

Using environmental metabarcoding to assess change associated with marine structures

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SAMS

Structure

- What is metabarcoding?
- Fives steps of metabarcoding
- Initial results
- Regulatory context

What is metabarcoding?

- Barcoding is the identification of taxa via their DNA sequence
- Meta – is the sequencing of numerous (millions) of DNA fragments, simultaneously.
- Metabarcoding is a rapidly evolving technology that enables ~high resolution taxonomic identification across broad taxonomic groups in ~100 samples simultaneously.

Five steps in metabarcoding

1. Sample collection
2. 'Wet lab'
3. Sequencing
4. Bioinformatics
5. Data interpretation (statistical modelling)

Benthic sample collection



- Sediment or filter papers from water samples
- Typically 5 – 20 g per sediment sample
- Filter ~litres of water

Wet-lab



- 1. DNA extraction, purification, marker PCR, tagging and quantification

‘Library’ preparation

Wet-lab

- Extracted DNA is a mixture of bacterial/protistan/meiobenthic cellular DNA and eDNA from all groups (including macro- and mega-benthos)
- From the extracted DNA can target regions within:
 - 16S (bacteria), 18S (general eukaryotes), COI (metazoa) others for plants, fungi and specialised groups
- Regions within markers are 'targeted' by primers, which can be bespoke designed, none are truly 'universal' (e.g. none cross all taxa)
- Advantages/disadvantages to all regions/markers and associated primers
- Sequencing - 'Illumina' or 'Oxford nanopore' dominate

Bioinformatics

- Shift from identifying 'operational taxonomic units' (OTUs) by clustering sequences to ASVs (amplicon sequence variants)
- Annotate sequences by comparison with databases
- Databases of various 'quality' – most charismatic species (e.g. whales, fish) sequenced for most markers
- Most meiobenthos not sequenced
- Databases are not necessarily well curated
- Bacterial databases are good and well curated

In a regulatory context, the question
is:

- Can we move from this:



Super-abundance
of the polychaete
Capitella sp



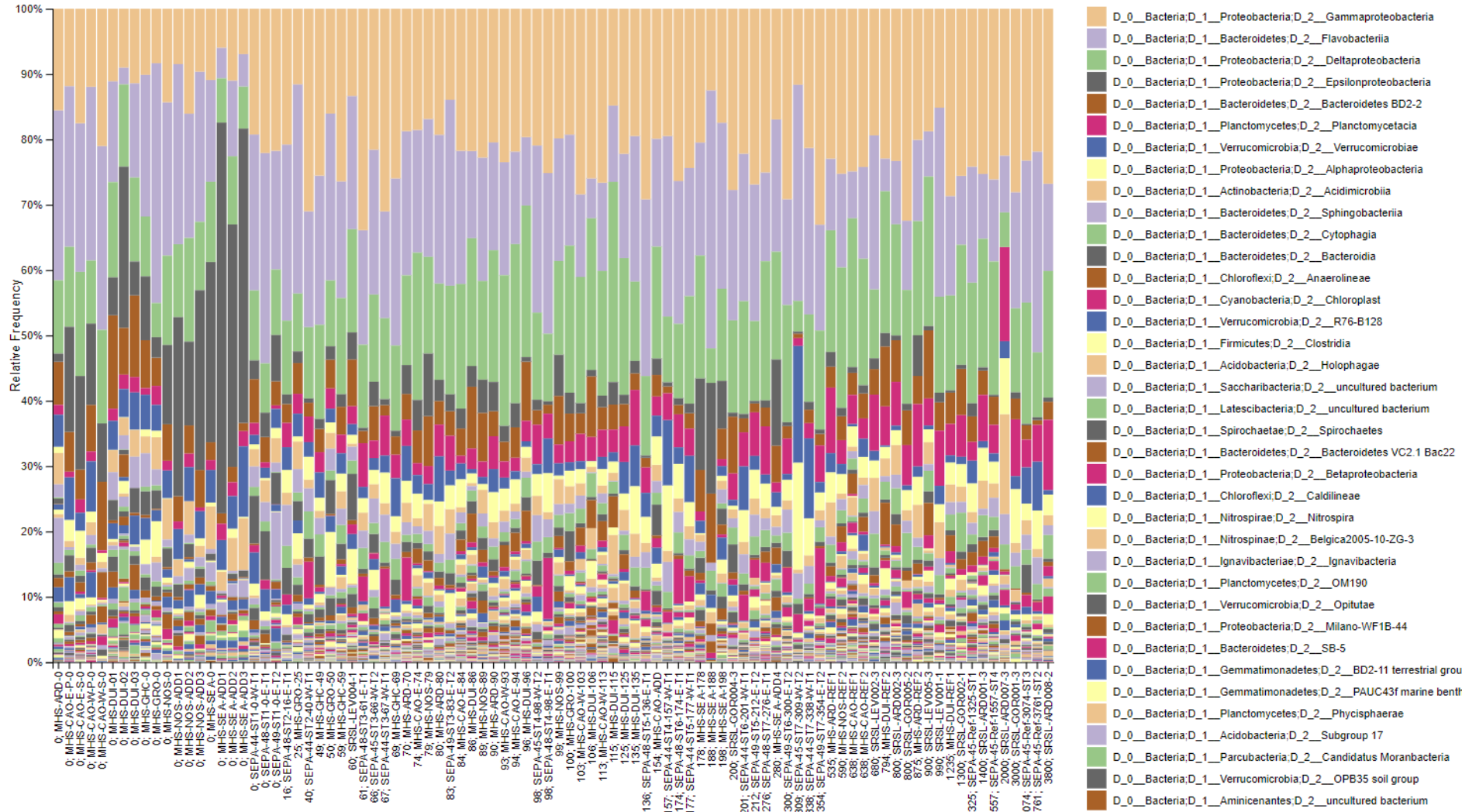
- And this ...



