the sulfide gradient: a novel strategy involving marine nematodes and chemoautotrophic ectosymbionts. *Marine Ecology* **12**: 261-279.

Pace, M.C., and Carman, K.R. (1996) Interspecific differences among meiobenthic copepods in the use of microalgal food resources. *Marine Ecology Progress Series* **143**: 77-86.

Parrish, C., Abrajano, T., Budge, S., Helleur, R., Hudson, E., Pulchan, K., and Ramos, C. (2000) Lipid and phenolic biomarkers in marine ecosystems: analysis and applications. In *Marine Chemistry.* Springer, Berlin.

Parrish, C.C., McKenzie, C., MacDonald, B., and Hatfield, E. (1995) Seasonal studies of seston lipids in relation to microplankton species composition and scallop growth in South Broad Cove, Newfoundland. *Marine Ecology Progress Series* **129**: 151-164.

Pascal, P.Y., Fleeger, J.W., Boschker, H.T.S., Mitwally, H.M., and Johnson, D.S. (2013) Response of the benthic food web to short- and long-term nutrient enrichment in saltmarsh mudflats. *Marine Ecology Progress Series* **474**: 27-41.

Pascal, P.Y., Dupuy, C., Richard, P., Rzeznik-Orignac, J., and Niquil, N. (2008) Bacterivory of a mudflat nematode community under different environmental conditions. *Marine Biology* **154**: 671-682.

Passarelli, C., Olivier, F., Paterson, D.M., and Hubas, C. (2012) Impacts of biogenic structures on benthic assemblages: microbes, meiofauna, macrofauna and related ecosystem functions. *Marine Ecology Progress Series* **465**: 85-97.

Passarelli, C., Olivier, F., Paterson, D.M., Meziane, T., and Hubas, C. (2014) Organisms as cooperative ecosystem engineers in intertidal flats. *Journal of Sea Research* **92**: 92-101.

Paterson, D., and Black, K. (1999) Water flow, sediment dynamics and benthic biology. *Advances in Ecological Research* **29**: 155-193.

Peters, K., Walters, C., and Moldowan, J. (2005) The biomarker guide. Cambridge University Press, Cambridge.

Peterson, B.J., and Fry, B. (1987) Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics* **18**: 293-320.

Pinckney, J., and Sandulli, R. (1990) Spatial autocorrelation analysis of meiofaunal and microalgal populations on an intertidal sandflat: scale linkage between consumers and resources. *Estuarine, Coastal and Shelf Science* **30**: 341-353.

Platt, H., and Warwick, R. (1980) The significance of free-living nematodes to the littoral ecosystem. In: Irvine DEG, Farn-ham WF (eds). In *The shore environment*. Vol 2: Ecosystems. Academic Press, Cambridge.

Platt, H.M., and Warwick, R.M. (1983) A synopsis of the freeliving marine nematodes. Part 1: British Enoplids. Cambridge University Press, Cambridge.

Polz, M.F., Felbeck, H., Novak, R., Nebelsick, M., and Ott, J.A. (1992) Chemoautotrophic, sulfur-oxidizing symbiotic bacteria on marine nematodes: morphological and biochemical characterization. *Microbial Ecology* **24**: 313-329.

Post, D.M. (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* **83**: 703-718.

Post, D.M., Pace, M.L., and Hairston Jr, N.G. (2000) Ecosystem size determines food-chain length in lakes. *Nature* **405**: 1047.

Post, K., Louwye, S., and Lambert, O. (2017) Scaldiporia vandokkumi, a new pontoporiid (Mammalia, Cetacea, Odontoceti) from the Late Miocene to earliest Pliocene of the Westerschelde estuary (The Netherlands). *PeerJ* **5**: e3991.

Powers, T. (2004) Nematode molecular diagnostics: from bands to barcodes. *Annual Review of Phytopathology* **42**: 367-383.

Pritchard, D.W. (1967) What is an estuary: physical viewpoint. In Lauff GH (ed). *Estuaries*. American Association for the Advancement of Science, Washington.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P. et al. (2012) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research* **41**: D590-D596.

R Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Viennva, Austria. URL http://www.R-project.org/.

Raffaelli, D., and Hawkins, S.J. (1999) Intertidal ecology. Kluwer Academic Publishers, Dordrecht.

