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BIOMARKER GENES FROM COASTAL ECOSYSTEM MONITORING: A STRONGHOLD FOR THE ASSESSMENT OF HEAVY METALS IMPACT

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WHY FOCUS ON MEDITERRANEAN DUNE ECOSYSTEM?

Coastal dunes are among the most unique habitats on Earth, characterized by complex environments, where flora and fauna exhibit highly specialized adaptations.

Coasts serve as ecotones between sea and land, creating dynamic ecosystems that interact with various land use systems and urban environments. These interactions increase human pressure on coastal areas.

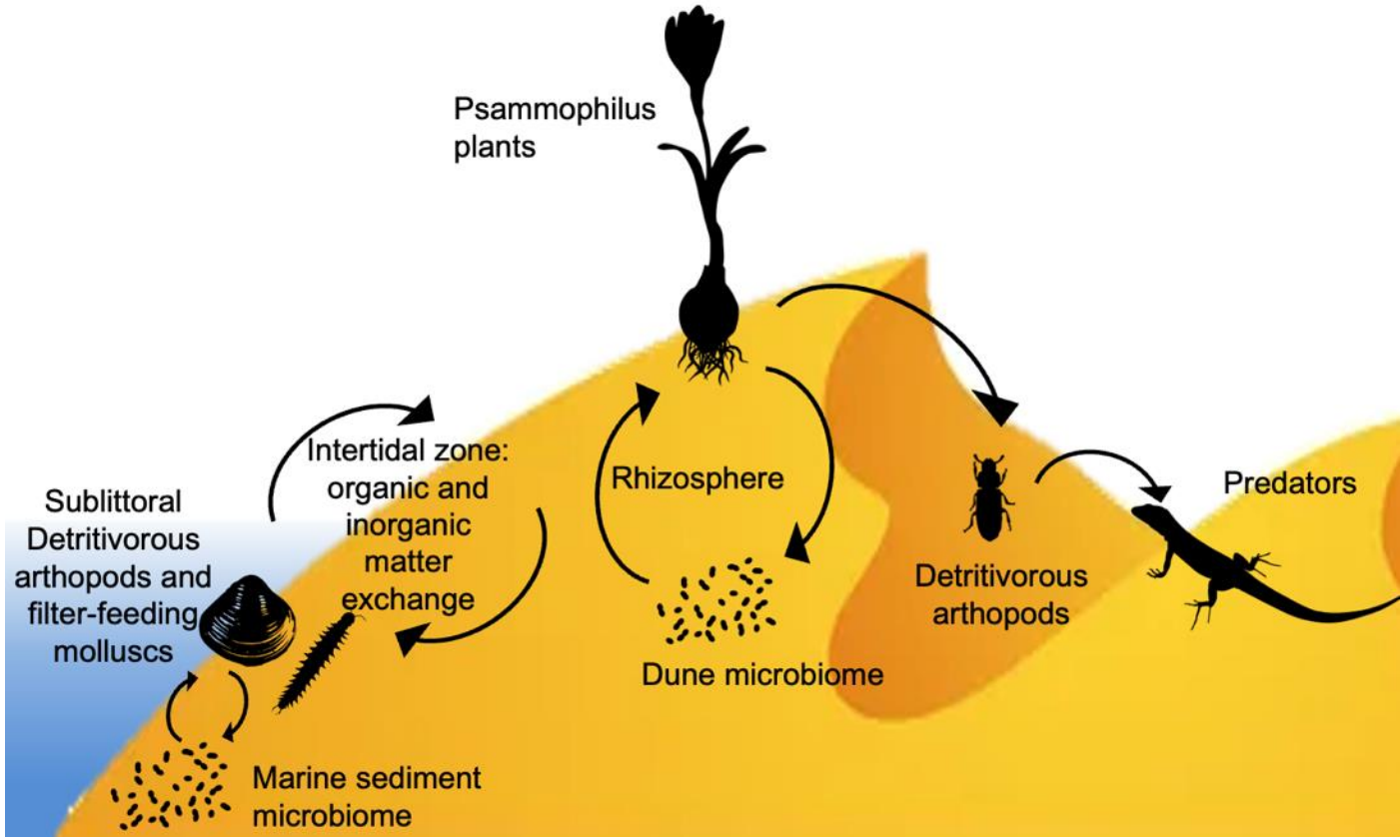
THREATS TO COASTAL DUNES

- Despite their ecological value and the services they provide, **coastal dunes are among the most endangered habitats**
- Increasing urbanization, chemical pollution, mass tourism, coastal erosion, sea-level rise, and the predicted increase in extreme climate events critically threaten this fragile ecosystem.
- **The fate of coastal dunes is particularly uncertain in the Mediterranean area**, where many habitats were recently classified as “threatened” and where the consequences of sea-level rise and extreme events are predicted to be especially severe.