More diverse, larger, less
abundant benthic infauna

- Via:

sample (taxa barplot)

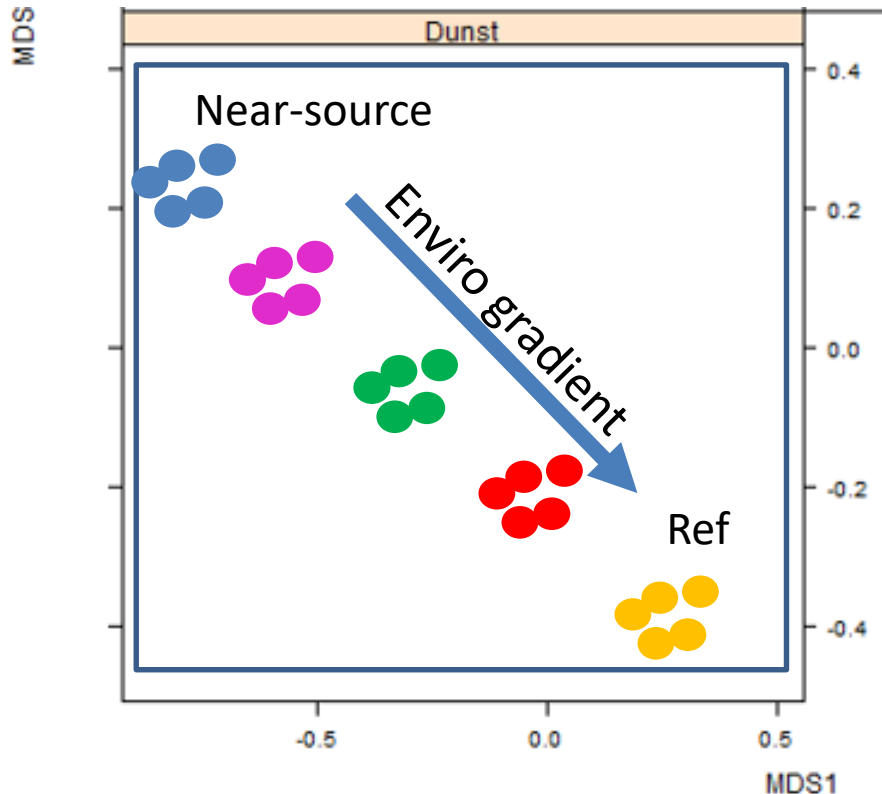


To effective regulatory compliance
assessments?