Riemann, F., and Helmke, E. (2002) Symbiotic relations of sediment-agglutinating nematodes and bacteria in detrital habitats: The enzyme-sharing concept. *Marine Ecology-Pubblicazioni Della Stazione Zoologica Di Napoli I* **23**: 93-113.

Riemann, F., and Schrage, M. (1978) The mucus-trap hypothesis on feeding of aquatic nematodes and implications for biodegradation and sediment texture. *Oecologia* **34**: 75-88.

Rodil, I.F., Lastra, M., and Lopez, J. (2007) Macroinfauna community structure and biochemical composition of sedimentary organic matter along a gradient of wave exposure in sandy beaches (NW Spain). *Hydrobiologia* **579**: 301-316.

Rodriguez, J.G., Lopez, J., and Jaramillo, E. (2001) Community structure of the intertidal meiofauna along a gradient of morphodynamic sandy beach types in southern Chile. *Revista Chilena De Historia Natural* **74**: 885-897.

Romeyn, K., and Bouwman, L. (1983) Food selection and consumption by estuarine nematodes. *Hydrobiological Bulletin* **17**: 103-109.

Rudnick, D.T. (1989) Time lags between the deposition and meiobenthic assimilation of phytodetritus. *Marine Ecology Progress Series Oldendorf* **50**: 231-240.

Rusch, A., Forster, S., and Huettel, M. (2001) Bacteria, diatoms and detritus in an intertidal sandflat subject to advective transport across the water-sediment interface. *Biogeochemistry* **55**: 1-27.

Rzeznik-Orignac, J., Boucher, G., Fichet, D., and Richard, P. (2008) Stable isotope analysis position of intertidal of food source and trophic nematodes and copepods. *Marine Ecology Progress Series* **359**: 145-150.

Rzeznik-Orignac, J., Fichet, D., and Boucher, G. (2004) Extracting massive numbers of nematodes from muddy marine deposits: efficiency and selectivity. *Nematology* **6**: 605-616.

Rzeznik-Orignac, J., Kalenitchenko, D., Mariette, J., Bodiou, J.-Y., Le Bris, N., and Derelle, E. (2017) Comparison of meiofaunal diversity by combined morphological and molecular approaches in a shallow Mediterranean sediment. *Marine Biology* **164**: 40.

Sabbe, K., and Vyverman, W. (1991) Distribution of benthic diatom assemblages in the Westerschelde (Zeeland, The Netherlands). *Belgian Journal of Botany* **124(2)**: 91-101.

Sahraean, N., Bezerra, T.C., Khanaghah, K.E., Mosallanejad, H., Van Ranst, E., and Moens, T. (2017) Effects of pollution on nematode assemblage structure and diversity on beaches of the northern Persian Gulf. *Hydrobiologia* **799**: 349-369.

Saidi, I., Essid, N., Boufahja, F., Nasri, A., Hannachi, A., Nefzi, B., and Beyrem, H. (2017) The effects of raw effluents from pulp and paper industry from Tunisia on marine nematodes: a microcosm bioassay. *Cahiers De Biologie Marine* **58**: 387-395.

Samuel, B.S., Rowedder, H., Braendle, C., Félix, M.-A., and Ruvkun, G. (2016) *Caenorhabditis elegans* responses to bacteria from its natural habitats. *Proceedings of the National Academy of Sciences* **113**: E3941-E3949.

Schiemer, F., Novak, R., and Ott, J. (1990) Metabolic studies on thiobiotic free-living nematodes and their symbiotic microorganisms. *Marine Biology* **106**: 129-137.

Schmidt, V.T., Smith, K.F., Melvin, D.W., and Amaral ‐ Zettler, L.A. (2015) Community assembly of a euryhaline fish microbiome during salinity acclimation. *Molecular Ecology* **24**: 2537-2550.

Scholz, D.S., Matthews, L.L., and Feller, R.J. (1991) Detecting selective digestion of meiobenthic prey by juvenile spot *Leiostomus xanthurus* (Pisces) using immunoassays. *Marine Ecology Progress Series* **72(1)**: 59-67.