Heavy metals & food webs

Among the main threats, heavy metals arising from anthropogenic sources enter food webs through soils and marine sediments.

Heavy metals may be transferred from soils to plants, invertebrates, and grazers, and may ultimately bioaccumulate in the organism at the higher levels of the trophic chains such as birds, reptiles and mammals



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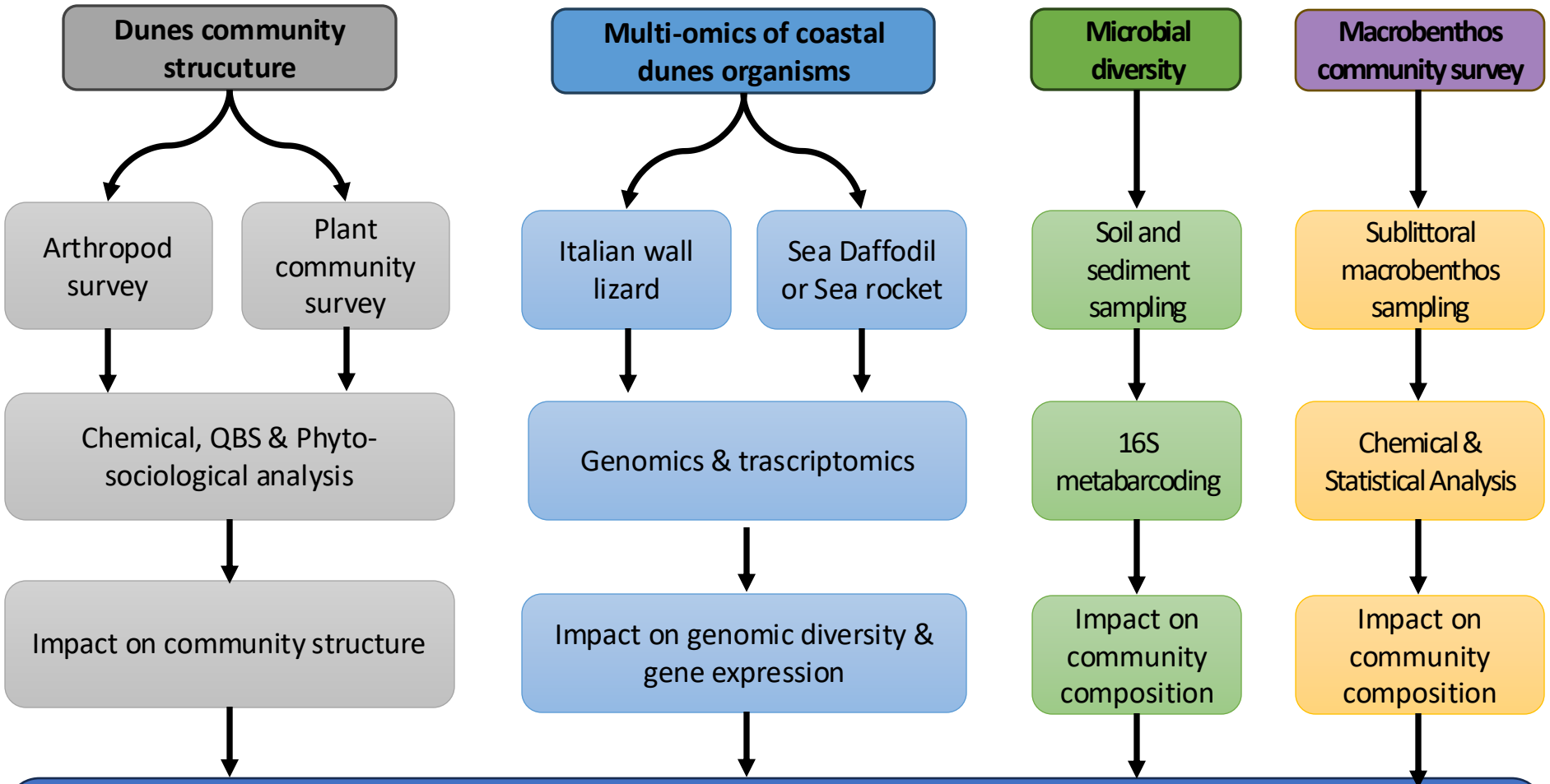
Koral - biomarker from coastal monitoring for the assessment of heavy metals impact

