Experimental elucidation of meiofaunal trophic interactions: From radioactive tracer techniques to next generation sequencing

Tom Moens, Nele De Meester, Katja Guilini, Anna-Maria Vafeiadou, Ann Vanreusel and Sofie Derycke

Elucidation of meiofaunal trophic interactions: Introduction

Meiofauna play also a key ecological role in linking detrital (and prokaryotic) resources with higher trophic levels: in fact most of the meiofaunal taxa eat microalgae, prokaryotes and detritus and, at the same time, it is known that meiofauna are a food source for macrofauna and fishes. Meiofauna and nematodes, based on laboratory and in situ experiments, are in fact able to influence microbial activities and to graze their production.
Pusceddu et al. (2014)

Meiofauna are ubiquitous in marine soft-sediment communities, and are an important link in transferring carbon primary and secondary production to higher trophic levels.
Baguley et al. (2008)

Grazers can affect bacterial communities at different levels. They are able to influence bacterial activity, either stimulatory or inhibitory. This can be a direct effect of grazing, but bioturbation and secretion of mucus trails by nematodes can also be important. Grazing impacts on the bacterial community structure have also been reported.
De Mesel et al. (2004)

Although their biomass is generally low, their high abundance and high metabolic and reproductive rates render them potentially important in benthic fluxes of carbon and nutrients (Kuipers et al., 1981, Coull, 1999).
Moens et al. (2005)

Elucidation of meiofaunal trophic interactions: Introduction

"Meiofauna matters: the roles of meiofauna in benthic ecosystems

(Schratzberger & Ingels, keynote at this conference)

Why?

- *non-trophic effects and interactions*
- *direct and indirect trophic interactions*

"Meiofauna people are fond of arm-waving to make speculations about how important meiofauna may be."

(anonymous reviewer, 2005)

Elucidation of meiofaunal trophic interactions: Introduction

Meiofauna play also a key ecological role in linking detrital (and prokaryotic) resources with higher trophic levels: in fact most of the meiofaunal taxa eat microalgae, prokaryotes and detritus and, at the same time, it is known that meiofauna are a food source for macrofauna and fishes. Meiofauna and nematodes, based on laboratory and in situ experiments, are in fact able to influence microbial activities and to graze their production.
Pusceddu et al. (2014)

Meiofauna are ubiquitous in marine soft-sediment communities, and are an important link in transferring carbon primary and secondary production to higher trophic levels.
Baguley et al. (2008)

Grazers can affect bacterial communities at different levels. They are able to influence bacterial activity, either stimulatory or inhibitory. This can be a direct effect of grazing, but bioturbation and secretion of mucus trails by nematodes can also be important. Grazing impacts on the bacterial community structure have also been reported.
De Mesel et al. (2004)

Although their biomass is generally low, their high abundance and high metabolic and reproductive rates render them potentially important in benthic fluxes of carbon and nutrients (Kuipers et al., 1981, Coull, 1999).
Moens et al. (2005)

Elucidation of meiofaunal trophic interactions: Content

Approaches to measure and quantify direct trophic interactions

from past to future

methodological constraints/problems

Are nematodes quantitatively important consumers?

controversy between and across different approaches

At what level should we measure (feeding types, families, species, populations, individuals…)?

Elucidation of meiofaunal trophic interactions: Approaches

1. Observations

A. Some problems

- time consuming
- observations in sediments?

→ under artificial conditions

→ artificial food 'availability'

→ what set of 'environmental' conditions?

- 'snapshot' of reality → anecdotal?
- largely qualitative

B. Some common practices

- no observations
- observations in artificial media

→ under artificial conditions

→ artificial food availability

→ most commonly at a constant temperature (often room), in light,…

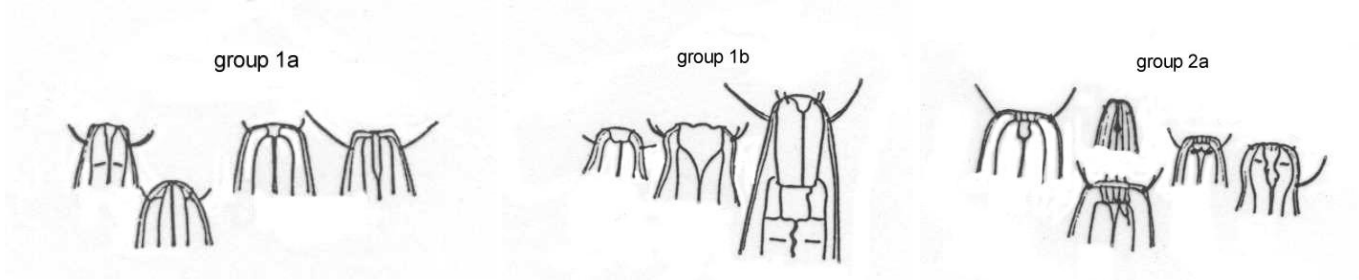
- observations of gut content → anecdotal and often inconclusive
- rely on morphological features

Elucidation of meiofaunal trophic interactions: Approaches

1. Observations

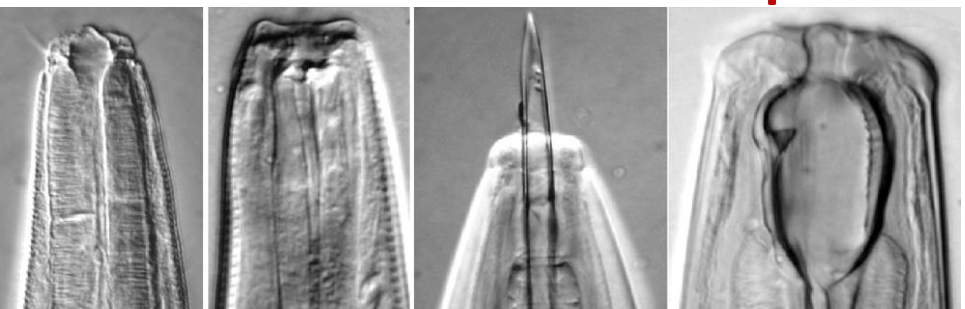
C. Some typical short-cuts

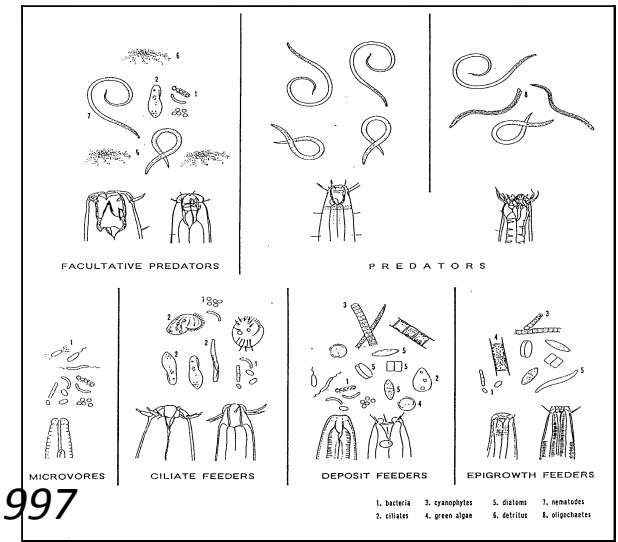
Wieser 1953



- **Black-box approach → 1 species, 1 feeding type**
- **Similar species do the same**