Schratzberger, M., and Ingels, J. (2018) Meiofauna matters: the roles of meiofauna in benthic ecosystems. *Journal of Experimental Marine Biology and Ecology* **502**: 12-25.

Schratzberger, M., Warr, K., and Rogers, S. (2007) Functional diversity of nematode communities in the southwestern North Sea. *Marine Environmental Research* **63**: 368-389.

Schrider, D.R., and Hahn, M.W. (2010) Gene copy-number polymorphism in nature. *Proceedings of the Royal Society B: Biological Sciences* **277**: 3213-3221.

Schuelke, T., Pereira, T.J., Hardy, S.M., and Bik, H.M. (2018) Nematode - associated microbial taxa do not correlate with host phylogeny, geographic region or feeding morphology in marine sediment habitats. *Molecular Ecology* **27**: 1930-1951.

Schulenburg, H., and Félix, M.-A. (2017) The natural biotic environment of *Caenorhabditis elegans*. *Genetics* **206**: 55-86.

Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W.S., and Huttenhower, C. (2011) Metagenomic biomarker discovery and explanation. *Genome Biology* **12**: R60.

Seinhorst, J. (1959) A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. *Nematologica* **4**: 67-69.

Shoemaker, K.M., and Moisander, P.H. (2017) Seasonal variation in the copepod gut microbiome in the subtropical North Atlantic Ocean. *Environmental Microbiology* **19(8)**: 3087-3097.

Sinnathamby, G., Henderson, G., Umair, S., Janssen, P., Bland, R., and Simpson, H. (2018) The bacterial community associated with the sheep gastrointestinal nematode parasite Haemonchus contortus. *PLoS One* **13(3)** :e0194378.

Sison-Mangus, M.P., Jiang, S., Tran, K.N., and Kudela, R.M. (2014) Host-specific adaptation governs the interaction of the marine diatom, Pseudo-nitzschia and their microbiota. *The ISME Journal* **8**: 63-76.

Snyder, R.E., and Chesson, P. (2003) Local dispersal can facilitate coexistence in the presence of permanent spatial heterogeneity. *Ecology Letters* **6**: 301-309.

Soetaert, K., Herman, P.M., and Kromkamp, J. (1994) Living in the twilight: estimating net phytoplankton growth in the Westerschelde estuary (The Netherlands) by means of an ecosystem model (MOSES). *Journal of Plankton Research* **16**: 1277-1301.

Soetaert, K., Muthumbi, A., and Heip, C. (2002) Size and shape of ocean margin nematodes: morphological diversity and depth-related patterns. *Marine Ecology Progress Series* **242**: 179-193.

Somerfield, P.J., Dashfield, S.L., and Warwick, R.M. (2007) Three-dimensional spatial structure: nematodes in a sandy tidal flat. *Marine Ecology Progress Series* **336**: 177-186.

Somerfield PJ, Warwick RM, and Moens M. 2005. Meiofauna techniques. In: *Methods for the study of marine benthos* (3rd Ed.), Blackwell Science Ltd, Malden.

Stal, L.J. (2010) Microphytobenthos as a biogeomorphological force in intertidal sediment stabilization. *Ecological Engineering* **36**: 236-245.

Steyaert, M. (2003) Spatial and temporal scales of nematode communities in the North Sea and Westerschelde. Dissertation, University of Ghent.

Steyaert, M., Herman, P.M.J., Moens, T., Widdows, J., and Vincx, M. (2001) Tidal migration of nematodes on an estuarine tidal flat (the Molenplaat, Schelde Estuary, SW Netherlands). *Marine Ecology Progress Series* **224**: 299-304.

Steyaert, M., Moodley, L., Nadong, T., Moens, T., Soetaert, K., and Vincx, M. (2007) Responses of intertidal nematodes to short-term anoxic events. *Journal of Experimental Marine Biology and Ecology* **345**: 175-184.

Steyaert, M., Vanaverbeke, J., Vanreusel, A., Barranguet, C., Lucas, C., and Vincx, M. (2003) The importance of fine-scale, vertical profiles in characterising nematode community structure. *Estuarine, Coastal and Shelf Science* **58**: 353-366.

Stock, W., Heylen, K., Sabbe, K., Willems, A., and De Troch, M. (2014) Interactions between benthic copepods, bacteria and diatoms promote nitrogen retention in Intertidal marine sediments. *PLoS One* **9(10)** :e111001.

