



Corso di Dottorato di Ricerca in Scienze della Vita e dell'Ambiente - Ciclo XXXVIII

Assessment of biodiversity metrics for restoration of macroalgal forests

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Several studies have shown that *Cystoseira sensu latu* forests are characterized by highly diverse associated macroalgal assemblages. Large-scale studies (Sales et al. 2012, Piazzzi et al. 2018) show that *Cystoseira s.l.* forests in the Mediterranean sea are often characterized by the presence of a common set of macroalgal epiphytes. The relative composition of the epiphytic assemblages associated to *Cystoseira s.l.* forests appears to be influenced by biogeographic patterns related to latitude and longitude, as reported by Sales et al. (2012) for 101 sites in the Mediterranean Sea. However, the relationship between conservation status of *Cystoseira s.l.* forests and composition and abundance of associated epiphytic assemblages has not been investigated yet.

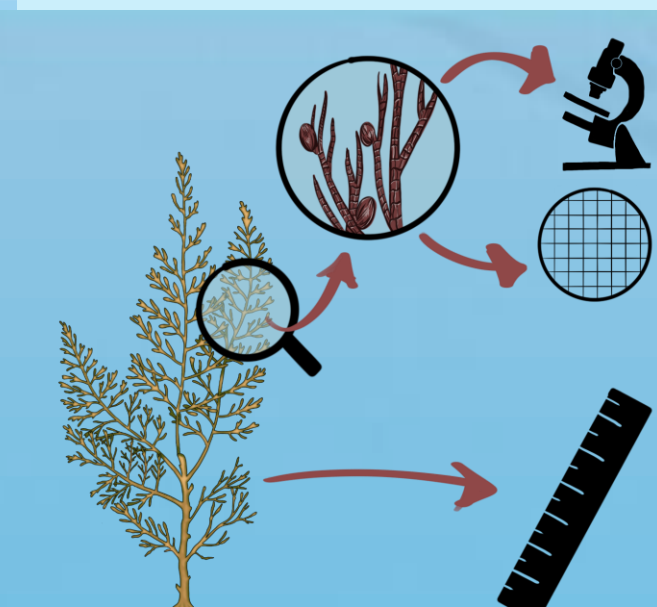


Hypothesis

Composition and abundance of algal epiphytes differ significantly among continuous and fragmented forests.

