Beyond taxonomy: Validating functional inference approaches in the context of fish-farm impact assessments

By

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Taxonomic & Functional profiling
(Differences & Advantages)

- Can exhibit different level of stochasticity and temporality;

- Can respond differently to:
    - Substrates;
    - Biogeography;
    - Abiotic environmental variables;
    - Community processes & interactions.

- Having both taxonomic and functional information also enables computation of functional redundancy within the community, which may help assess its resilience;

- Provide complementary information and increase our understanding of how microbial communities are being affected.

Functional profiling
(Approaches)

- Two main options:
  - Shotgun Metagenomics
    - Relatively low-throughput;
    - Expensive;
    - High computational & storage requirements.
  - 16S rRNA-based inference methods (hidden-state prediction based [HSP])
    - Economic alternative to metagenomics;
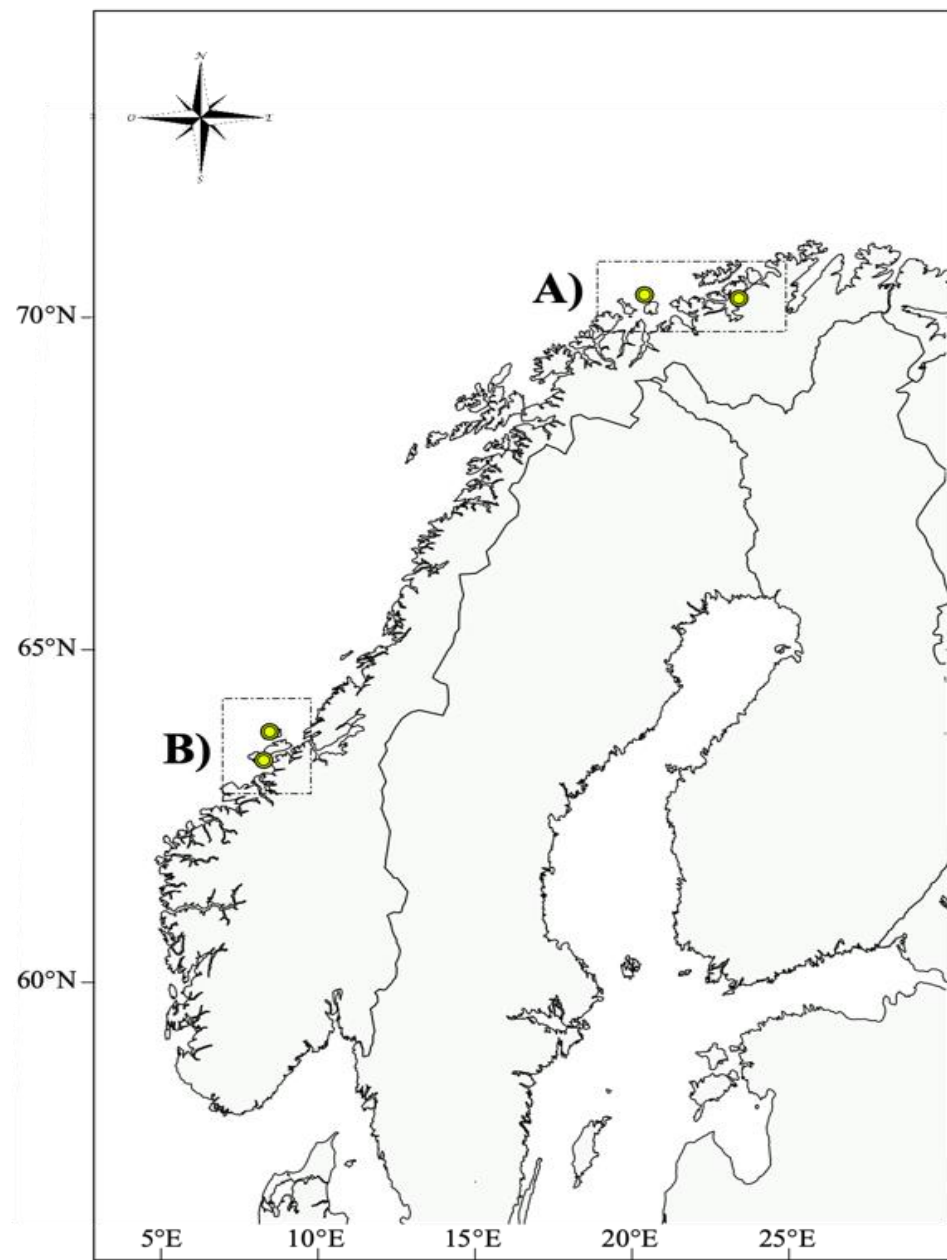   - More sensitive;
    - Less accurate.