Preliminary results

- Variability between replicates within grab

Assess within grab variability as a function of marker

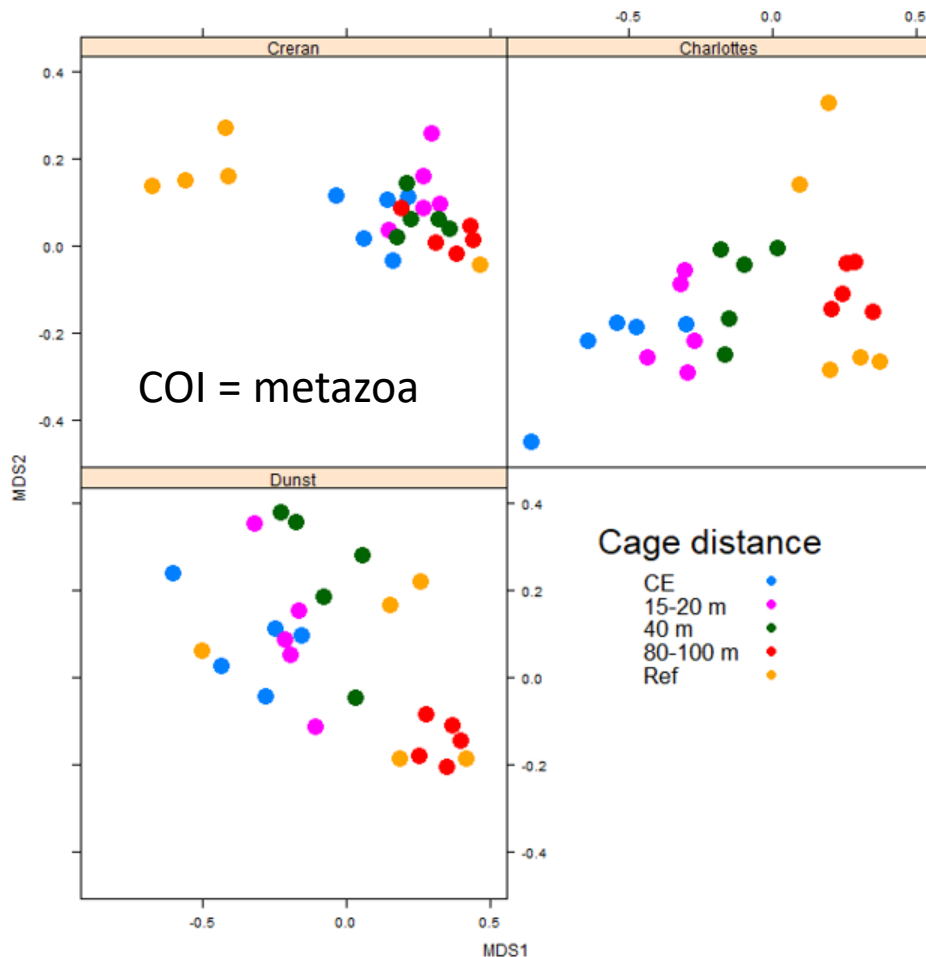


MDS plot of taxa by site

Ideally

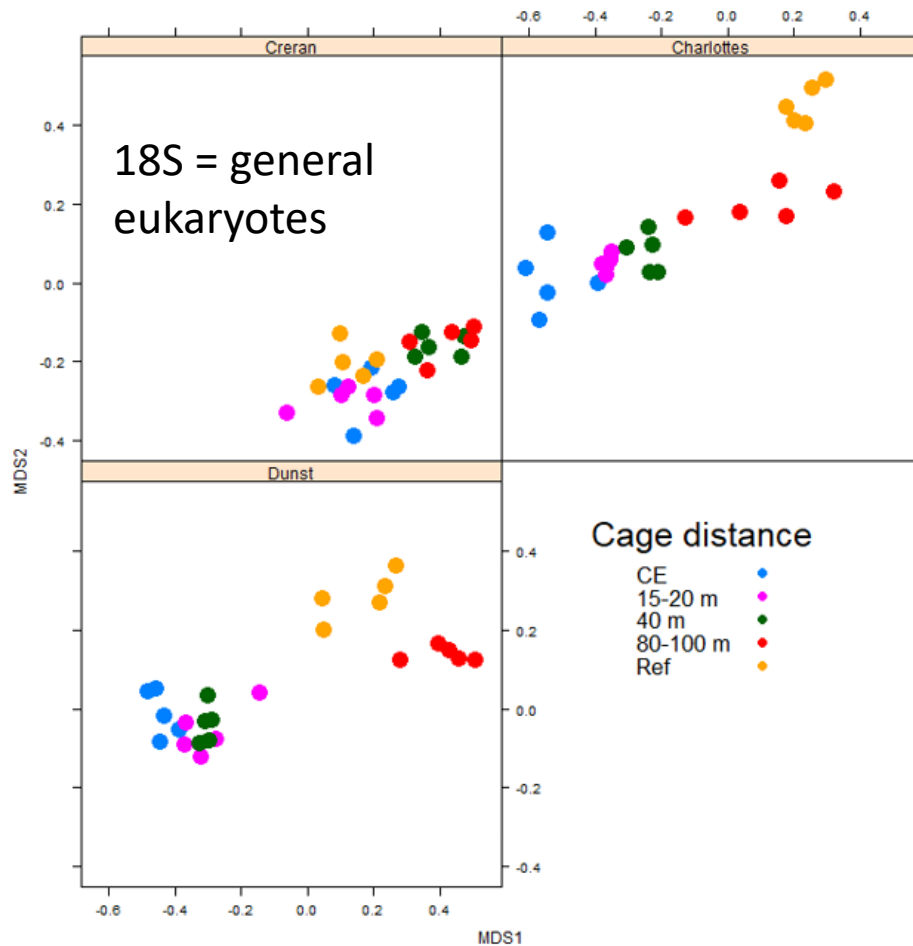