Traunspurger 1997





Moens & Vincx 1997

Elucidation of meiofaunal trophic interactions: Approaches

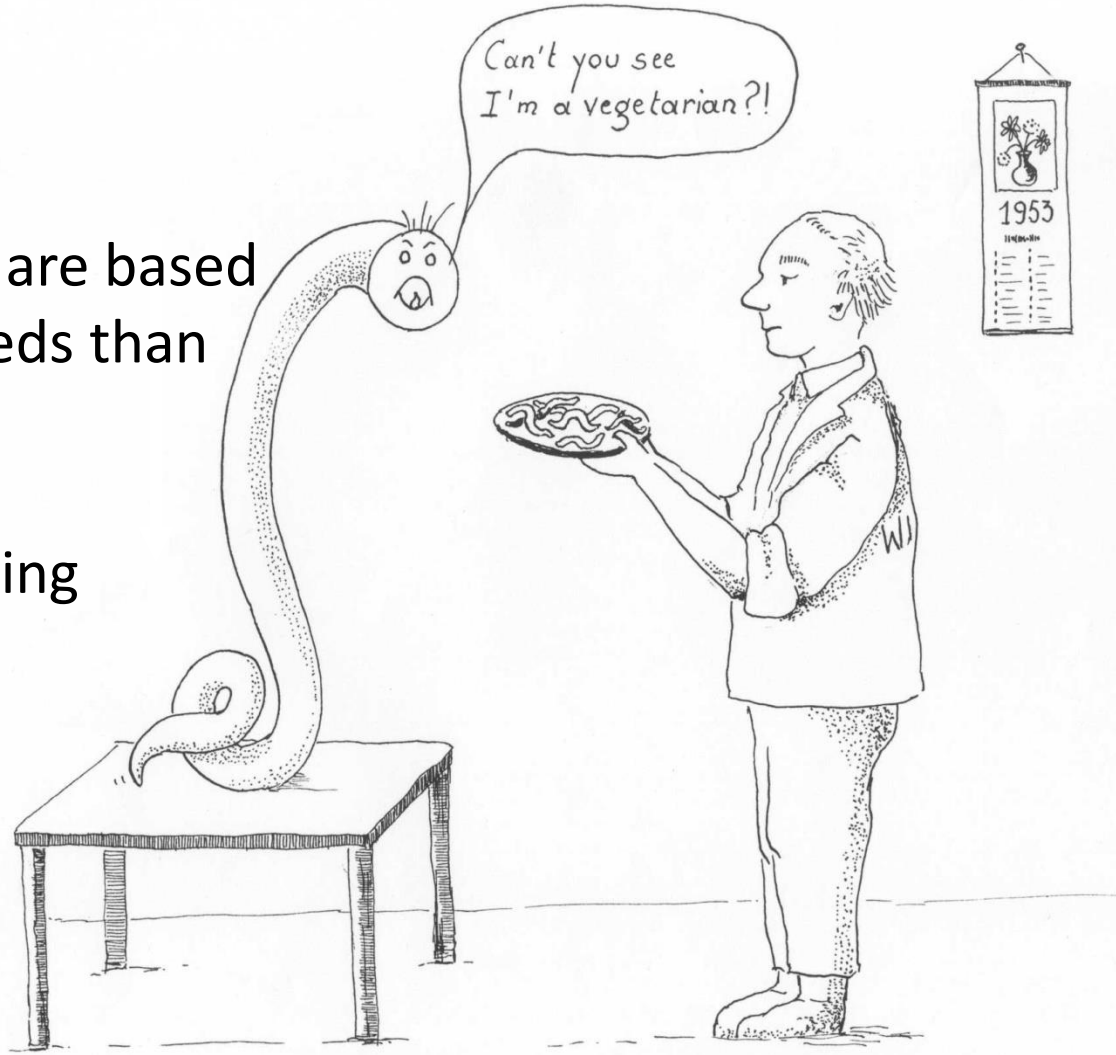
1. Observations

C. Some typical short-cuts

- Feeding-type classifications are based more on how a nematode feeds than on what it eats

- Morphology can be misleading

Hypodontolaimus & Metachromadora have a muscular pharynx and prominent tooth, but they are not predators



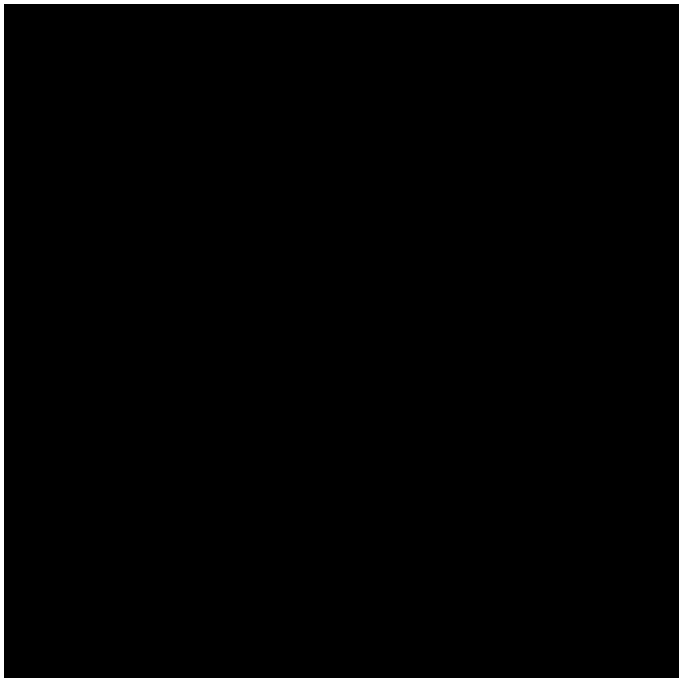
Elucidation of meiofaunal trophic interactions: Approaches

1. Observations

D. Some future directions

- **behavioral observations**

courtesy An-Sofie D'Hondt



on artificial media, e.g. movement towards/selection of food *in the sediment matrix*

courtesy Luana Monteiro

Elucidation of meiofaunal trophic interactions: Approaches

2. Tracer experiments

A. Fluorescence **B. Radioactive tracers** **C. Stable isotopes**

‘in situ’ **‘ex situ’**

add prelabelled food **add label ‘on the spot’ (pulse-chase)**

many methodological issues

Elucidation of meiofaunal trophic interactions: Approaches

2. Tracer experiments

methodological issues

Use of prelabelled food

- choice of food (usually single species)
- alive, dead, preserved (how)?
- no realistic food distribution
- no realistic food-sediment ‘interaction’

Pulse-chase

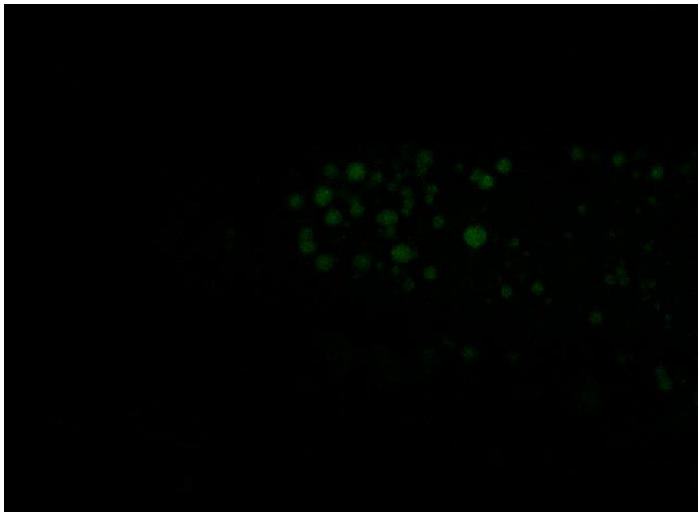
- not only the intended food can get labelled
- multiple non-grazing routes of label uptake
- how to properly administer and distribute label?

Elucidation of meiofaunal trophic interactions: Approaches

2. Tracer experiments

methodological issues in <u>fluorescent tracer</u> uptake experiments

- Pretty much the same as on the previous slide, BUT in addition, nematode autofluorescence greatly hampers proper quantification of ingested particles



courtesy Ineke Dhondt

- Any preservation method can lead to gut evacuation and several preservatives (e.g. glutaraldehyde) greatly add to the problem of autofluorescence

Elucidation of meiofaunal trophic interactions: Approaches

2. Tracer experiments

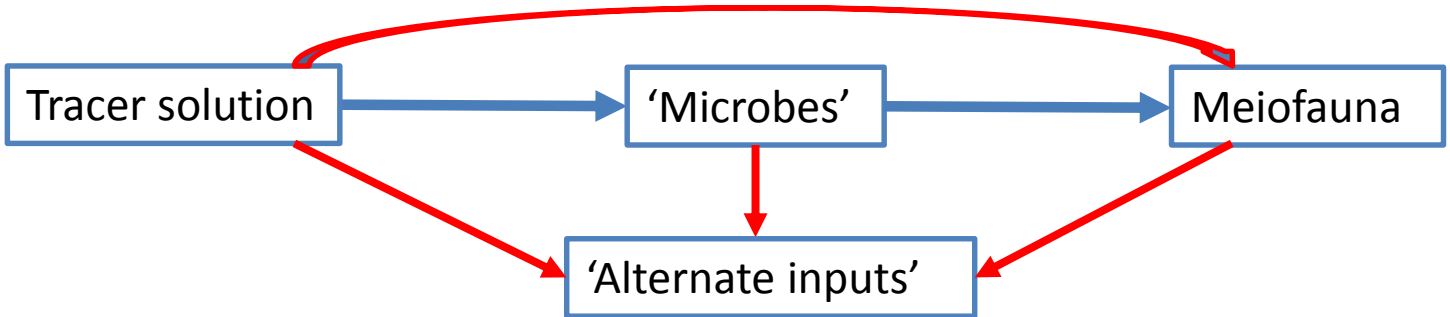
methodological issues in radioactive tracer experiments

(pulse-chase) → serious risk of overestimating uptake

- extensive methodological work done by Paul Montagna, J.E. Bauer, Kevin Carman to control for