Main objective

Evaluate the performance of 3 metabolic inference methods, PAPRICA, PICRUST2 and TAX4FUN2, against metagenomics and environmental data, in the context of salmon farm benthic surveys
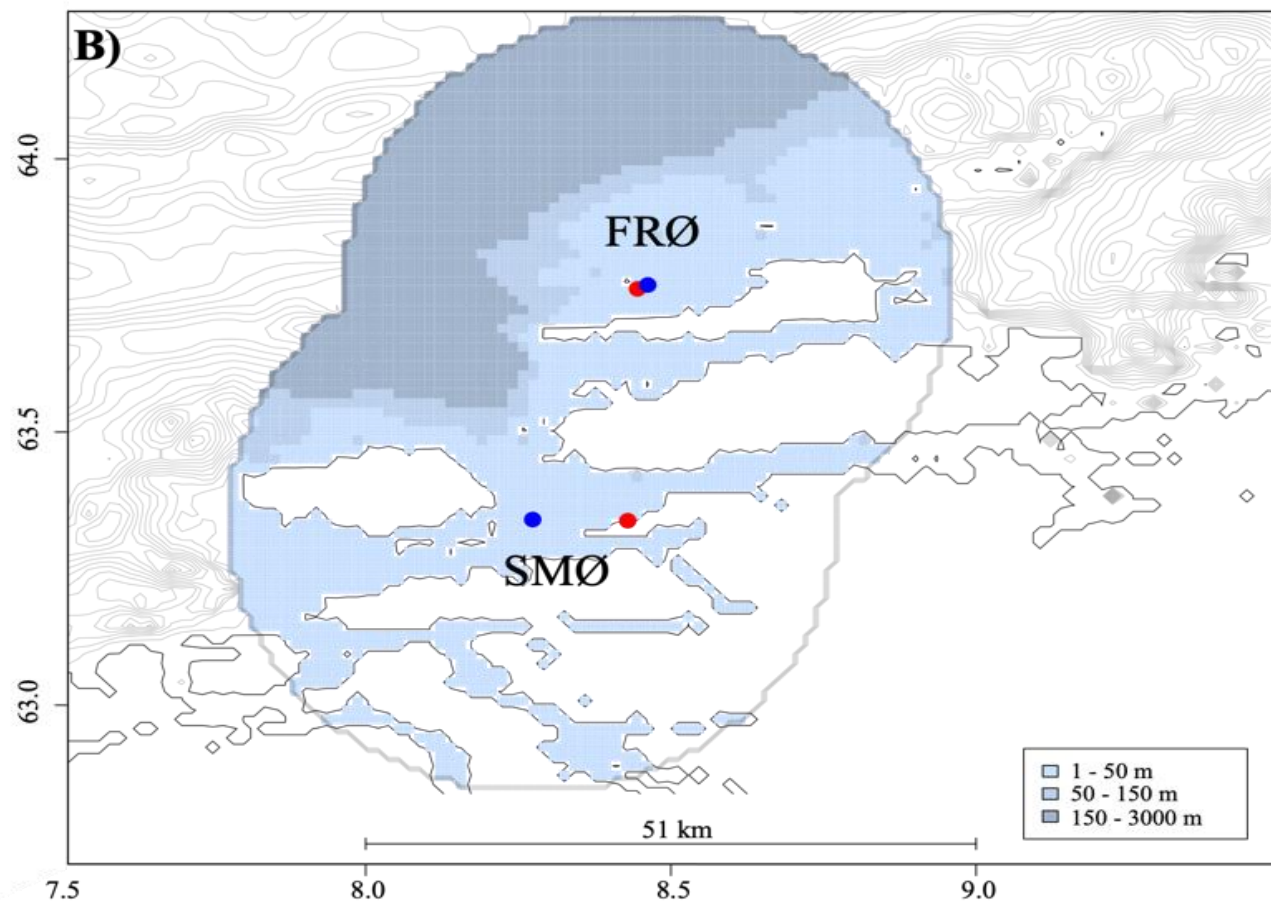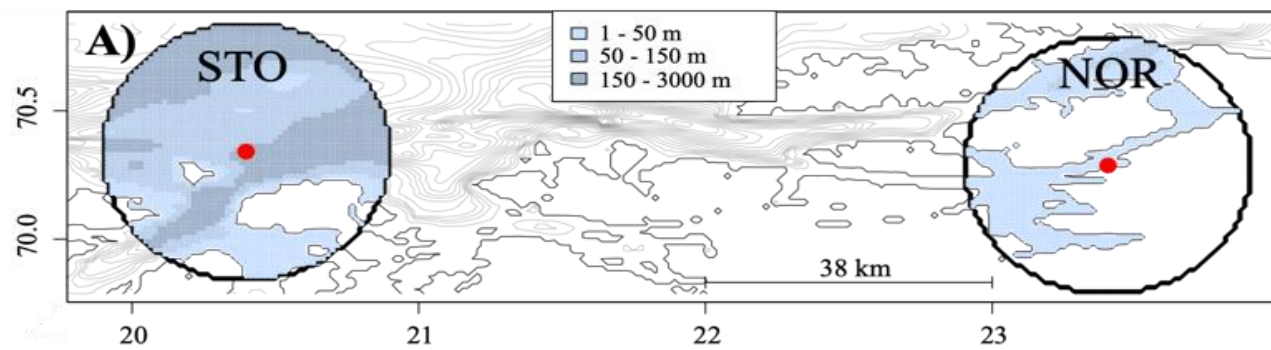
Specific objectives