The project Koral is based on four main pillars:

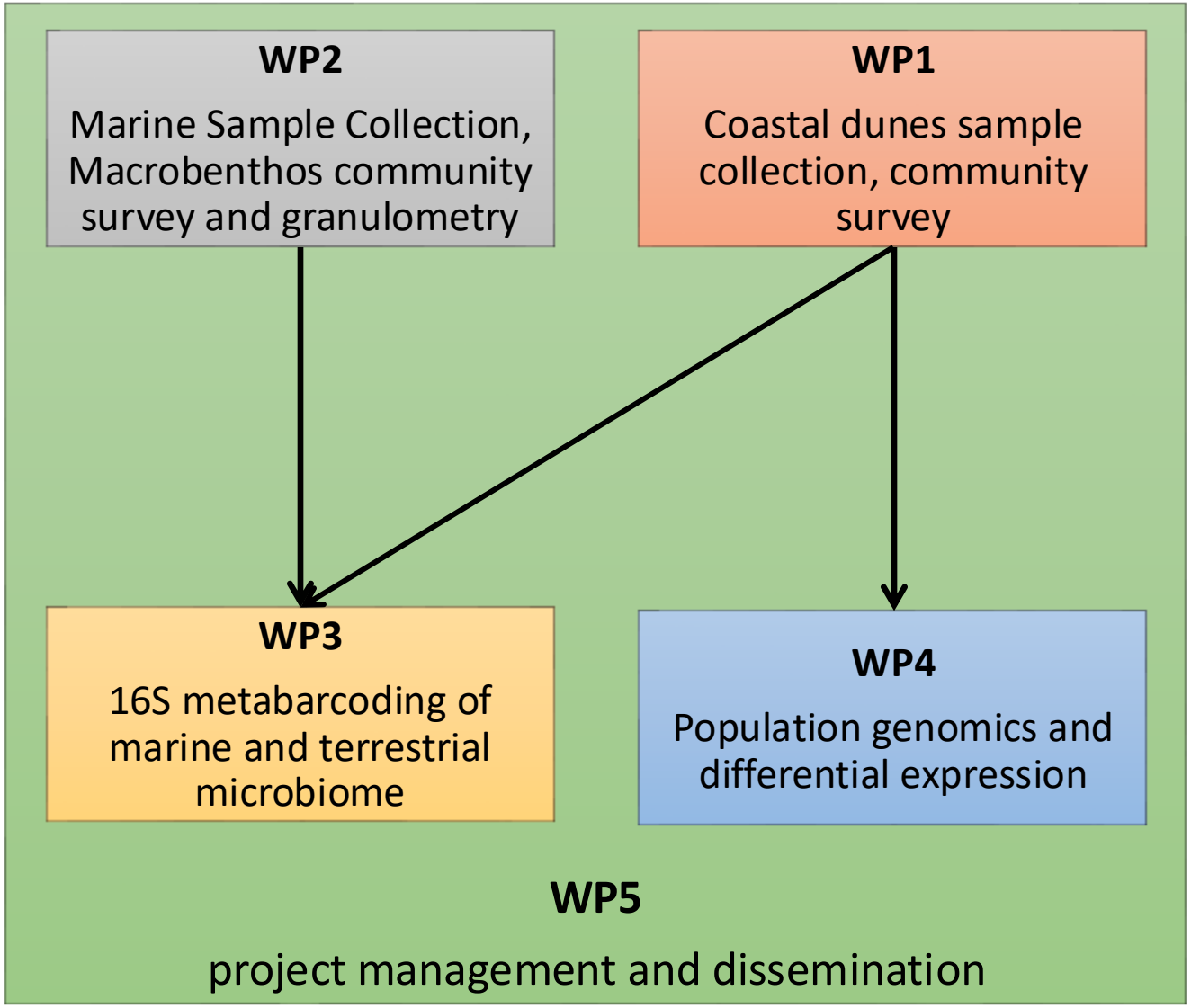
- **Ecosystemic Approach**
  We adopt a holistic perspective by analyzing both marine and terrestrial ecosystems, focusing on different levels of the food chain.
- **Interdisciplinarity**
  Our work integrates traditional ecological surveys with advanced molecular tools to gain a more comprehensive understanding of environmental processes.
- **Innovative Technology**
  We employ emerging techniques for environmental monitoring and focus on developing novel early warning biomarkers to detect ecosystem changes.
- **Knowledge Advancement**
  The project aims to generate new data that will enhance scientific research and support evidence-based decision-making for environmental management.