+ alternative routes of label uptake + adsorption to body surfaces

+ homogeneous distribution of label



Elucidation of meiofaunal trophic interactions: Important results

2. Radioactive tracer experiments → <u>conclusions</u>

- Montagna (1995) 'Large variation between studies, but on average meiofauna ***graze ca 1% of microbial production*** h^{-1}*'*

- Blanchard (1990), Montagna & Yoon (1991) 'Meiofaunal grazing temporarily exceeds microbial production'

Elucidation of meiofaunal trophic interactions: Approaches

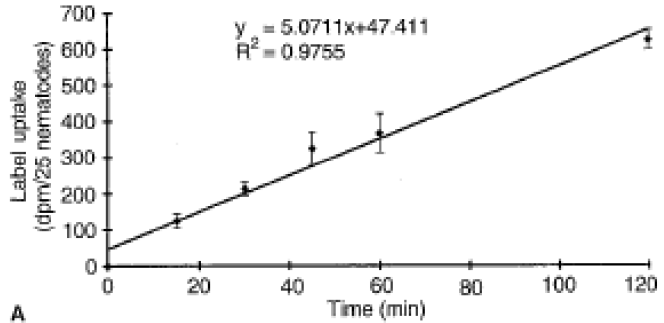
2. Tracer experiments

methodological issues

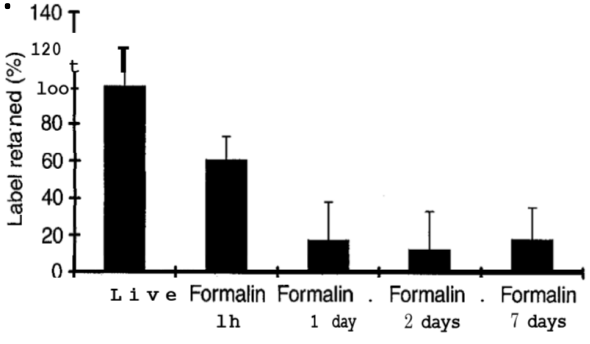
Use of prelabelled food | **Pulse-chase**

Duration of experimental incubation → do we measure ingestion, absorption/assimilation, …?

Preservation and subsequent handling?



Serious risk of underestimating uptake (Moens et al. 1999)

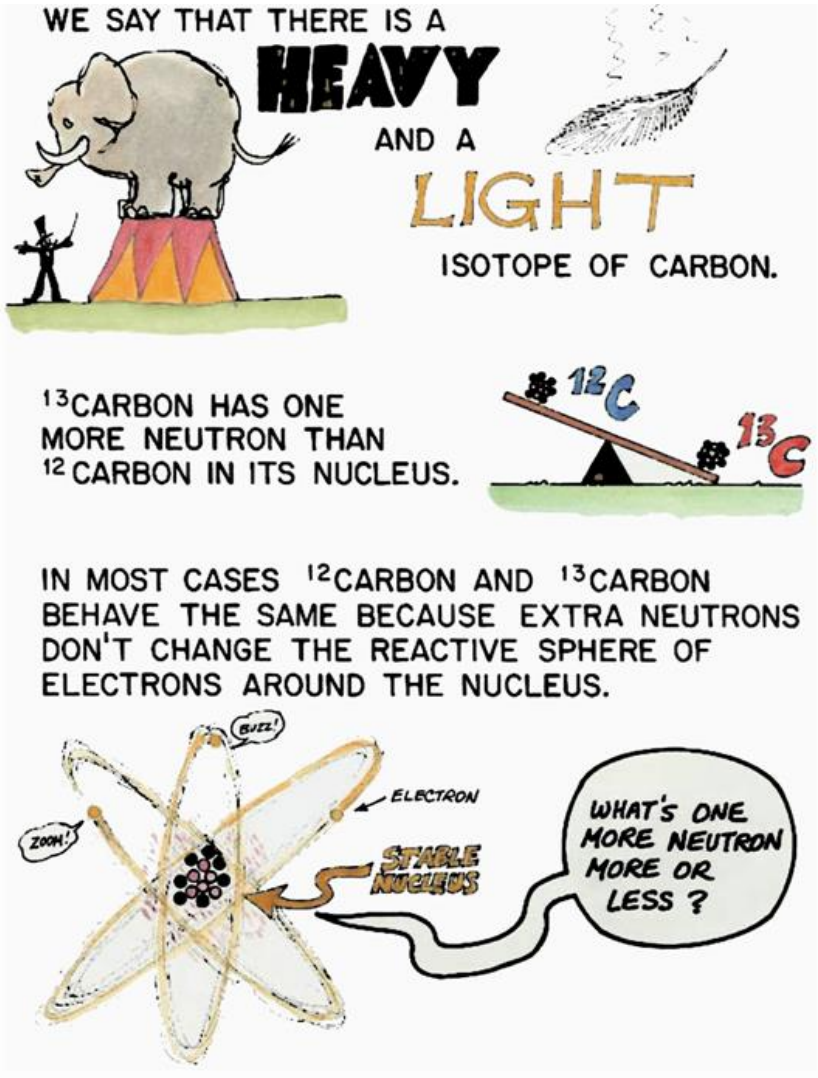


Elucidation of meiofaunal trophic interactions: Approaches

3. Stable isotopes

A: as tracers in enrichment exps

Fig. An extra neutron in the ^{13}C isotope makes the nucleus more massive or "heavier" than the ^{12}C isotope, but **does not affect most chemistry** that is related to reactions in the electron shell.
Fry (2008)



Elucidation of meiofaunal trophic interactions: Approaches

in kinetic reactions, lighter isotopes usually react faster, while in exchange reactions, heavy isotopes concentrate where bonds are strongest

→ Both processes lead to isotopic **fractionation**, and isotopic fractionation leads to

- different isotopic ratios between sources
- different isotopic ratios between consumer & resource

H																	He
Li	Be											B	C	N	O	F	Ne
Na	Mg											Al	Si	P	S	Cl	Ar
K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr
Rb	Sr	Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe
Cs	Ba	La	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn
Fr	Ra	Ac															

Elucidation of meiofaunal trophic interactions: Important results

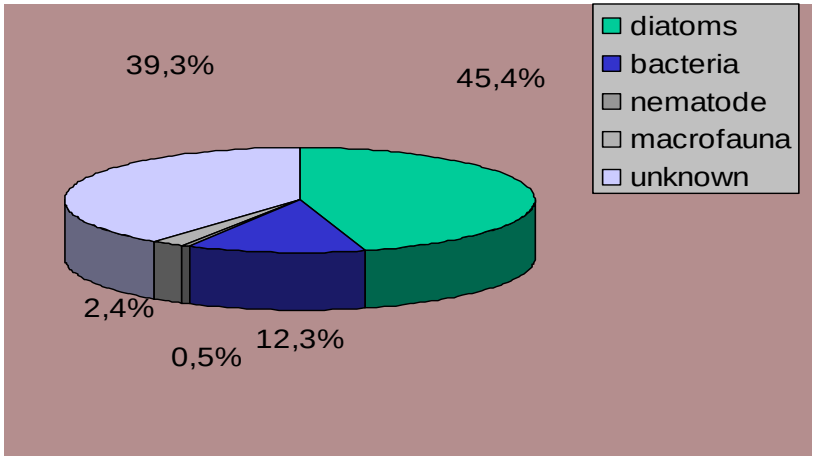
3. Stable isotopes: A: tracer experiments

Nowadays, most radioactive tracer work has been replaced by stable isotope tracers

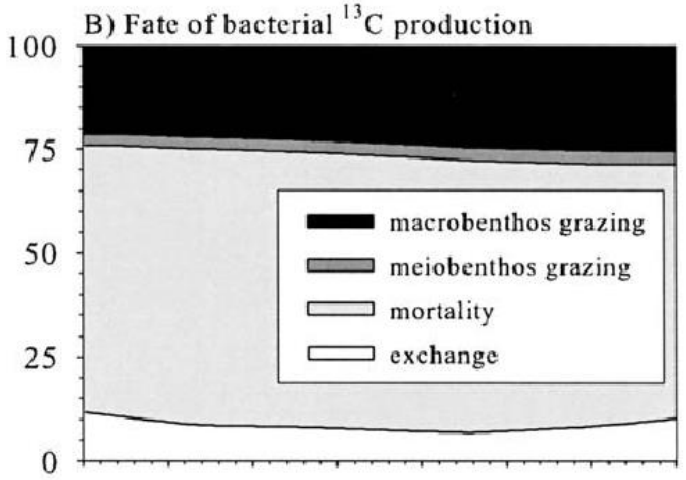
Main conclusions:

More often than not, the results indicate that meiofauna ***graze an insignificant fraction*** *of microbial production/biomass*