Sturgeon, A., Pinder, S., Costa, M., and Weese, J. (2014) Characterization of the oral microbiota of healthy cats using next-generation sequencing. *The Veterinary Journal* **201**: 223-229.

Sudhaus, W. (2011) Phylogenetic systematisation and catalogue of paraphyletic" Rhabditidae"(Secernentea, Nematoda). *Journal of Nematode Morphology and Systematics* **14**: 113-178.

Taberlet, P., Coissac, E., Pompanon, F., Brochmann, C., and Willerslev, E. (2012) Towards next-generation biodiversity assessment using DNA metabarcoding. *Molecular Ecology* **21**: 2045-2050.

Taipale, S., Strandberg, U., Peltomaa, E., Galloway, A.W., Ojala, A., and Brett, M.T. (2013) Fatty acid composition as biomarkers of freshwater microalgae: analysis of 37 strains of microalgae in 22 genera and in seven classes. *Aquatic Microbial Ecology* **71**: 165-178.

Thistle, D., and Sedlacek, L. (2004) Emergent and non-emergent species of harpacticoid copepods can be recognized morphologically. *Marine Ecology Progress Series* **266**: 195-200.

Thomas, M.C., and Lana, P.C. (2011) A new look into the small-scale dispersal of free-living marine nematodes. *Zoologia (Curitiba)* **28**: 449-456.

Tietjen, J.H., and Lee, J.J. (1973) Life history and feeding habits of the marine nematode, Chromadora macrolaimoides Steiner. *Oecologia* **12**: 303-314.

Traunspurger, W. (2000) The biology and ecology of lotic nematodes. *Freshwater Biology* **44**: 29-45.

Tremaroli, V., and Backhed, F. (2012) Functional interactions between the gut microbiota and host metabolism. *Nature* **489**: 242-249.

Turnbaugh, P.J., and Gordon, J.I. (2008) An invitation to the marriage of metagenomics and metabolomics. *Cell* **134**: 708-713.

Turnbaugh, P.J., Ley, R.E., Hamady, M., Fraser-Liggett, C.M., Knight, R., and Gordon, J.I. (2007) The human microbiome project. *Nature* **449**: 804.

Turner, J.T. (2002) Zooplankton fecal pellets, marine snow and sinking phytoplankton blooms. *Aquatic Microbial Ecology* **27**: 57-102.

Underwood, A., Chapman, M., and Connell, S. (2000) Observations in ecology: you can't make progress on processes without understanding the patterns. *Journal of Experimental Marine Biology and Ecology* **250**: 97-115.

Underwood, G., and Kromkamp, J. (1999) Primary production by phytoplankton and microphytobenthos in estuaries. *Advances in Ecological Research* **29**: 93-153.

Urban-Malinga, B., and Moens, T. (2006) Fate of organic matter in Arctic intertidal sediments: Is utilisation by meiofauna important? *Journal of Sea Research* **56**: 239-248.

Urkmez, D., Brennan, M.L., Sezgin, M., and Bat, L. (2015) A brief look at the free-living Nematoda of the oxic/anoxic interface with a new genus record (*Trefusia*) for the Black Sea. *Oceanological and Hydrobiological Studies* **44**: 539-551.

Uye, S.-i. (2011) Human forcing of the copepod–fish–jellyfish triangular trophic relationship. *Hydrobiologia* **666**: 71-83.

Vafeiadou, A.-M., Materatski, P., Adão, H., De Troch, M., and Moens, T. (2014) Resource utilization and trophic position of nematodes and harpacticoid copepods in and adjacent to *Zostera noltii* beds. *Biogeosciences* **11**: 4001-4014.

Valiela, I. (1984) Marine ecological processes. Springer-Verlag, New York.

Van Campenhout, J., and Vanreusel, A. (2016) Closely related intertidal and deep-sea *Halomonhystera* species have distinct fatty acid compositions. *Helgoland Marine Research* **70**: 8.

Van Colen, C., Underwood, G.J., Serôdio, J., and Paterson, D.M. (2014) Ecology of intertidal microbial biofilms: Mechanisms, patterns and future research needs. *Journal of Sea Research* **92**: 2-5.