How much and at what organization level (molecular up to communities) heavy metals impact on Mediterranean Coastal ecosystems? Is it possible to identify new early warning biomarkers?



Ecosystem understanding at different organization level (from molecules to communities) of the impact on heavy metals on Mediterranean Coastal ecosystems.
Identification and characterization of new putative biomarkers

Working packages



Field activities

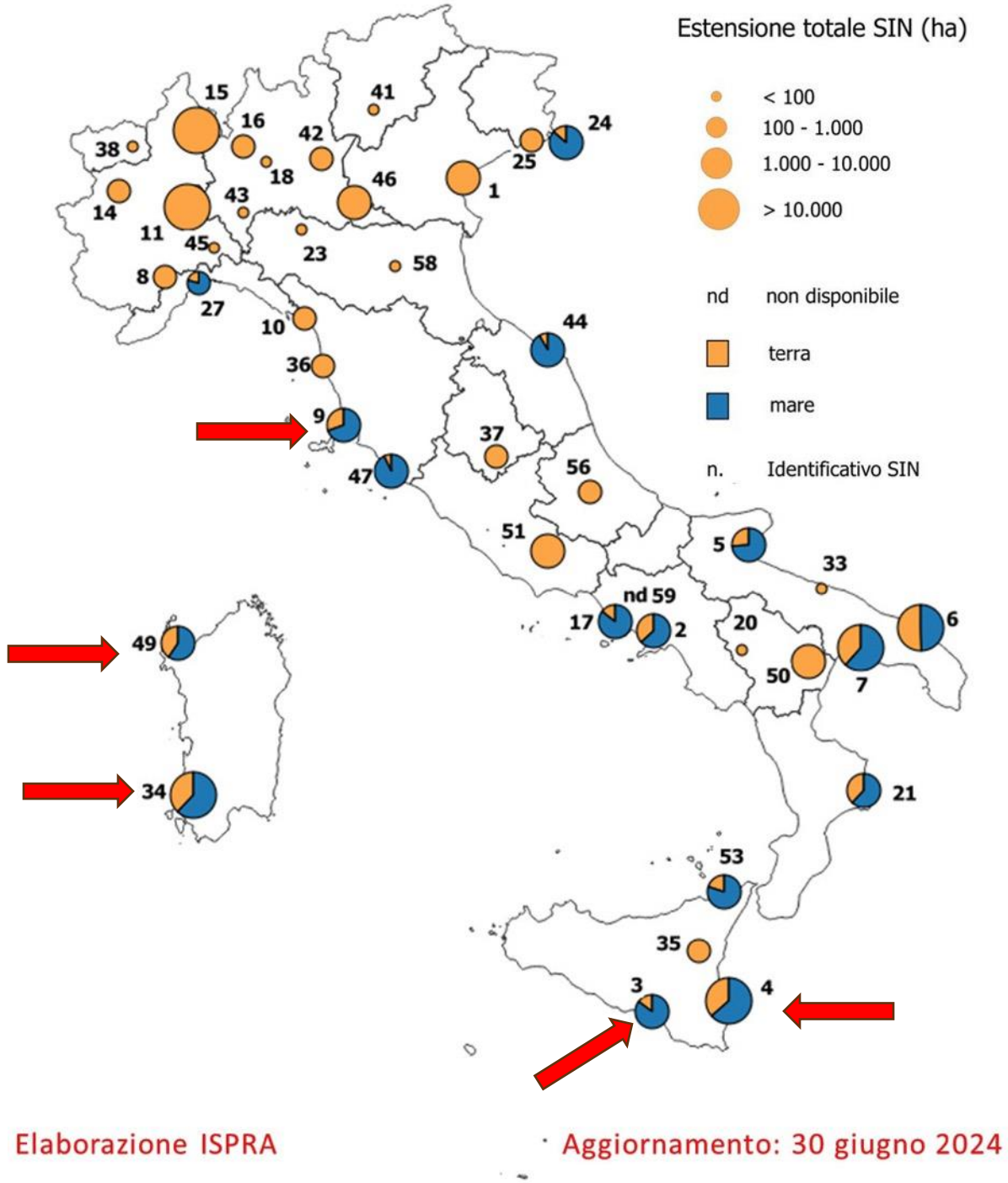