- Low variation (reps within grab)
- Clear gradient
- No overlapping stations
- Consistent trend between Sites

Within grab variability depends on the marker - COI



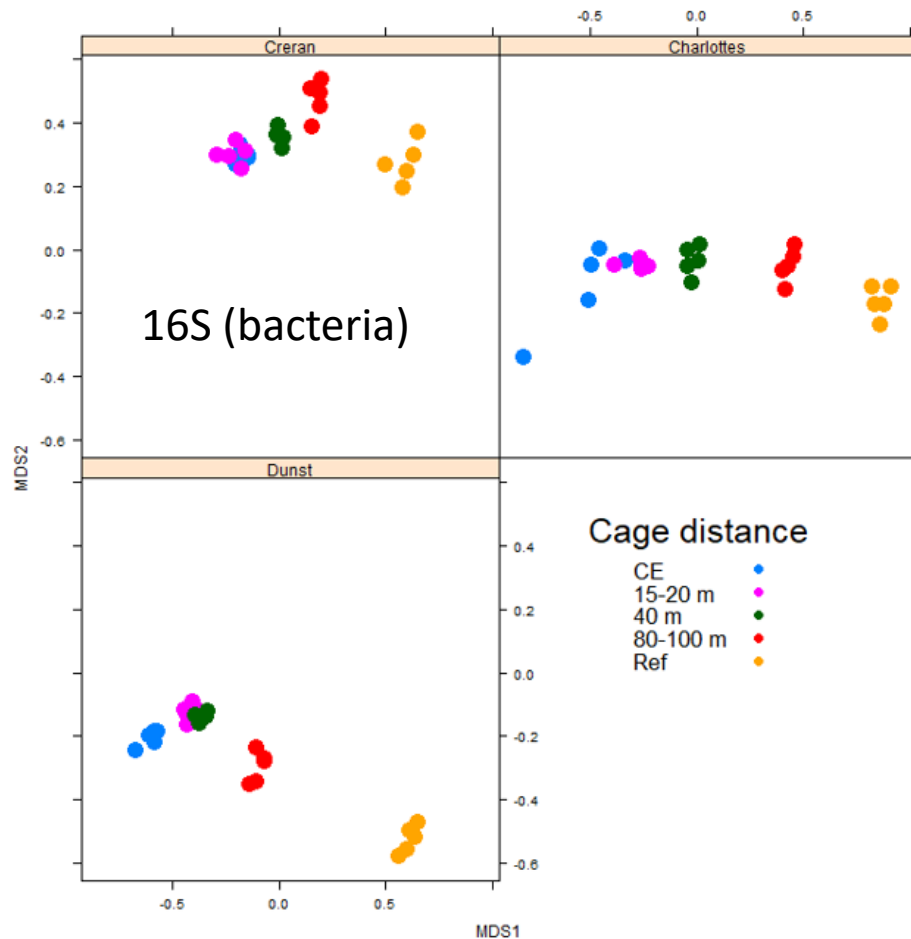
- Lots of noise
- Overlapping
- But still some intuitive trends