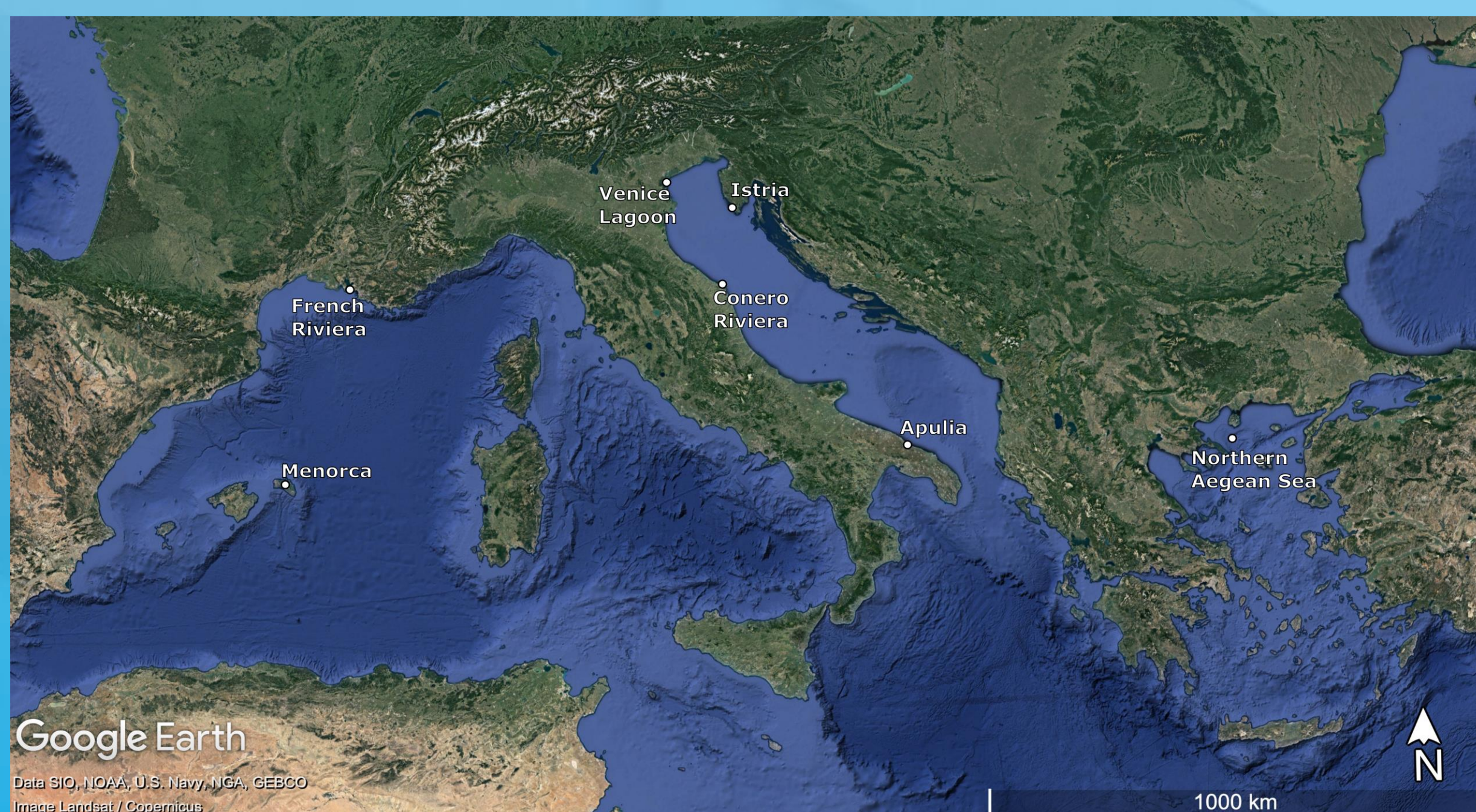


Traditional microscopy analysis



Epiphytic assemblages associated with forests of *Gongolaria barbata* and *Ericaria amentacea* and *E. mediterranea*. under different conditions of density (continuous to fragmented) are being investigated in 7 regions.

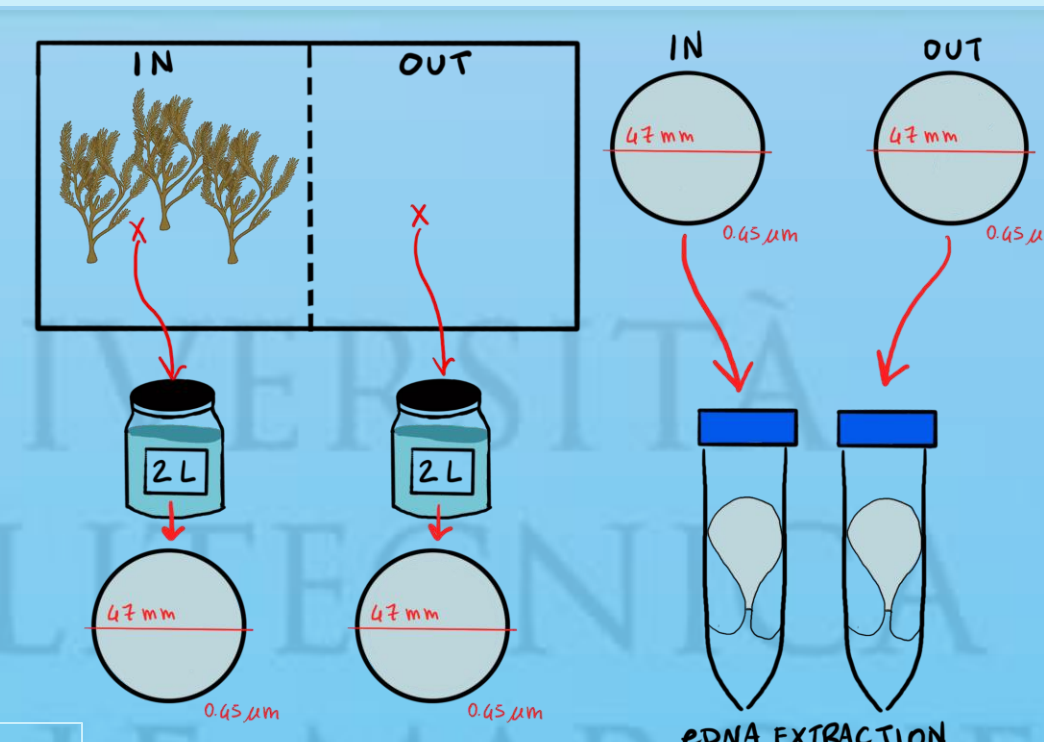
Nested Sampling Design: 2 plots randomly selected in each site, 3 thalli randomly selected in each plot, 3 branches (~20 cm long) collected from each thallus.