Lab activities

Siti di Interesse Nazionale (SIN)

SINs are extensive portions of the national territory of environmental value

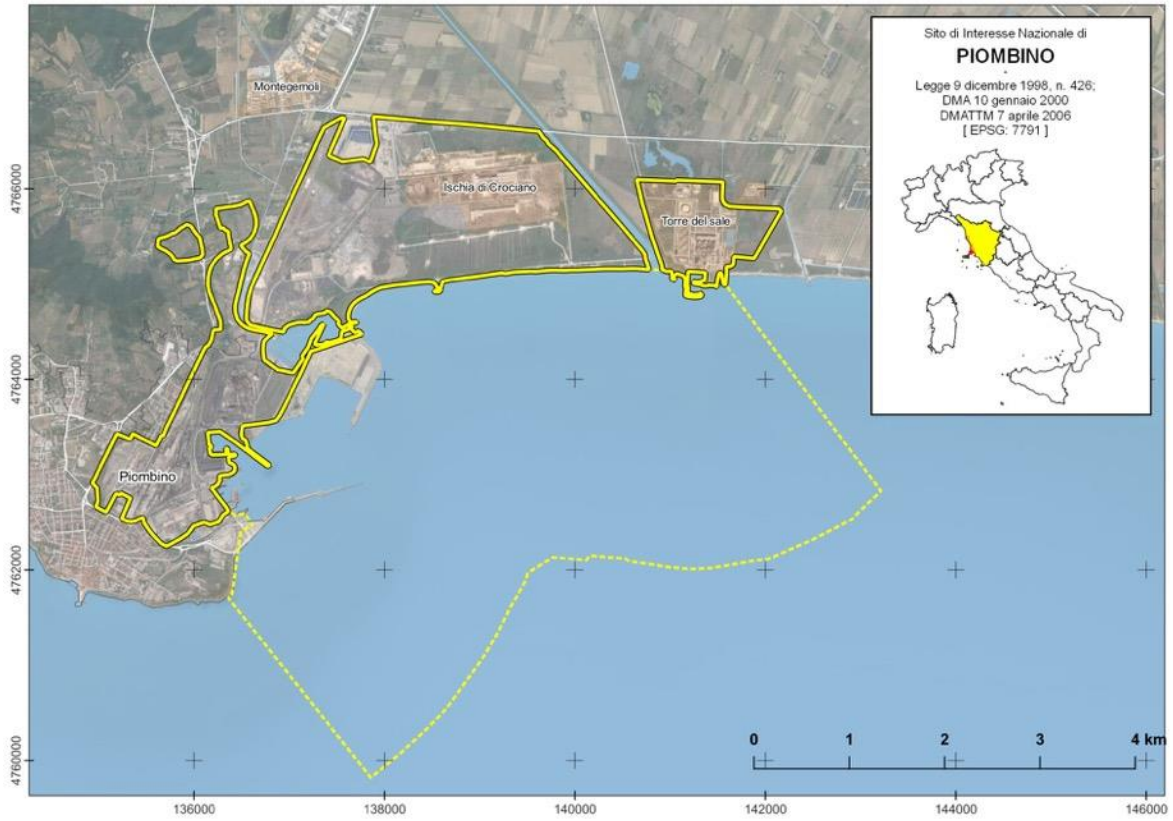
These sites are identified by law for the purpose of remediation, based on characteristics that pose a high health and ecological risk due to population density or the extent of the site itself.

They also have a significant socio-economic impact and pose a risk to assets of historical and cultural interest.