Within grab variability depends on the marker – 18S



- Reasonable resolution
- Clear patterns of 'distance-effect'
- Some overlap between stations

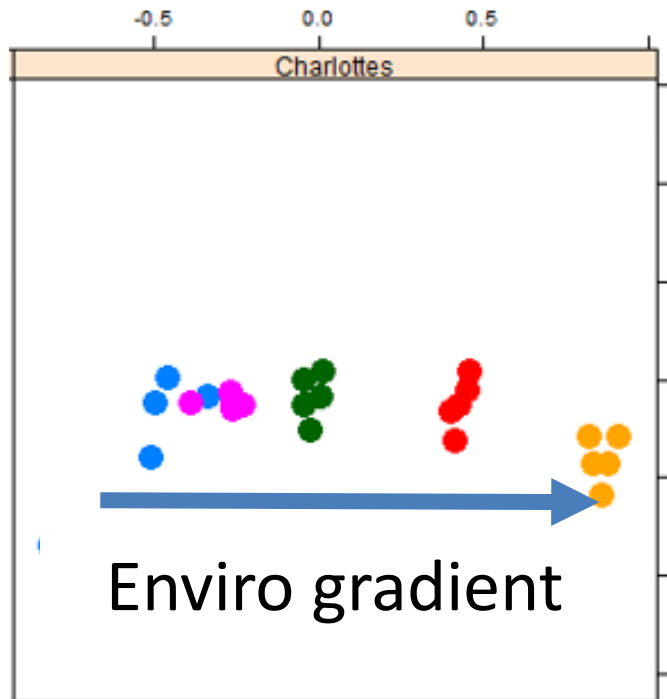
Within grab variability depends on the marker - 16S



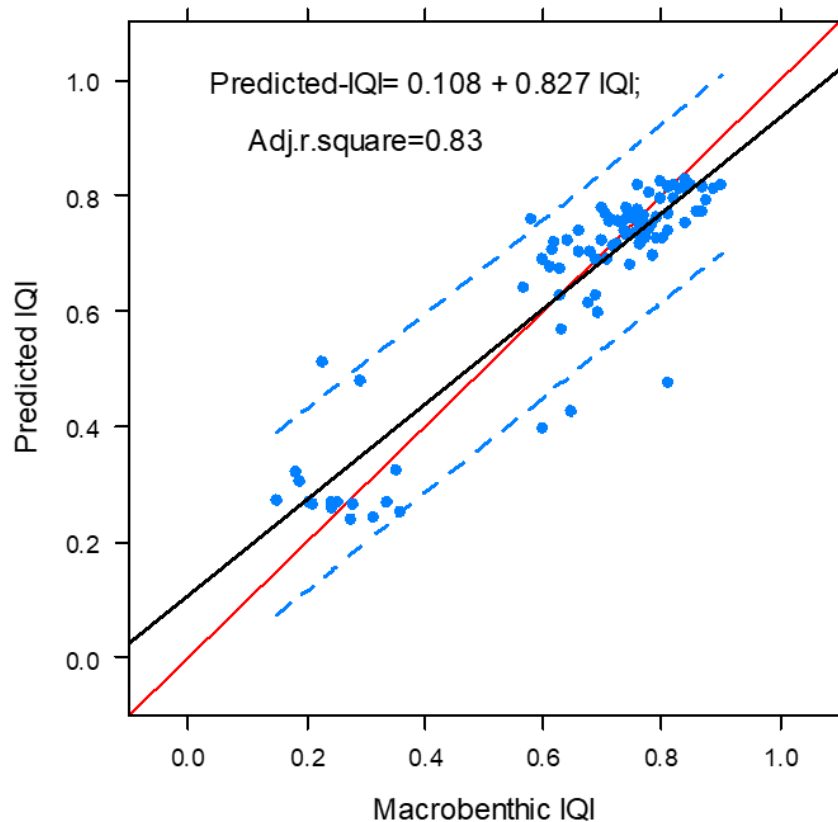
- Very tight clustering of replicates from within the same grab
- Stations very clearly separated
- Low stress and intuitive patterns

Figure 23 Marker:16S, Taxon: Species, Make Unique: FALSE, Remove unassigned: YES, Presence/absence: NO. Stress=0.0904

How good are bacteria at predicting traditional morphology-based metrics and what taxa are driving these patterns?