Contrast the taxonomic and functional microbial diversity recovered from metabarcoding and metagenomics;

Compare predictions of HSP methods against metagenomics;

Compare the sensitivity of functional communities derived from HSP methods and metagenomics towards fish farm activities, and correlation with macro-fauna and physico-chemical data.
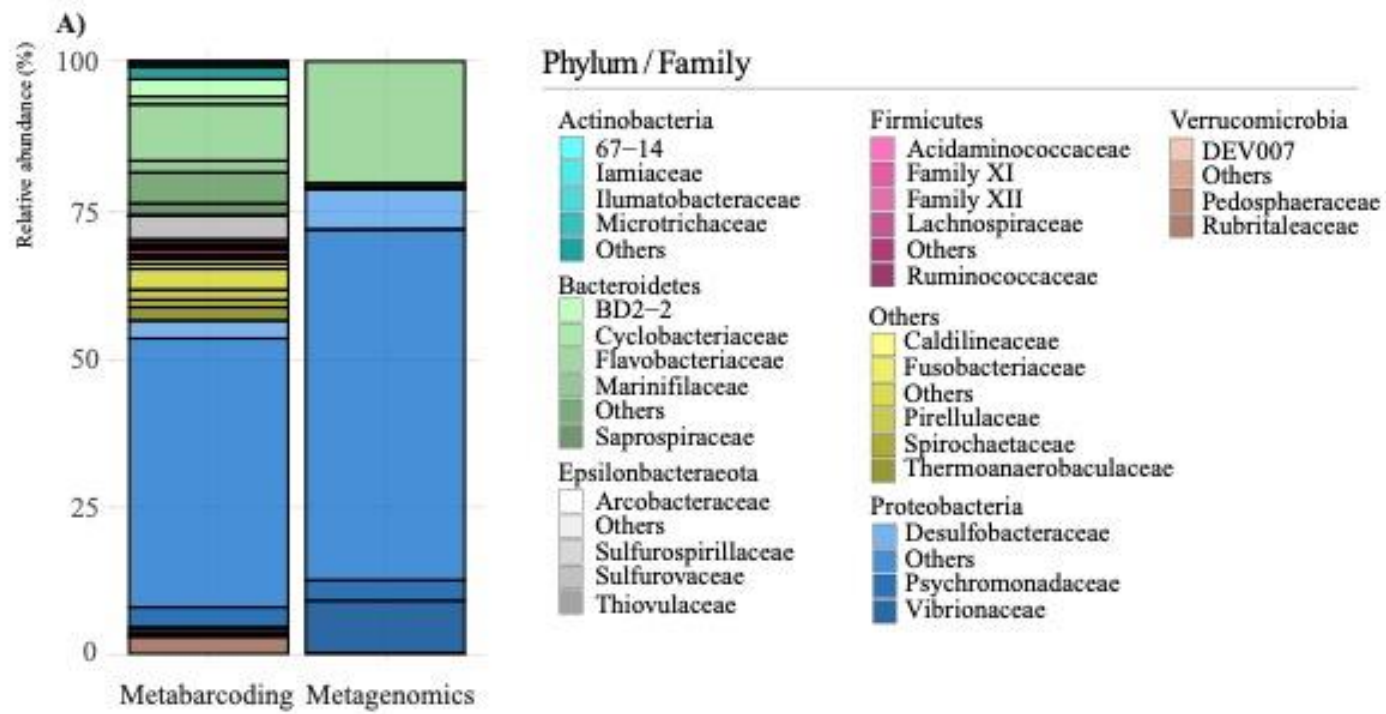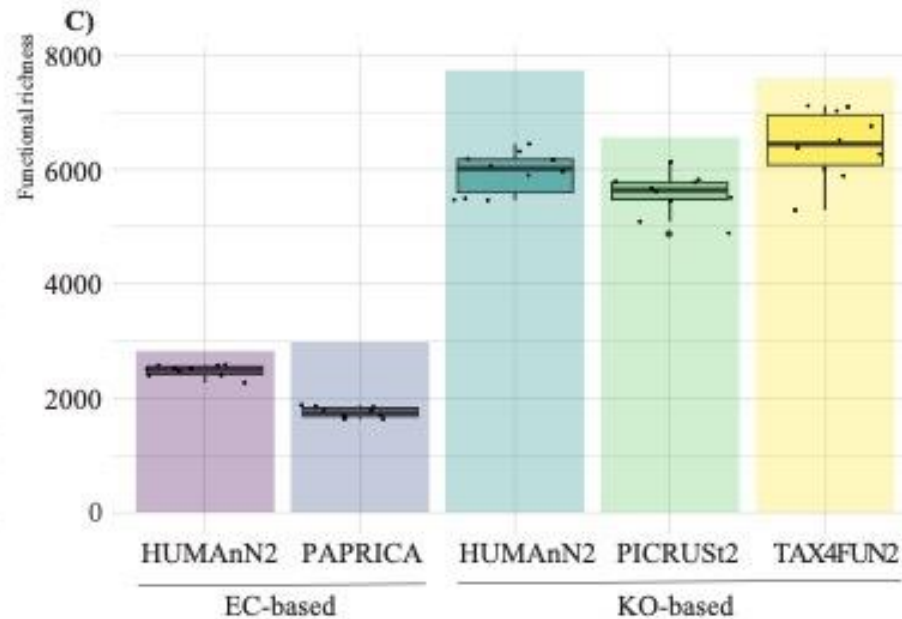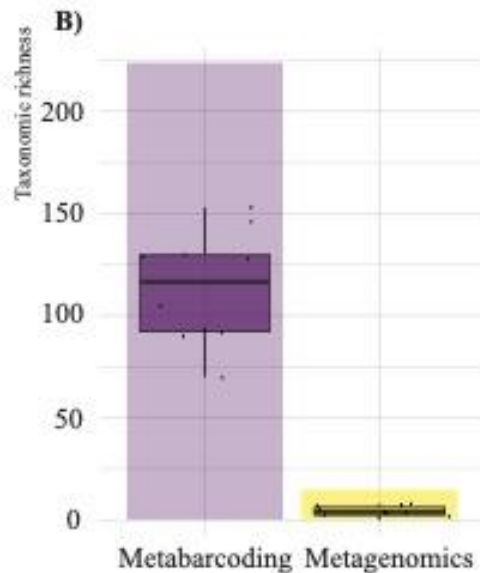
Farm