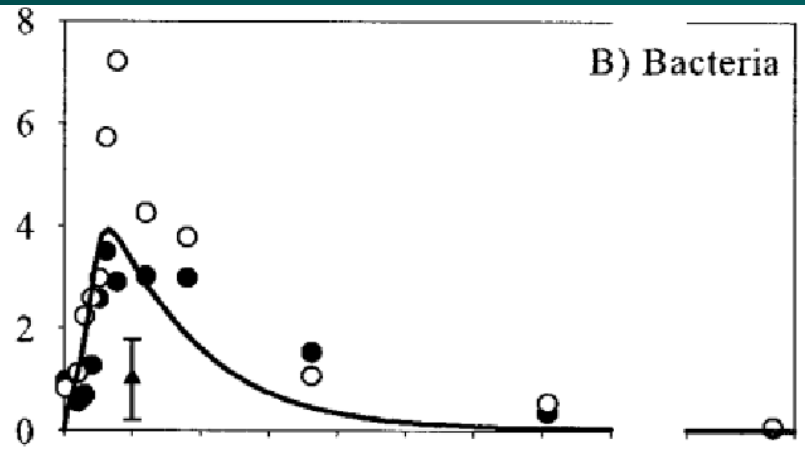
Based on original data from Middelburg et al. (2000)

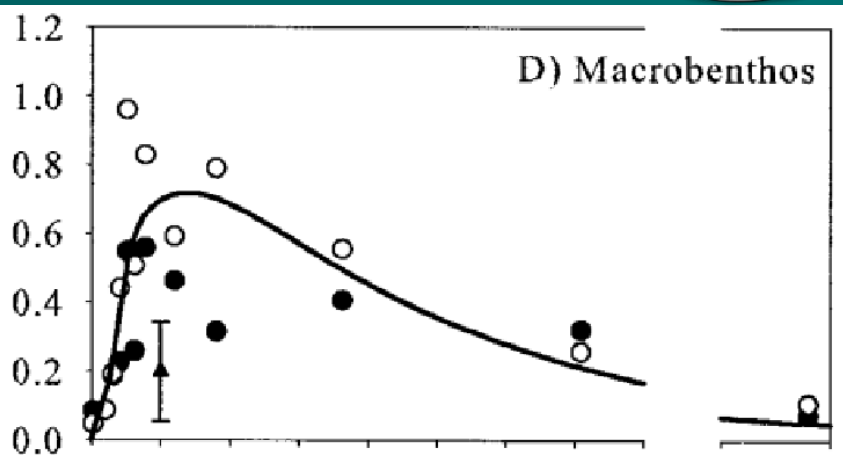


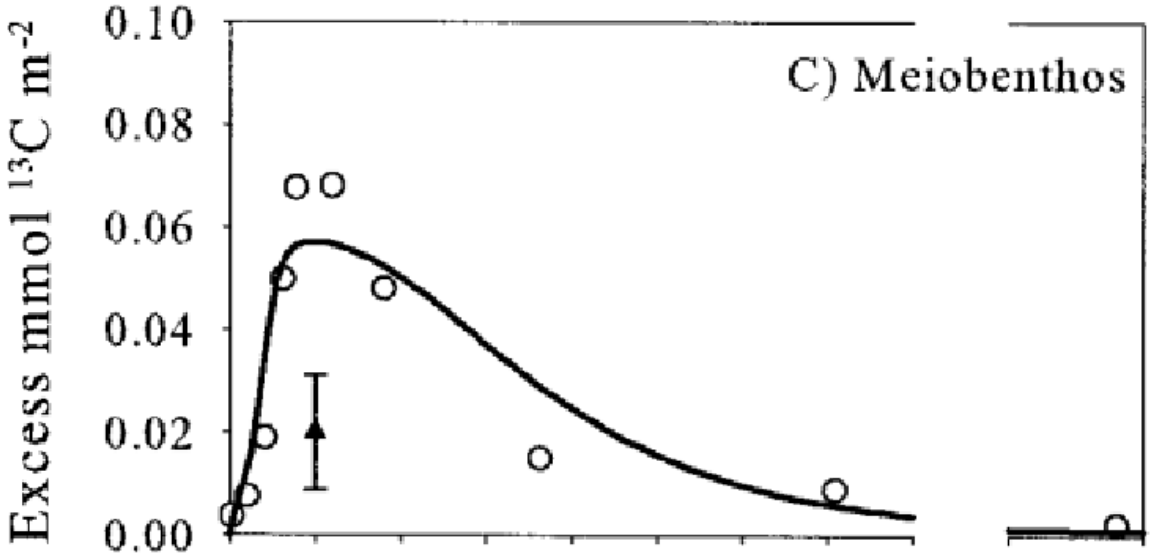
Van Oevelen et al. (2006)



Elucidation of meiofaunal trophic interactions: Important results

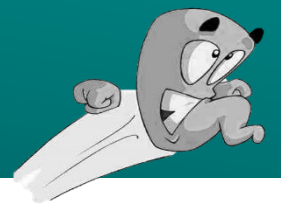






Van Oevelen et al. (2006)

Elucidation of meiofaunal trophic interactions: 'Novel' approaches

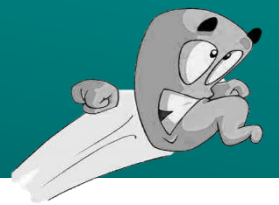


Absolute quantifications: difficult and often with conflicting results

Characterizing resource utilization and trophic position: we are pretty much addressing the same questions as 40 years ago

let's observe but in different ways

Elucidation of meiofaunal trophic interactions: 'Novel' approaches



X. Gut content analysis in a different way:

phytopigment analysis

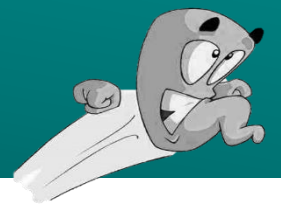
Pioneered for meiofauna by Lidia Souza-Santos, Paulo Santos & Jacques Castel (mid '90s)

Applied more recently for epilithic meiofauna by Nabil Majdi et al.

Interesting enough, but…

(high biomass requirements, issues with preservations, etc…)

Elucidation of meiofaunal trophic interactions: 'Novel' approaches



3B. Stable isotopes: natural abundances

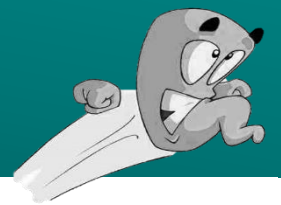
A. Some possibilities

- an **integrated picture of diet** over the past days/weeks
- information on **resources** (mostly C isotopes, S would be useful but is too 'rare') → *you are what you eat*
- information on **trophic level** (cf. trophic-level fractionation) → mostly N
- **metrics** based on isotopes allow assessment of niche width and overlap

B. Some problems

- only really useful if *different resources differ* enough in their isotopic composition
- resource resolution limited
- *substantial biomass* (ca 5 µg of an element) required for reproducible measurements
- *trophic-level fractionation appears far from constant*