Molecular barcoding and metabarcoding

Barcoding analysis from macroalgal tissues with specific primers:

- *tufA* (plastid elongation factor)
- *rbcl* (rubisco large subunit)
- ITS (internal transcribe spacer)
- *psbA* (photosystem II protein D1)
- **23S** (flank Domain V of the 23S plastid rRNA gene).



23S sequences used to create a **high-quality database** for metabarcoding analysis.

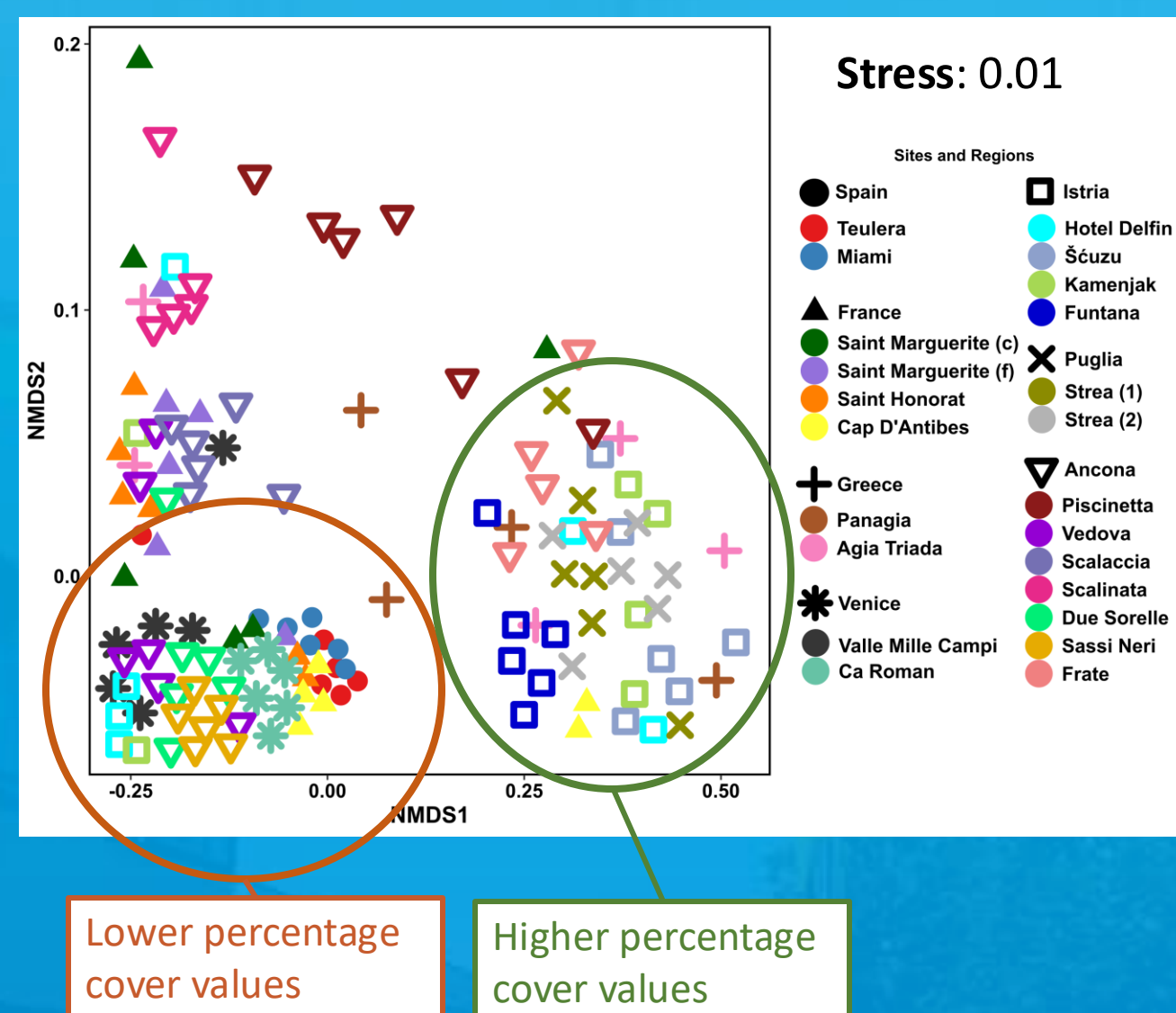
Water samples:

4 *G. barbata* forests.
2L collected INSIDE the forests + 2L collected OUTSIDE the forests

Samples filtration with **EMC filters** (47 mm; 0.45 µm)