Pen   Reference site

A) Relative abundance (%) — Metabarcoding vs Metagenomics

Phylum / Family

Actinobacteria
- 67−14
- Iamiaceae
- Ilumatobacteraceae
- Microtrichaceae
- Others

Bacteroidetes
- BD2−2
- Cyclobacteriaceae
- Flavobacteriaceae
- Marinifilaceae
- Others
- Saprospiraceae

Epsilonbacteraeota
- Arcobacteraceae
- Others
- Sulfurospirillaceae
- Sulfurovaceae
- Thiovulaceae

Firmicutes
- Acidaminococcaceae
- Family XI
- Family XII
- Lachnospiraceae
- Others
- Ruminococcaceae

Others
- Caldilineaceae
- Fusobacteriaceae
- Others
- Pirellulaceae
- Spirochaetaceae
- Thermoanaerobaculaceae

Proteobacteria
- Desulfobacteraceae
- Others
- Psychromonadaceae
- Vibrionaceae

Verrucomicrobia
- DEV007
- Others
- Pedosphaeraceae
- Rubritaleaceae

B) Taxonomic richness — Metabarcoding, Metagenomics

C) Functional richness — HUMAnN2, PAPRICA (EC-based); HUMAnN2, PICRUSt2, TAX4FUN2 (KO-based)