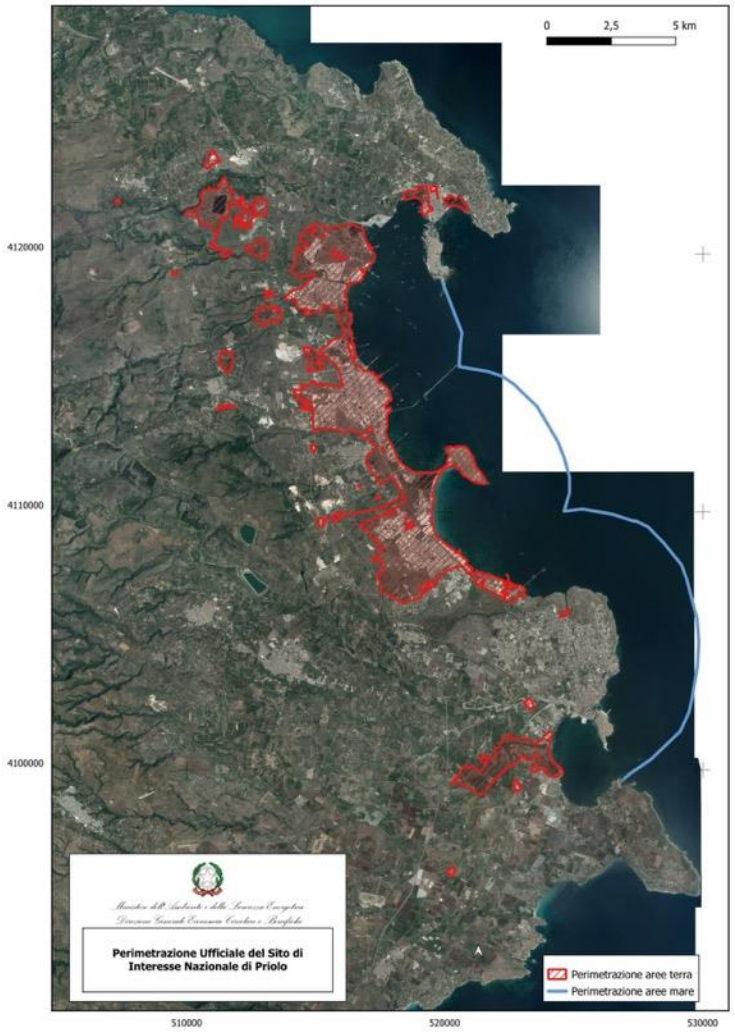


Sites of National Interest (SIN)

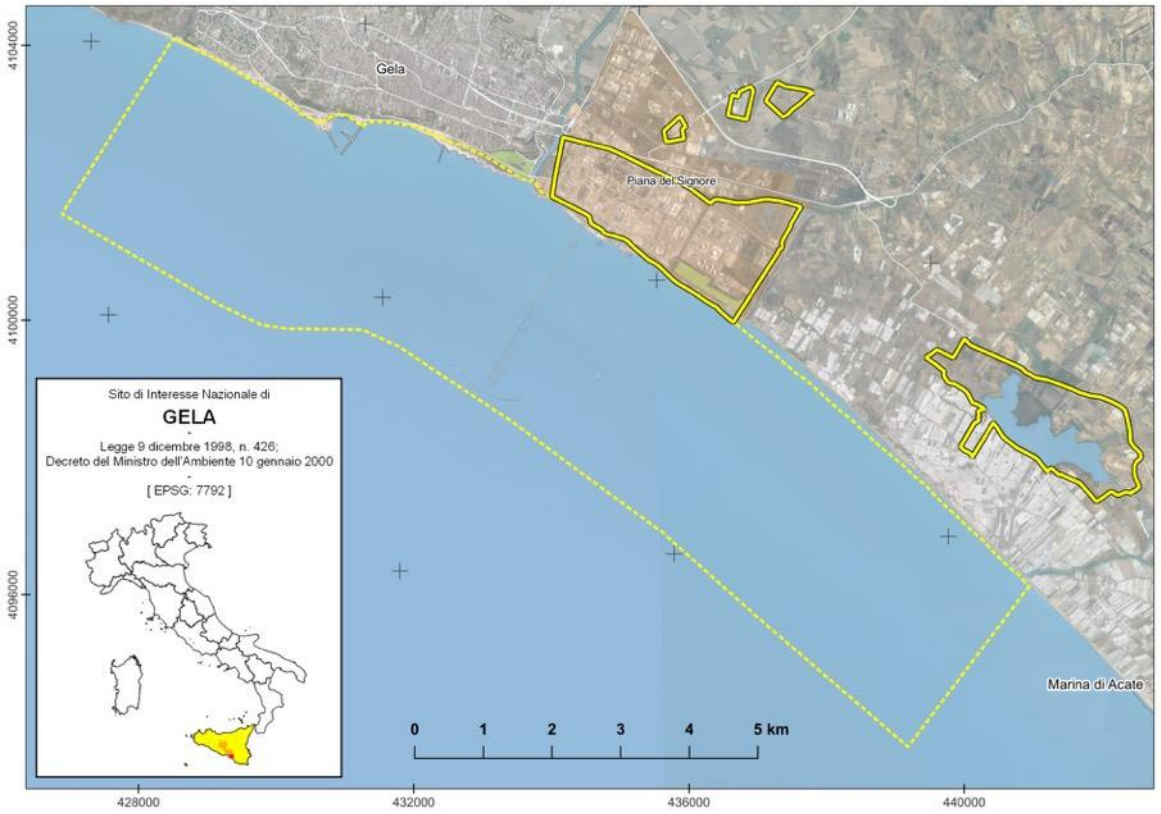
Piombino (Tuscany)