DNA extraction
DNA quantification

QIIME2 and **Rstudio** for bioinformatic analysis



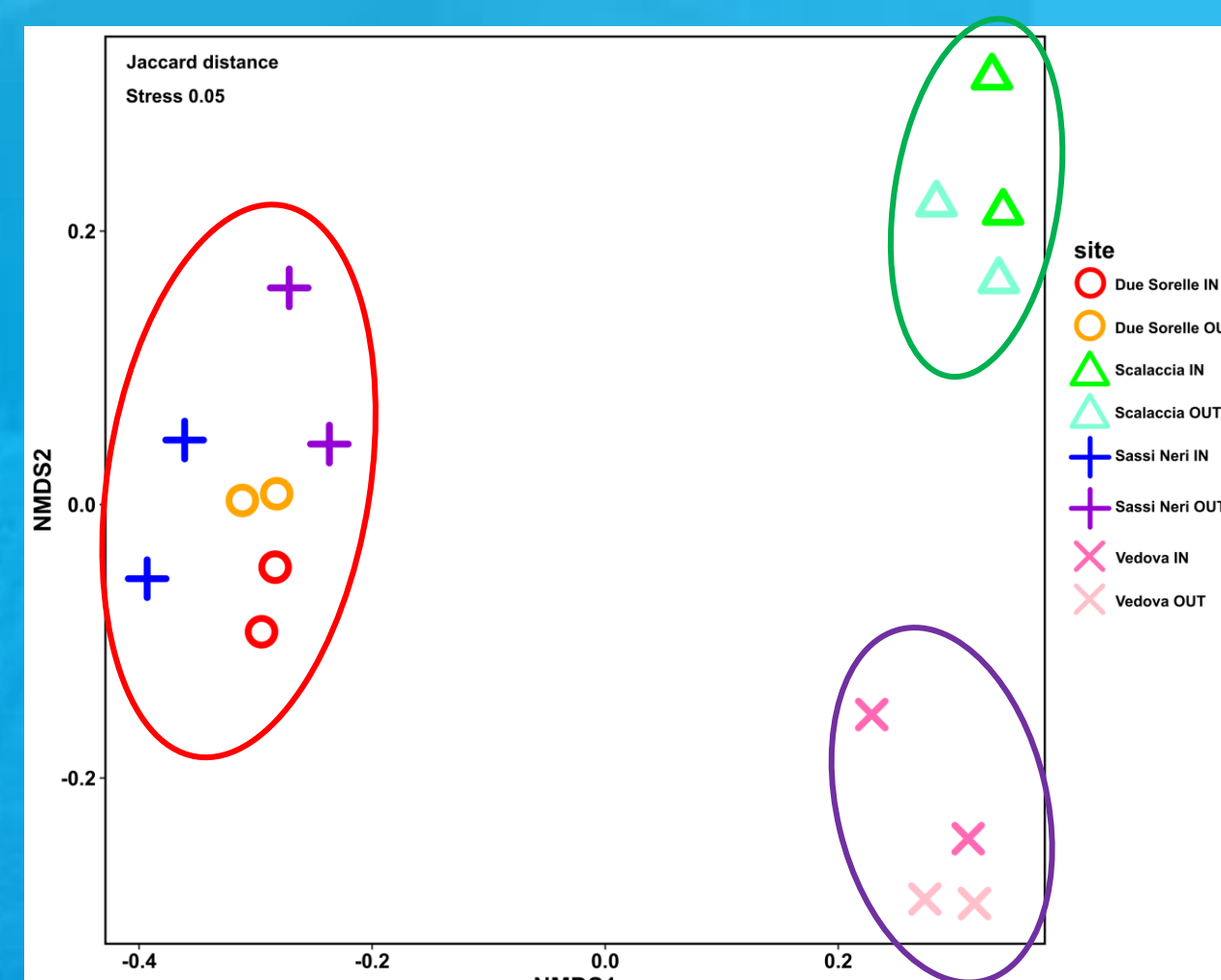
Gongolaria barbata, NMDS

- number of epiphytes species
- epiphytes percentage cover
- number of functional groups

Are data grouped by sites?

Anosim R 0.49 ; $p < 0.0001$

In **both** plots, the **distribution** of samples appears to be **mainly driven** by the **percentage cover** of epiphytes on the basiphytes.



NMDS: OTU presence/absence

Are data grouped by sites?

R 0.92 ; $p < 0.0001$

The distribution of samples appears to reflect the **geographical distribution** of the sites, with **no evident differences** between samples collected **inside** and **outside** the macroalgal forests.