Higher taxonomic diversity recovered by 16S rRNA metabarcoding;
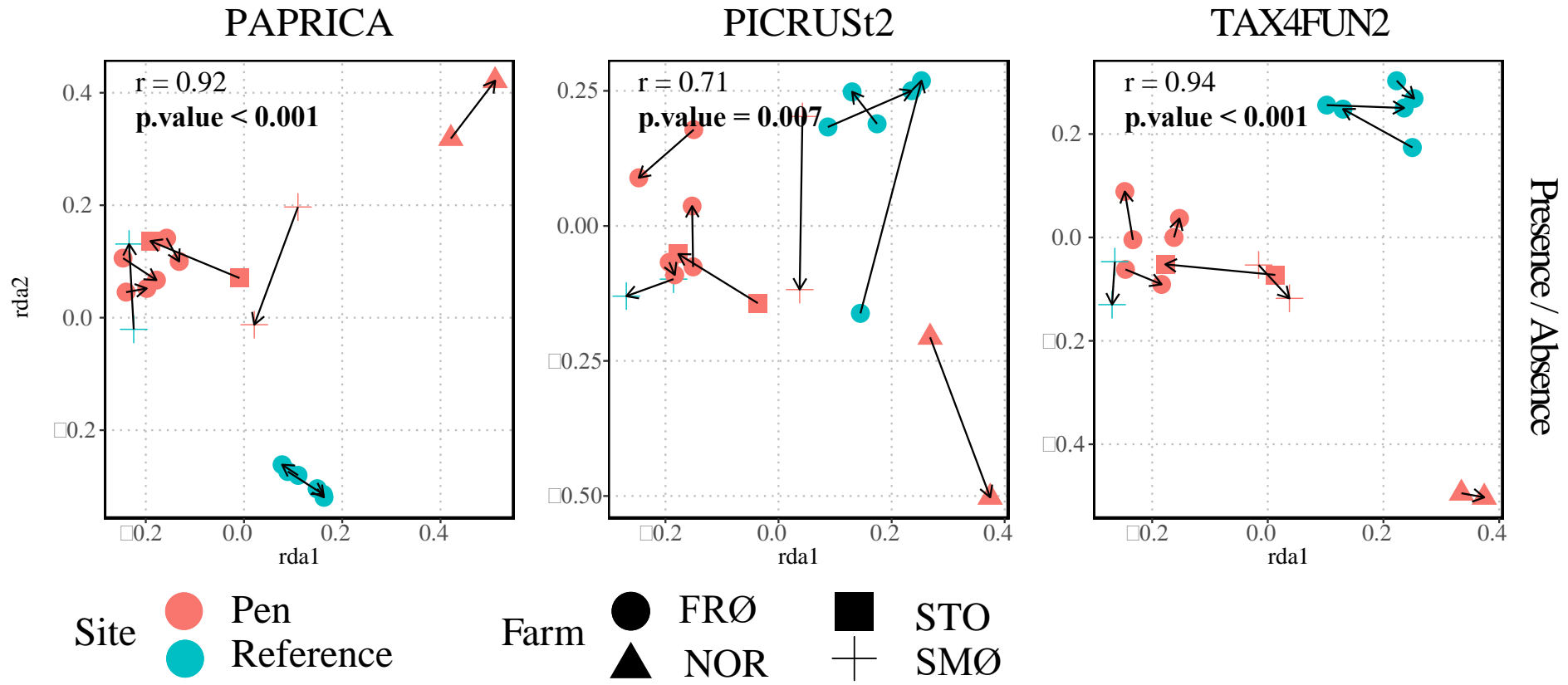
Similar functional richness