Elucidation of meiofaunal trophic interactions: 'Novel' approaches

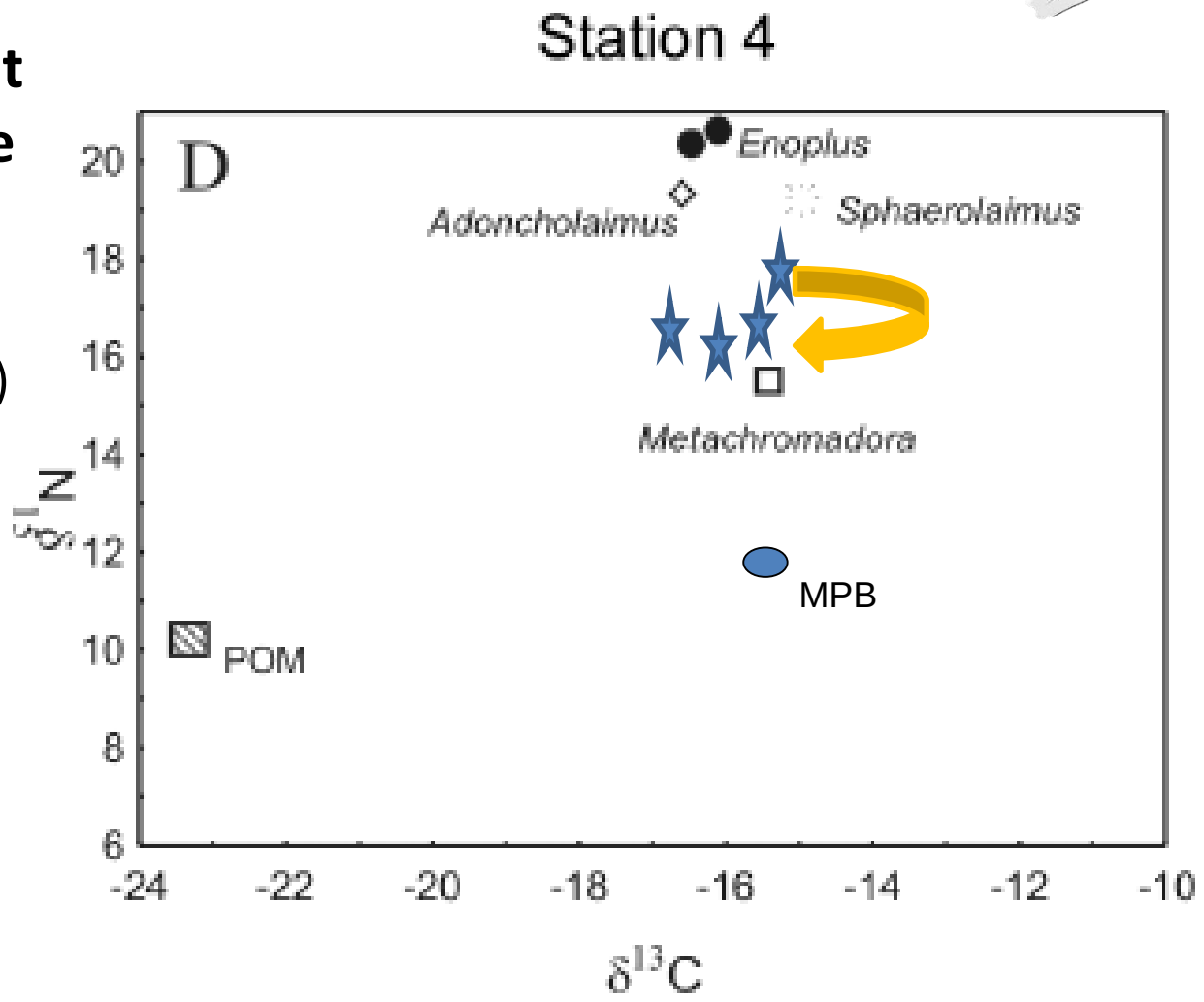


Community values not always representative

From left to right:
Daptonema (1B)
Praeacanthonchus (2A/1B)
Bathylaimus (ciliate feeder/1B)
Enoploides (2B)

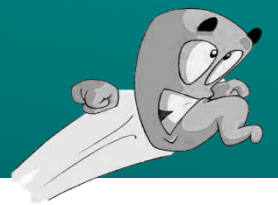
→ **High degree of omnivory!**

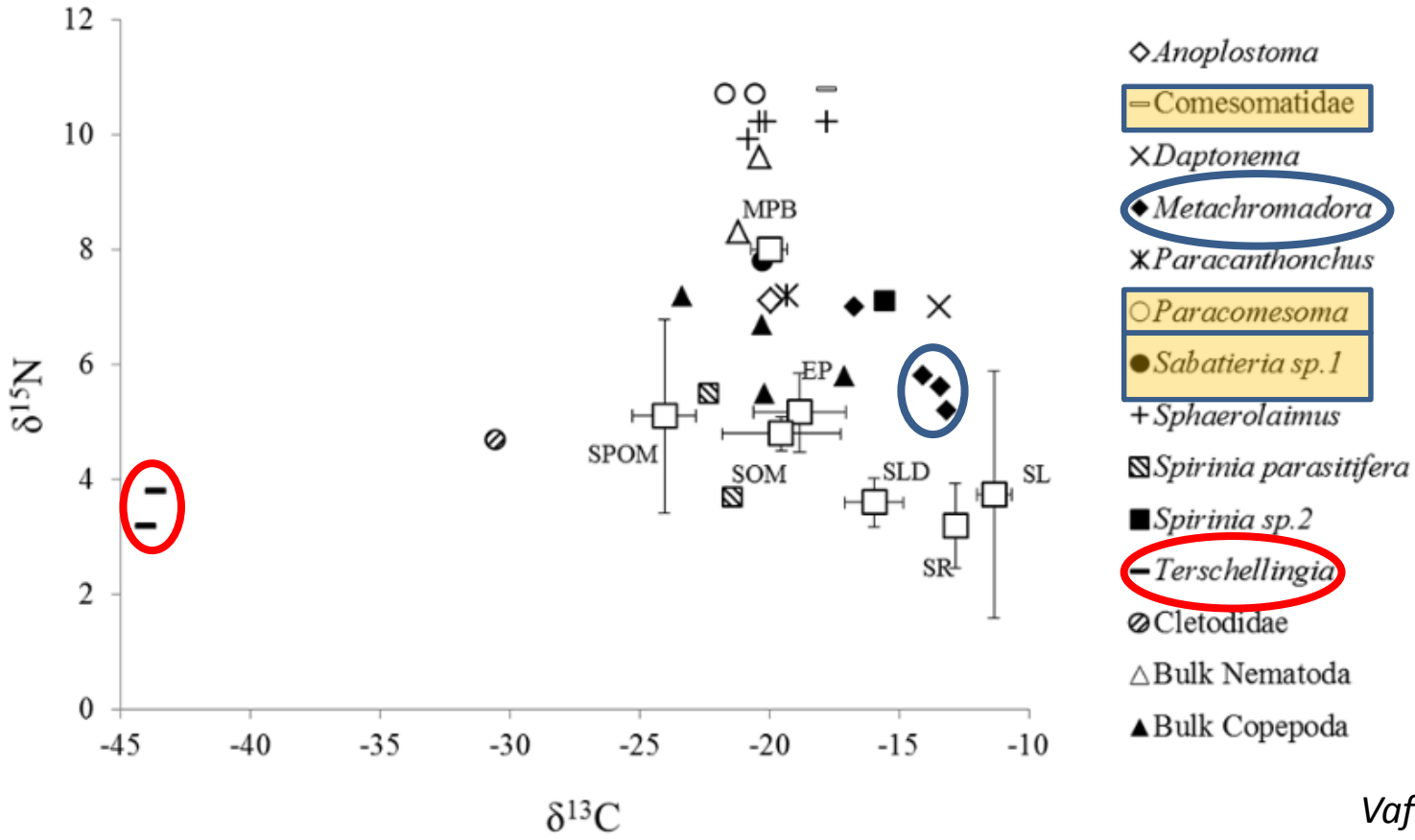
→ **Our black-box approach needs revision!**



Moens et al. (2005) with additional data from Tania Bezerra & T.M.

Elucidation of meiofaunal trophic interactions: 'Novel' approaches



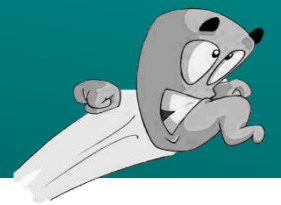


Never trust morphology or taxonomic relatedness alone when dealing with functional traits!

Vafeiadou et al. (2014)

Figure 3. Biplots of $\delta^{13}C$ / $\delta^{15}N$ of meiobenthos from the upper 2 cm and their potential resources in seagrass beds **(A)** and bare sediments **(B)**. Resource data are mean values (±SD) of all replicate samples per source material. Abbreviations used: SL, SR and SLD for seagrass leaves, roots and detritus, respectively; EP for epiphytes, MPB for microphytobenthos, SPOM for suspended particulate organic matter and SOM for bulk sediment organic matter.

Elucidation of meiofaunal trophic interactions: 'Novel' approaches



4. Fatty acid profiles

Pioneering work on meiofauna by Daniel Leduc, Marleen De Troch and in several papers on deep-sea nematodes in group of Ann Vanreusel

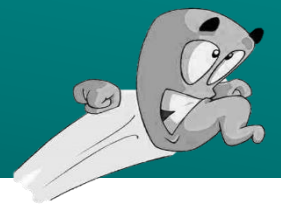
Complementary info to SI

Similar limitations

Bioconversion as an additional issue

We need sufficient biomass → pooling of many tens of inds.

Elucidation of meiofaunal trophic interactions: 'Novel' approaches

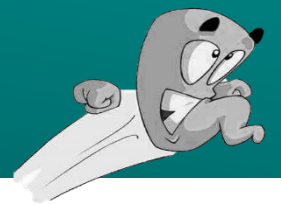


interindividual variation codetermines a population's niche width and hence its environmental tolerance range (Violle et al. 2012).

interindividual variation is key to understanding competitive interactions (both intra- and interspecific) (Violle et al. 2011) and hence community assembly and structure.

So where does that leave us?