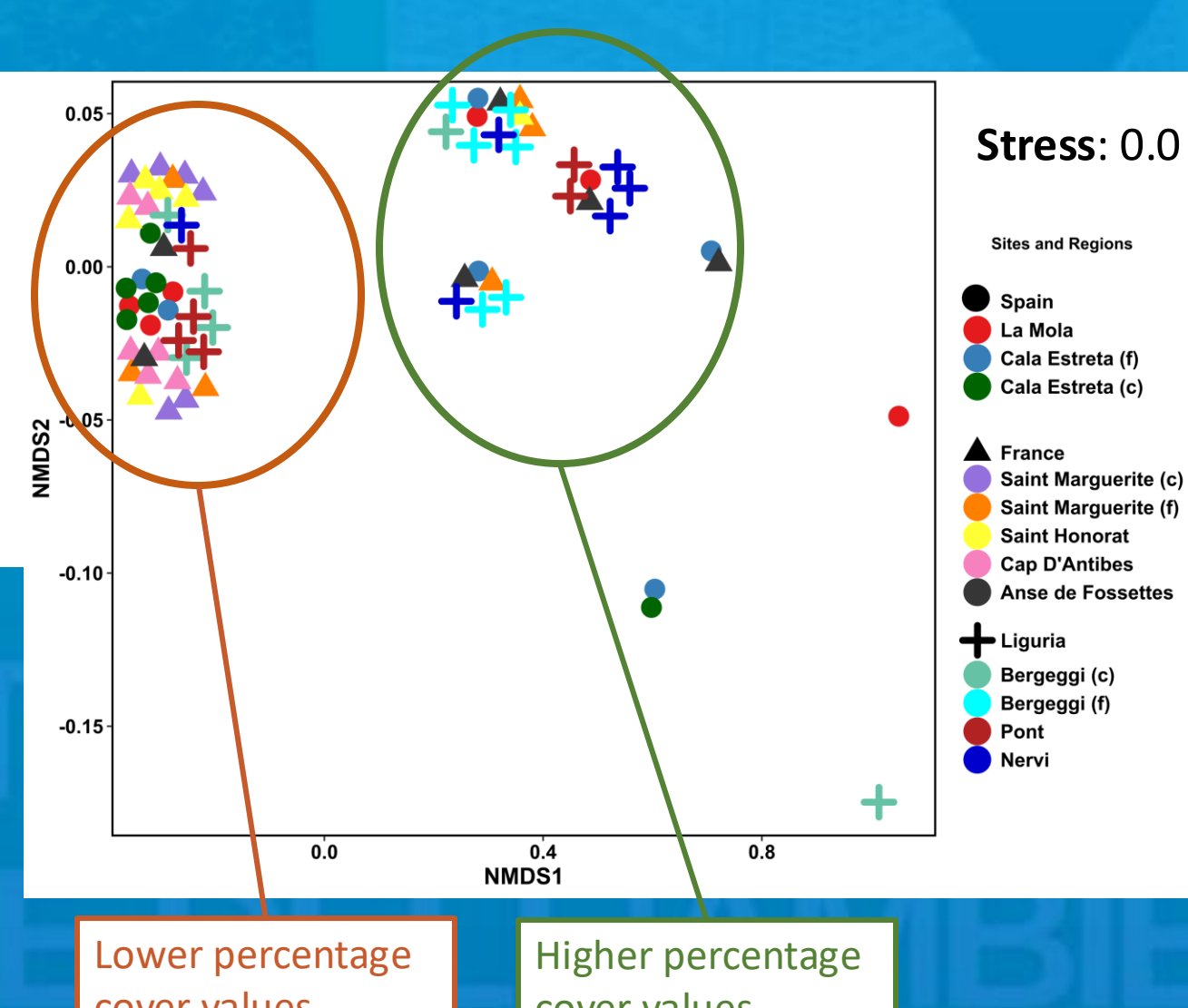
Ericaria spp., NMDS

- number of epiphytes species
- epiphytes percentage cover
- number of functional groups

Are data grouped by sites?

Anosim R 0.14 ; $p < 0.0001$

The analysis did **not** reveal evident **differences** in the abundance and composition of the epiphytic community in **relation to macroalgal forest density**.



NMDS: identified TAXA presence/absence in each site and each sampling method

Are data grouped by sites?

R -0.004 ; $p > 0.4$

Are data grouped by sampling method?

R 0.98 ; $p > 0.002$

A **separation** of the data is **evident** depending on the **analysis method**: samples identified by traditional microscopy are grouped on the right side of the plot, while those identified by metabarcoding are grouped on the left.