Van De Koppel, J., Herman, P.M.J., Thoolen, P., and Heip, C.H.R. (2001) Do alternate stable states occur in natural ecosystems? Evidence from a tidal flat. *Ecology* **82**: 3449-3461.

Van Gaever, S., Moodley, L., Pasotti, F., Houtekamer, M., Middelburg, J.J., Danovaro, R., and Vanreusel, A. (2009) Trophic specialisation of metazoan meiofauna at the Håkon Mosby Mud Volcano: fatty acid biomarker isotope evidence. *Marine Biology* **156**: 1289-1296.

van Oevelen, D., Middelburg, J.J., Soetaert, K., and Moodley, L. (2006a) The fate of bacterial carbon in an intertidal sediment: Modeling an *in situ* isotope tracer experiment. *Limnology and Oceanography* **51**: 1302-1314.

Van Oevelen, D., Soetaert, K., Middelburg, J.J., Herman, P.M., Moodley, L., Hamels, I. et al. (2006b) Carbon flows through a benthic food web: Integrating biomass, isotope and tracer data. *Journal of Marine Research* **64**: 453-482.

Vanaverbeke, J., Franco, M., Van Oevelen, D., Leon, M., Provoost, P., Steyaert, M. et al. (2008) Benthic responses to sedimentation of phytoplankton on the Belgian Continental Shelf. In Rousseau V, Lancelot C, Cox D, (eds). *Current Status of Eutrophication in the Belgian Coastal Zone*. Presses Universitaires de Bruxelles, Brussels.

Vanaverbeke, J., Merckx, B., Degraer, S., and Vincx, M. (2011) Sediment-related distribution patterns of nematodes and macrofauna: Two sides of the benthic coin? *Marine Environmental Research* **71**: 31-40.

Vanaverbeke, J., Soetaert, K., and Vincx, M. (2004) Changes in morphometric characteristics of nematode communities during a spring phytoplankton bloom deposition. *Marine Ecology Progress Series* **273**: 139-146.

Vandamme, P., Opelt, K., Knöchel, N., Berg, C., Schönmann, S., De Brandt, E. et al. (2007) *Burkholderia bryophila* sp. nov. and *Burkholderia megapolitana* sp. nov., moss-associated species with antifungal and plant-growth-promoting properties. *International Journal of Systematic and Evolutionary Microbiology* **57**: 2228-2235.

Vander Zanden, M.J., and Rasmussen, J.B. (2001) Variation in $\delta^{15}N$ and $\delta^{13}C$ trophic fractionation: implications for aquatic food web studies. *Limnology and Oceanography* **46**: 2061-2066.

Vanfleteren, J. (1980) Nematodes as nutritional models. In: Zuckerman BM (ed) *Nematodes as biological models*, Vol 2, p47-79. Academic Press, New York.

vanTamelen, P.G. (1996) Algal zonation in tidepools: Experimental evaluation of the roles of physical disturbance, herbivory and competition. *Journal of Experimental Marine Biology and Ecology* **201**: 197-231.

Vecchi, M., Newton, I.L., Cesari, M., Rebecchi, L., and Guidetti, R. (2018) The Microbial Community of Tardigrades: Environmental Influence and Species Specificity of Microbiome Structure and Composition. *Microbial Ecology* **76(2)**: 467-481.

Vredenburg, V.T., Briggs, C., and Harris, R. (2011) Host-pathogen dynamics of amphibian chytridiomycosis: the role of the skin microbiome in health and disease. The National Academies Press, Washington.

Vieira, D.C., and Fonseca, G. (2013) The Importance of Vertical and Horizontal Dimensions of the Sediment Matrix in Structuring Nematodes Across Spatial Scales. *PLoS One* **8 (10)**: e77704.

Violle, C., Enquist, B.J., McGill, B.J., Jiang, L., Albert, C.H., Hulshof, C. et al. (2012) The return of the variance: intraspecific variability in community ecology. *Trends in Ecology & Evolution* **27**: 244-252.

Voulgaris, G. (1999) Sedimentary Processes in the Intertidal Zone. *Estuaries* **22(3)**: 728-730.