Elucidation of meiofaunal trophic interactions: 'Novel' approaches



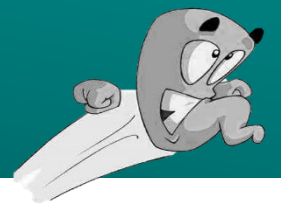
5. Stable isotope analysis in a different way:

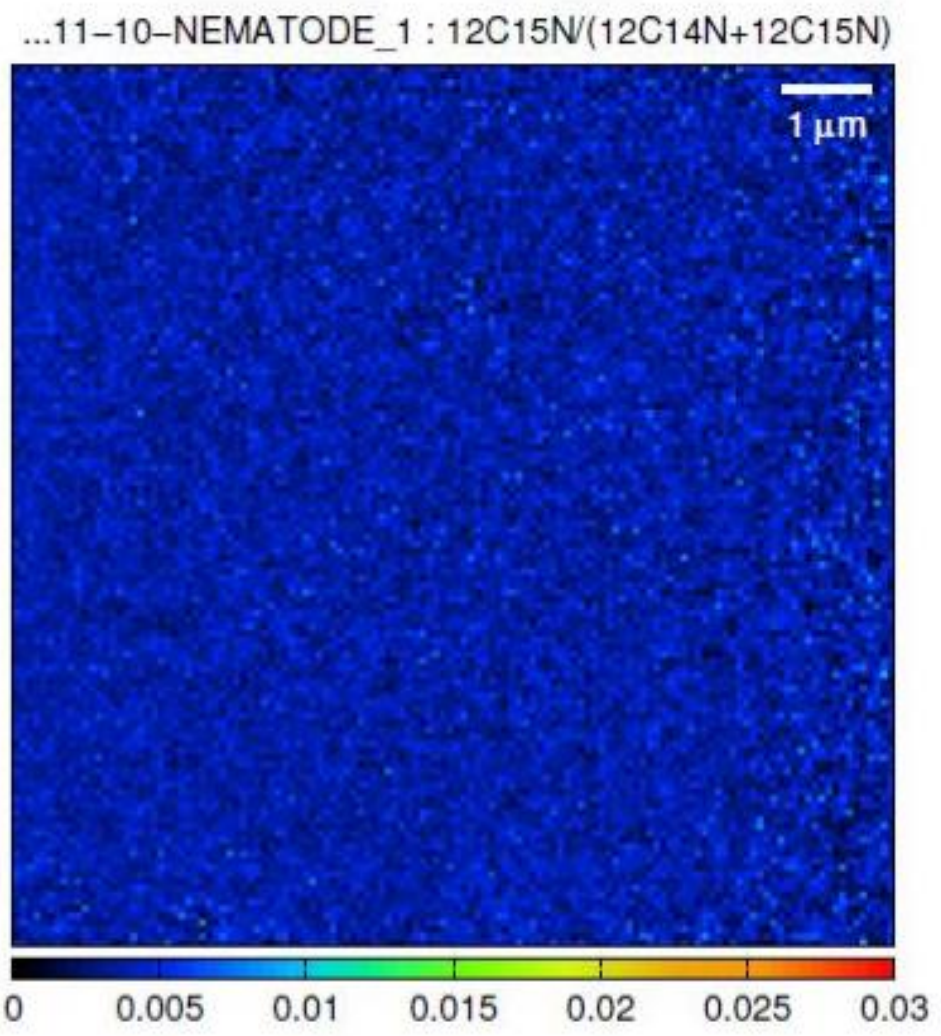
NanoSIMS allows assessment of all types of isotopic ratios even at the level of single cells

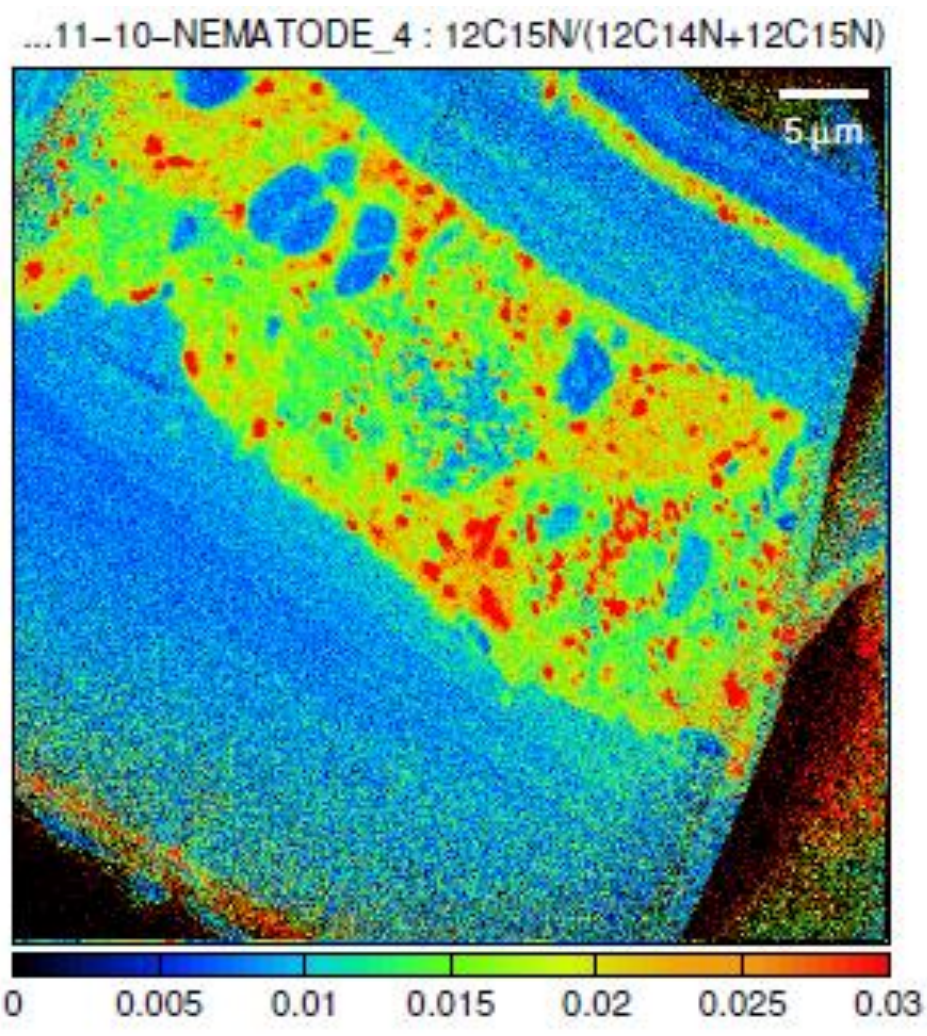
NanoSIMS

Courtesy of Katja Guilini et al.

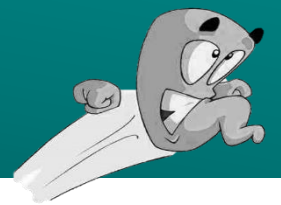
Elucidation of meiofaunal trophic interactions: ‘Novel’ approaches







Elucidation of meiofaunal trophic interactions: 'Novel' approaches



5. Stable isotope analysis in a different way: NanoSIMS

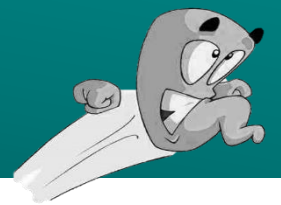
A. Some possibilities

- measurements at the level of individuals, tissues, and even single cells
- uptake and assimilation can be visualized
- many isotopic combinations possible → S isotopes can for the first time be used in our analyses of resource use