between HSP methods and metagenomics.
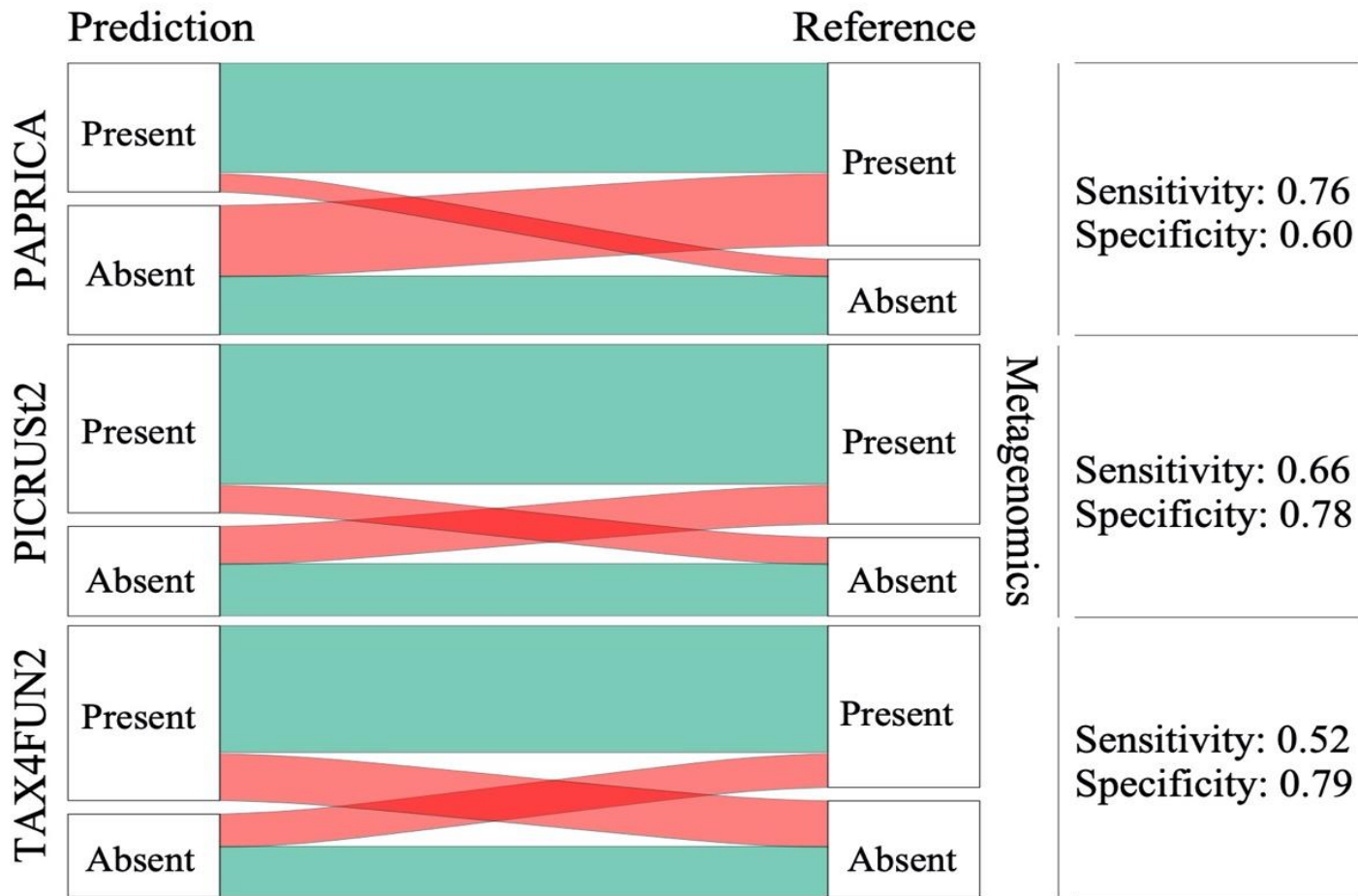
Functional inference methods versus metagenomics data