Vranken, G., and Heip, C. (1986) The productivity of marine nematodes. *Ophelia* **26**: 429-442.

Warwick, R.M., Platt, H.M., and Somerfield, P.J. (1998) Freeliving marine nematodes: part III. Monhysterida. Synopses of the British Fauna no. 53. Field Studies Council, Shrewsbury.

Wasmuth, J., Schmid, R., Hedley, A., and Blaxter, M. (2008) On the extent and origins of genic novelty in the phylum Nematoda. *PLoS neglected tropical diseases* **2**: e258.

Watermann, F., Hillebrand, H., Gerdes, G., Krumbein, W.E., and Sommer, U. (1999) Competition between benthic cyanobacteria and diatoms as influenced by different grain sizes and temperatures. *Marine Ecology Progress Series* **187**: 77-87.

Weber, S., and Traunspurger, W. (2013) Food choice of two bacteria-feeding nematode species dependent on food source, food density and interspecific competition. *Nematology* **15**: 291-301.

Weber, S., and Traunspurger, W. (2016) Influence of the ornamental red cherry shrimp *Neocaridina davidi* (Bouvier, 1904) on freshwater meiofaunal assemblages. *Limnologica-Ecology and Management of Inland Waters* **59**: 155-161.

Weiss, S., Xu, Z.Z., Peddada, S., Amir, A., Bittinger, K., Gonzalez, A. et al. (2017) Normalization and microbial differential abundance strategies depend upon data characteristics. *Microbiome* **5**: 27.

Wentworth, C.K. (1922) A scale of grade and class terms for clastic sediments. *The Journal of Geology* **30**: 377-392.

Widdows, J., and Brinsley, M. (2002) Impact of biotic and abiotic processes on sediment dynamics and the consequences to the structure and functioning of the intertidal zone. *Journal of Sea Research* **48**: 143-156.

Wieser, W. (1953) Die Beziehungen zwichen Mundhohlengestalt, Ernahrungsweise und Vorkommen bei freilebenden marinen Nematoden. *Arkiv for Zoologi (Ser 2)* **4**: 439-484.

Williams, B., Schrank, B., Huynh, C., Shownkeen, R., and Waterston, R. (1992) A genetic mapping system in *Caenorhabditis elegans* based on polymorphic sequence-tagged sites. *Genetics* **131**: 609-624.

Wright, S., and Jeffrey, S. (1997) High-resolution HPLC system for chlorophylls and carotenoids of marine phytoplankton. In Jeffrey S, Mantoura R, Wright S. (eds). *Phytoplankton pigments in oceanography: guidelines to modern methods*. UNESCO, Paris.

Yeates, G.W., Ferris, H., Moens, T., and Van der Putten, W. (2009) The role of nematodes in ecosystems. In Wilson MJ, Kakouli-Duarte T. (eds). *Nematodes as Environmental Bioindicators*. CAB International, Wallingford.

Yoder, M., De Ley, I.T., King, I.W., Mundo-Ocampo, M., Mann, J., Blaxter, M. et al. (2006) DESS: a versatile solution for preserving morphology and extractable DNA of nematodes. *Nematology* **8**: 367-376.

Ysebaert, T., Fettweis, M., Meire, P., and Sas, M. (2005) Benthic variability in intertidal soft-sediments in the mesohaline part of the Schelde estuary. *Hydrobiologia* **540**: 197-216.

Yu, X.-J., and Walker, D.H. (2006) The order rickettsiales. In *The prokaryotes*. Springer, Singapore.

Zhang, F., Berg, M., Dierking, K., Félix, M.-A., Shapira, M., Samuel, B.S., and Schulenburg, H. (2017) *Caenorhabditis elegans* as a model for microbiome research. *Frontiers in Microbiology* **8**: 485.

Zhang, J., Kobert, K., Flouri, T., and Stamatakis, A. (2014) PEAR: a fast and accurate Illumina Paired-End reAd mergeR. *Bioinformatics* **30**: 614-620.

Zimmermann, J., Wentrup, C., Sadowski, M., Blazejak, A., Gruber-Vodicka, H.R., Kleiner, M. et al. (2016) Closely coupled evolutionary history of ecto- and endosymbionts from two distantly related animal phyla. *Molecular Ecology* **25**: 3203-3223.