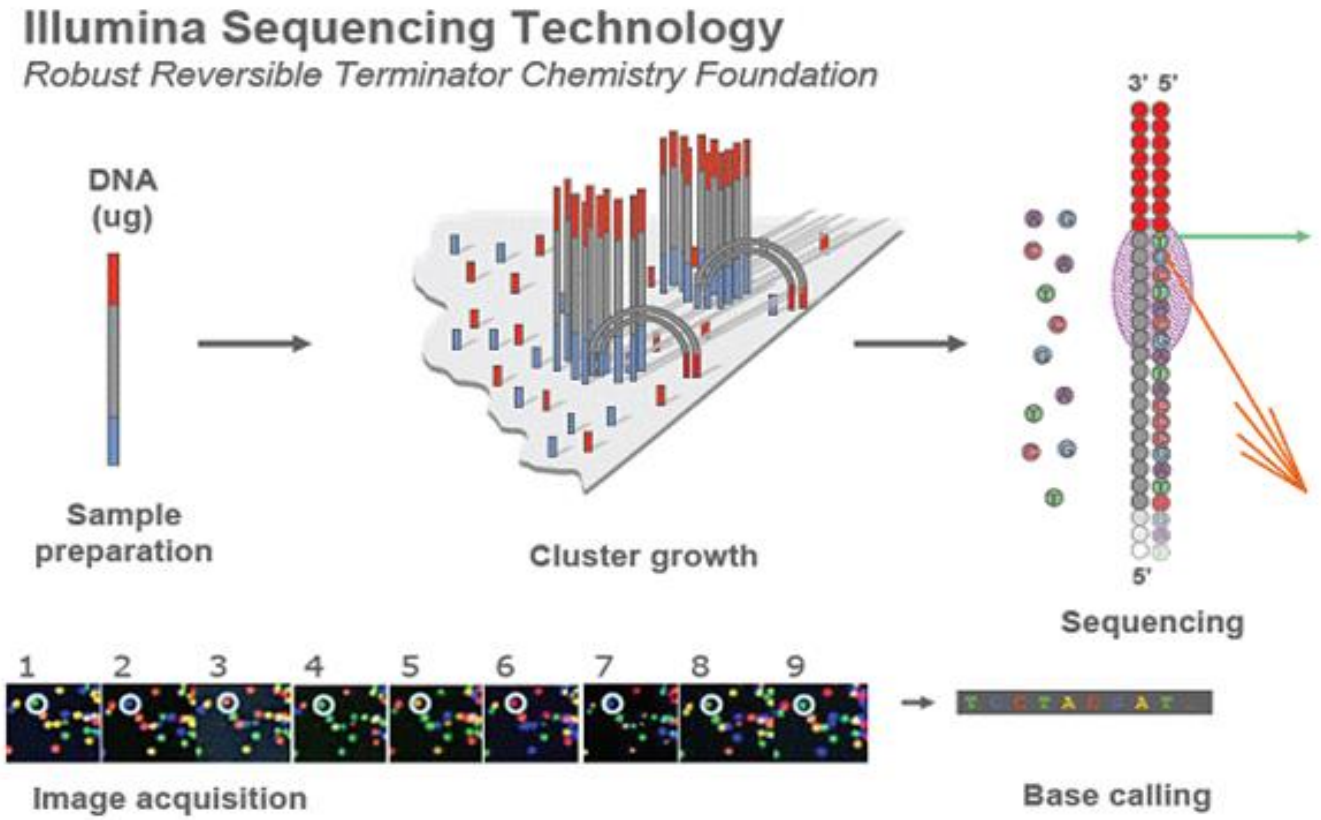
B. Some problems

- much specialized preparatory work
- much specialized work to analyse the data and decide on how to select the right info from the wealth of data
- extremely expensive and high-tech equipment
- analyses for the moment 20-50 times more expensive than (bulk) EA-IRMS

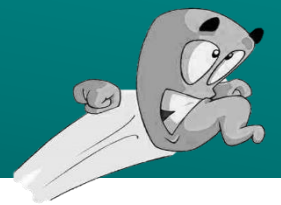
Elucidation of meiofaunal trophic interactions: 'Novel' approaches



6. Next Generation Sequencing to analyse 'gut content'

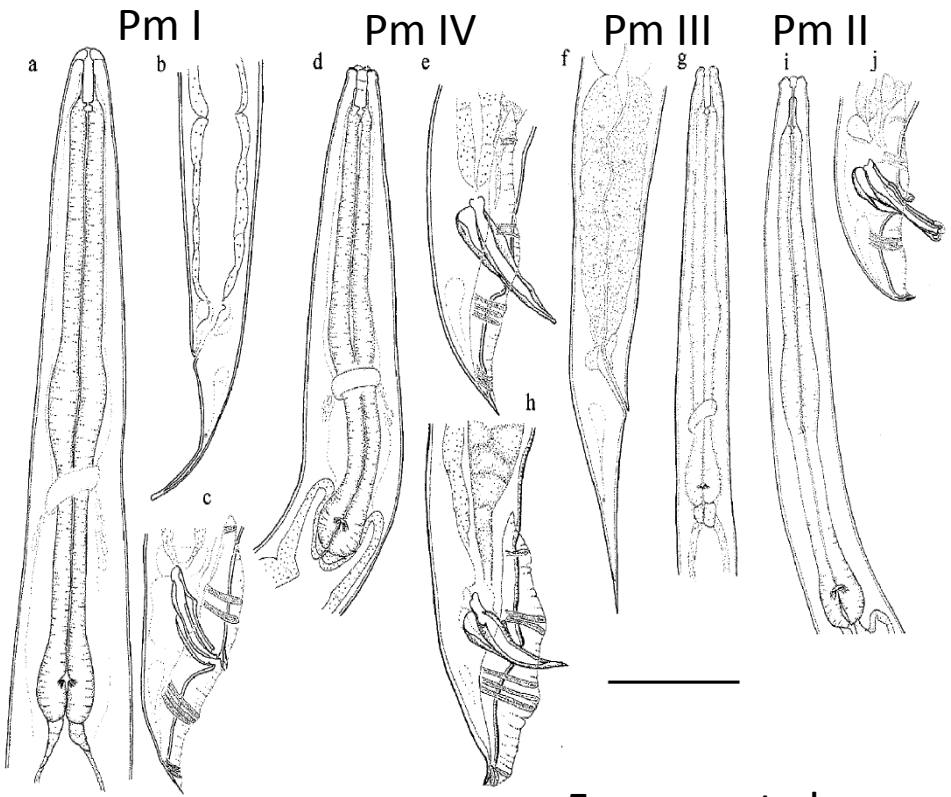


Elucidation of meiofaunal trophic interactions: 'Novel' approaches



Unravelling coexistence of cryptic ***Litoditis marina*** species

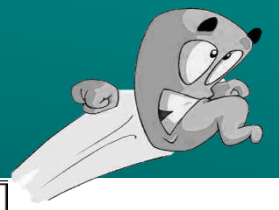
De Meester N. (2016) – PhD
Is niche-differentiation important?
Is resource differentiation important in separating niches?

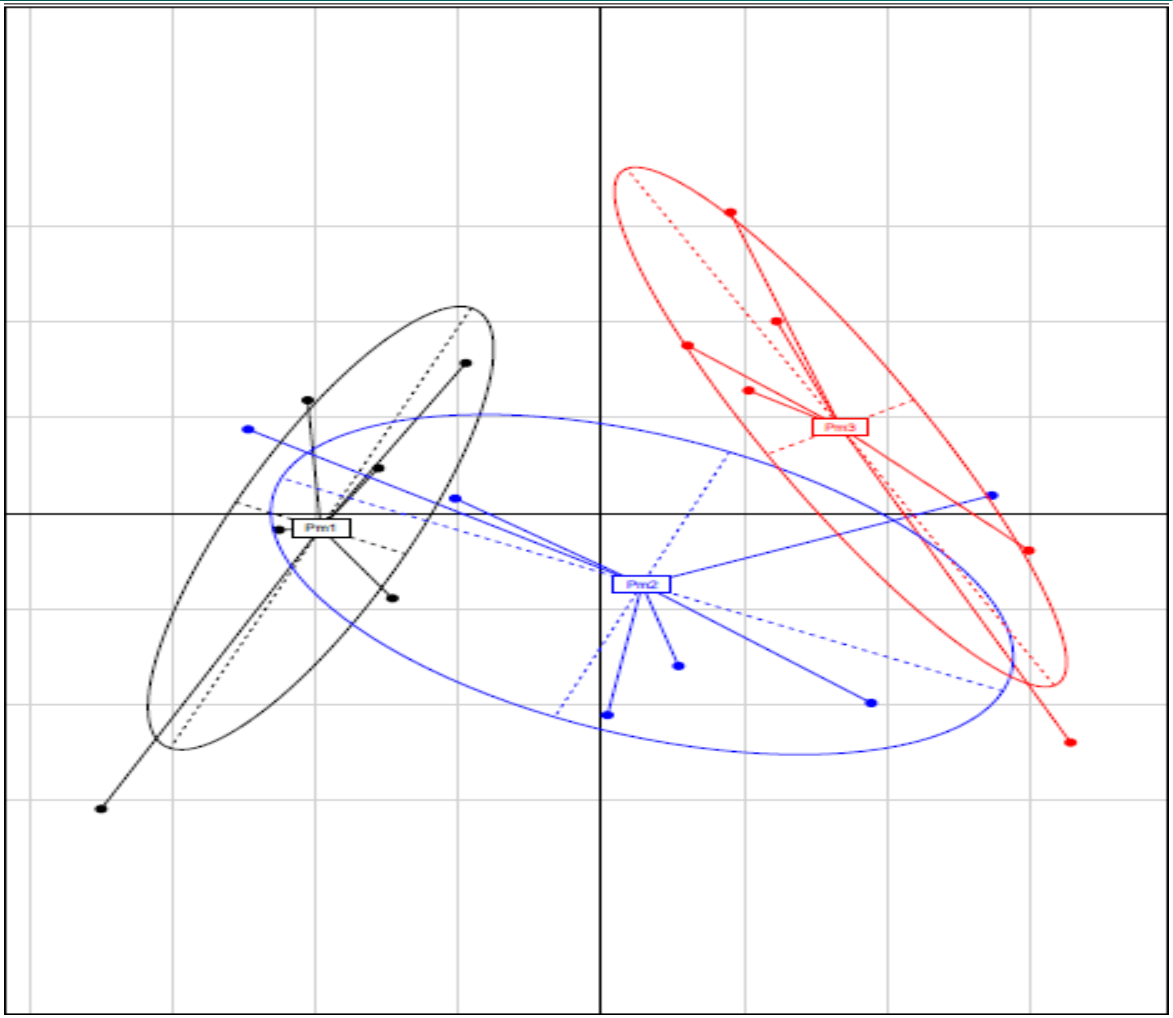


Fonseca et al, 2008

Derycke et al. (2016) – Mol Ecol
'Coexisting cryptic species of the *Litoditis marina* complex (Nematoda) have distinct microbiomes with high intraspecific variability'