- True sensitivity of inference methods likely underestimated;
- Specificity affected by incompleteness of reference database AND by functions occurring at shallow phylogenetic depth.

High and significant correspondence between response of functional communities between HSP and metagenomic data.

Procrustes analysis

Protest analysis of the 16S rRNA gene metabarcoding-based and metagenomics data with macrofauna and physico-chemical data

Transformation	Method	Macrofauna		Physico-chemical	
		r	p.value	r	p.value
	ASVs	0.41	0.453	0.72	0.111
	PAPRICA	0.90	**<0.001**	0.98	**0.048**
Presence Absence	PICRUSt2	0.69	**0.011**	0.50	0.126
	TAX4FUN2	0.95	**<0.001**	0.74	0.126
	HUMAnN2 (EC)	0.90	**<0.001**	0.96	**0.004**
	HUMAnN2 (KO)	0.89	**<0.001**	0.67	0.211

Permutational analysis of variance of the taxonomic (amplicon sequence variant; ASVs) and functional communities between distance categories (pen versus reference sites) per methodology

		Distance from pen	
Transformation	Method	R^2	p.value
Presence Absence	ASVs	0.17	**0.014**
	PAPRICA	0.39	**0.012**
	PICRUSt2	0.26	**0.025**
	TAX4FUN2	0.40	**0.008**
	HUMAnN2 (EC)	0.24	**0.045**
	HUMAnN2 (KO)	0.24	**0.029**

Take home message

- Although functions captured by both approaches slightly differed, they responded similarly to environmental changes. This indicate that HSP methods represent a valuable tool in detecting and evaluating the effects of salmon farming on benthic ecosystems.

- Functional profiles may be slightly more robust and sensitive in detecting environmental alterations than taxonomic profiles as they appear less affected by biogeography, community processes and interactions, and more so by environmental conditions.