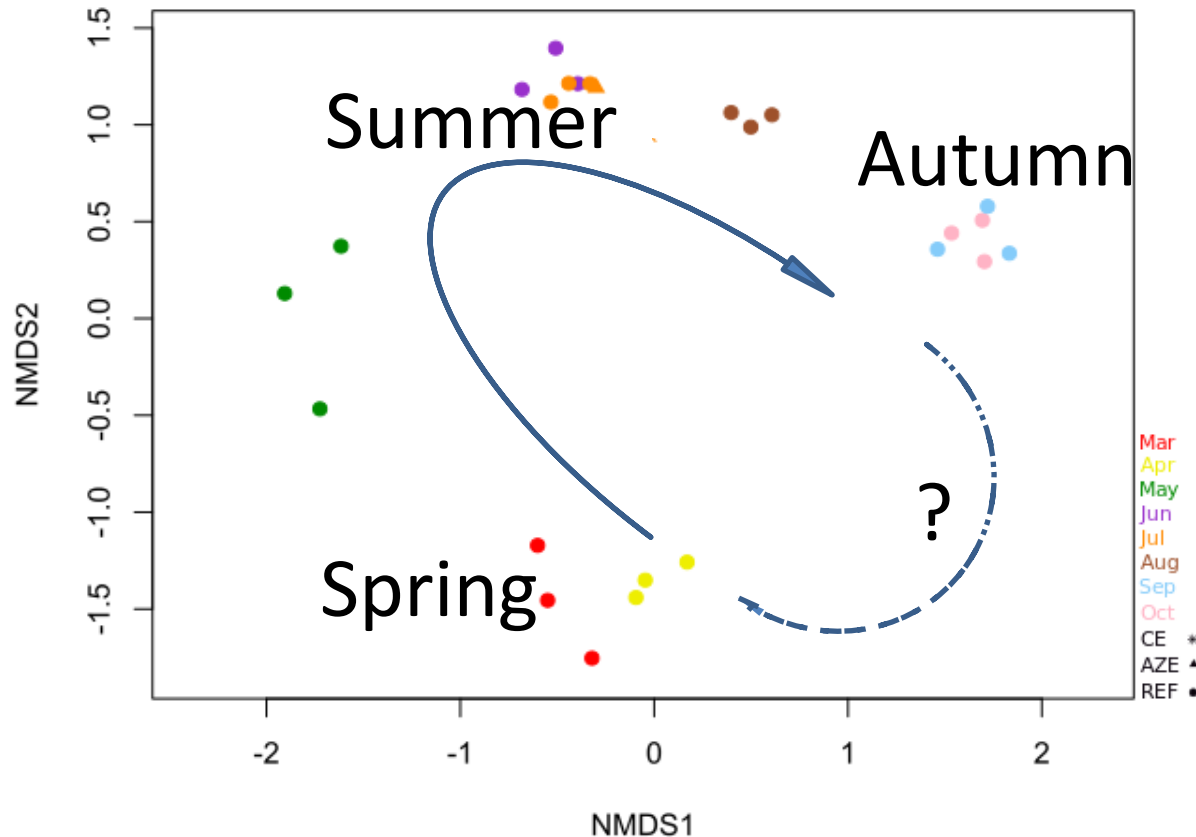


Supervised machine learning - RandomForest



- Ideally a 1:1 relationship between traditional and predicted, with no error
- Already reasonable predictive power (prediction interval shown)
- Primary drivers include member of sulphur metabolising families (Granulosicoccaceae), Xanthomonadales JTB255 group, Flammeovirgaceae and 'unknowns'

Temporal variability (16S)



- Collaboration with Thorsten Stoeck
- Clear seasonal cycle at the reference stations.
- Natural cycles do exist

What does metabarcoding offer?

- Molecular approaches are cheaper, faster , more comprehensive and objective cf macrobenthos
- Can target any species or group (e.g. whales to bacteria)
- Increasingly useful concurrence between macrobenthic and molecular indices (pattern-matching)

Will it be used in regulation/compliance monitoring?

- Yes, molecular will largely replace traditional approaches over the next 3 years.

My thanks to the following for
supporting this research

Questions?