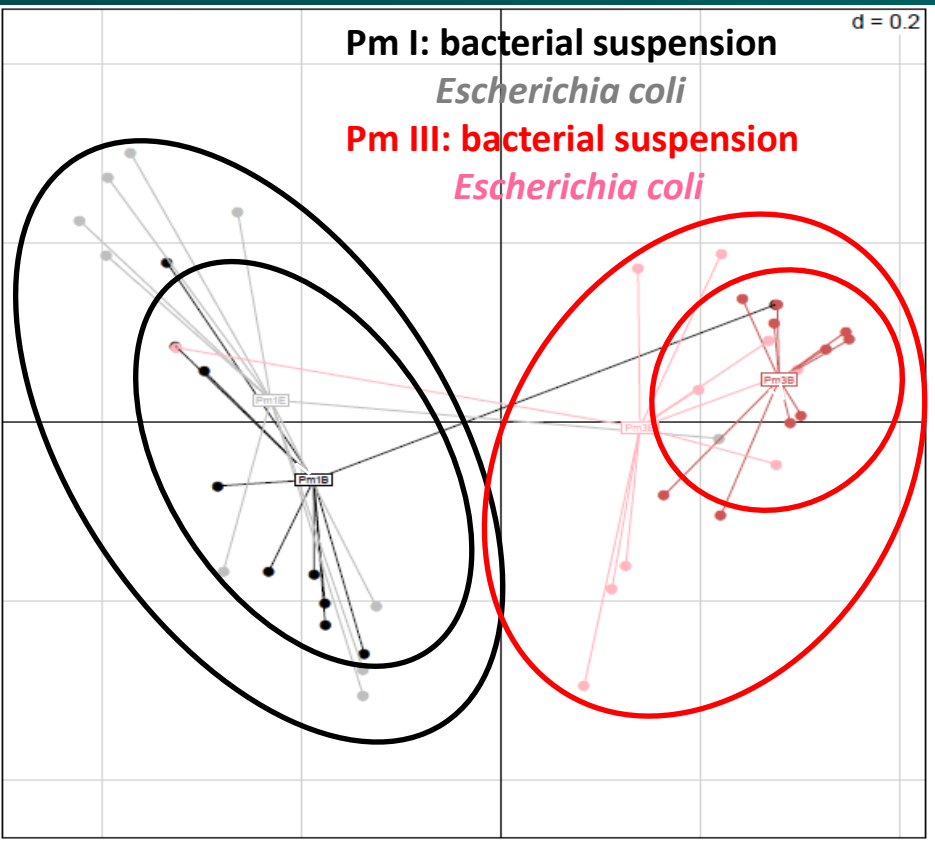
Elucidation of meiofaunal trophic interactions: 'Novel' approaches





Derycke et al. (2016) – Mol Ecol

Elucidation of meiofaunal trophic interactions: 'Novel' approaches

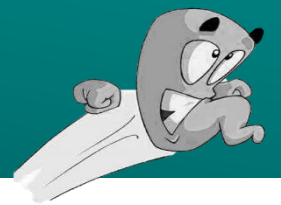


Derycke et al. (2016) – Mol Ecol

Table 2 Summary of the PERMDISP and PERMANOVA statistics between the microbiomes of the four food experiment treatments (Pm1B, Pm1E, Pm3B and Pm3E) for the data set containing all OTUs and for the core OTUs. For the pairwise comparisons, significant *P*-values after Bonferroni correction are indicated in bold.

		All OTUs		Core Genome	
Food experiment		Pseudo-*F*	*P* value	Pseudo-*F*	*P* value
PERMDISP	Species	9.04	<0.001	7.11	0.011
	Food	2.94	0.095	1.57	0.22
	Species*food	6.80	<0.001	6.65	0.001
PERMANOVA	Species	10.97	0.001	16.56	0.001
	Food	3.10	0.005	3.59	0.008
	Species*food	2.02	0.049	2.46	0.043
Pairwise test	Pm1B-Pm1E	1.65	0.236	1.62	0.13
Pairwise test	Pm3B-Pm3E	3.98	**0.004**	5.50	**0.001**
Pairwise test	Pm1B-Pm3B	8.78	**0.004**	14.71	**0.001**
Pairwise test	Pm1E-Pm3E	4.81	**0.004**	6.1	**0.002**

Elucidation of meiofaunal trophic interactions: 'Novel' approaches



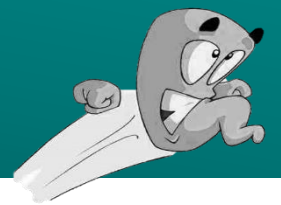
So we can analyse prokaryotic 'diets' of meiofauna at the level of individuals

Variability among individuals is large → consequences at the population level?

Differences between species can clearly be analysed

We should be able to analyse eukaryotic diets in much the same way, but so far not successful

Elucidation of meiofaunal trophic interactions: 'Novel' approaches



More group-specific predator-prey relationships can be analysed if suitable prey-specific primers can be developed.

Invertebrate Biology 133(2): 121–127.

DOI: 10.1111/ivb.12048

Diagnostic PCR can be used to illuminate meiofaunal diets and trophic relationships

Hanna Maghsoud,[1] Austin Weiss,[2] Julian P.S. Smith III,[2,a] Marian K. Litvaitis,[3] and Stephen R. Fegley[4]

and Smith et al. (2016) *poster 76, this conference*.

Elucidation of meiofaunal trophic interactions: Conclusions

- This keynote has focused on a limited number of trophic interactions. A.o., meiofauna-ciliate/flagellate and meiofauna-fungi interactions deserve more attention.

- Despite substantial efforts and different methodological approaches, **some qualitative and nearly all quantitative key questions remain under debate**.

- We have to **observe** again, though with different means, before quantifying.

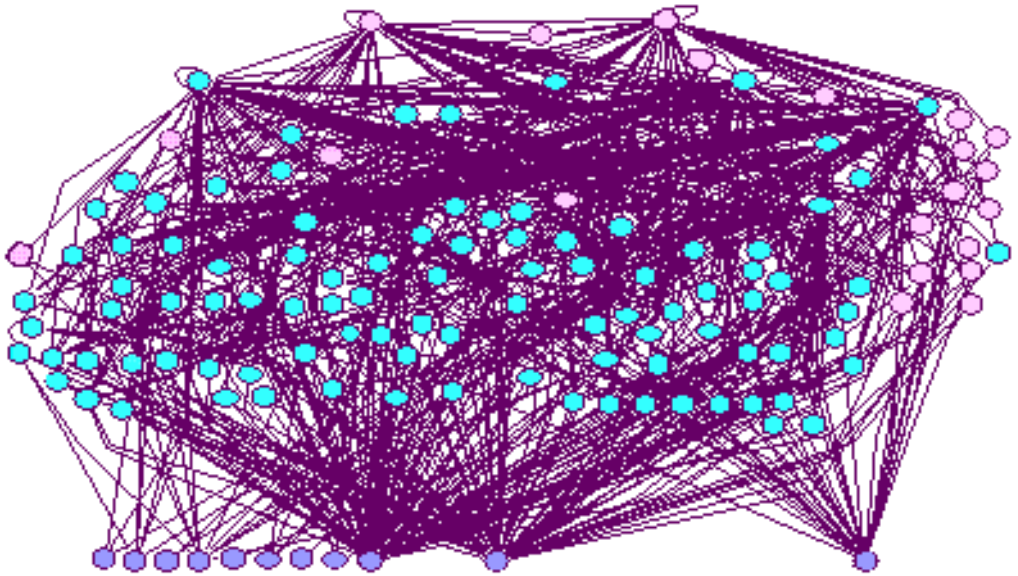
Elucidation of meiofaunal trophic interactions: Conclusions

- Novel technological advances open up **unprecedented opportunities** to study trophic interactions, including under natural conditions.

- They all do have their **caveats**, and some simple issues such as sample preservation effects on gut content become more pressing than ever.

- **Little, if any, additional understanding on food web interactions is to be expected from analyses lumping organisms** at the community, guild or family level.

Elucidation of meiofaunal trophic interactions: Conclusions



Jenny Schmid-Araya et al. (2002)

THANK YOU ALL !