Sites of national Interest (SIN)



Priolo and Gela (Sicily)



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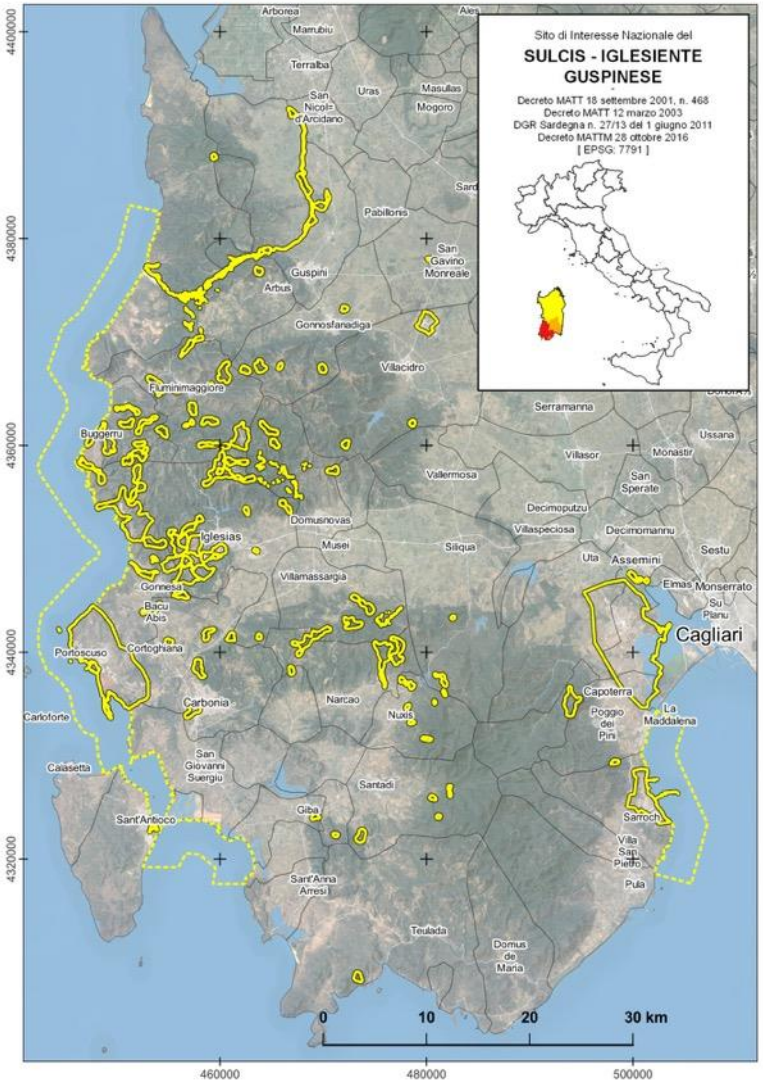
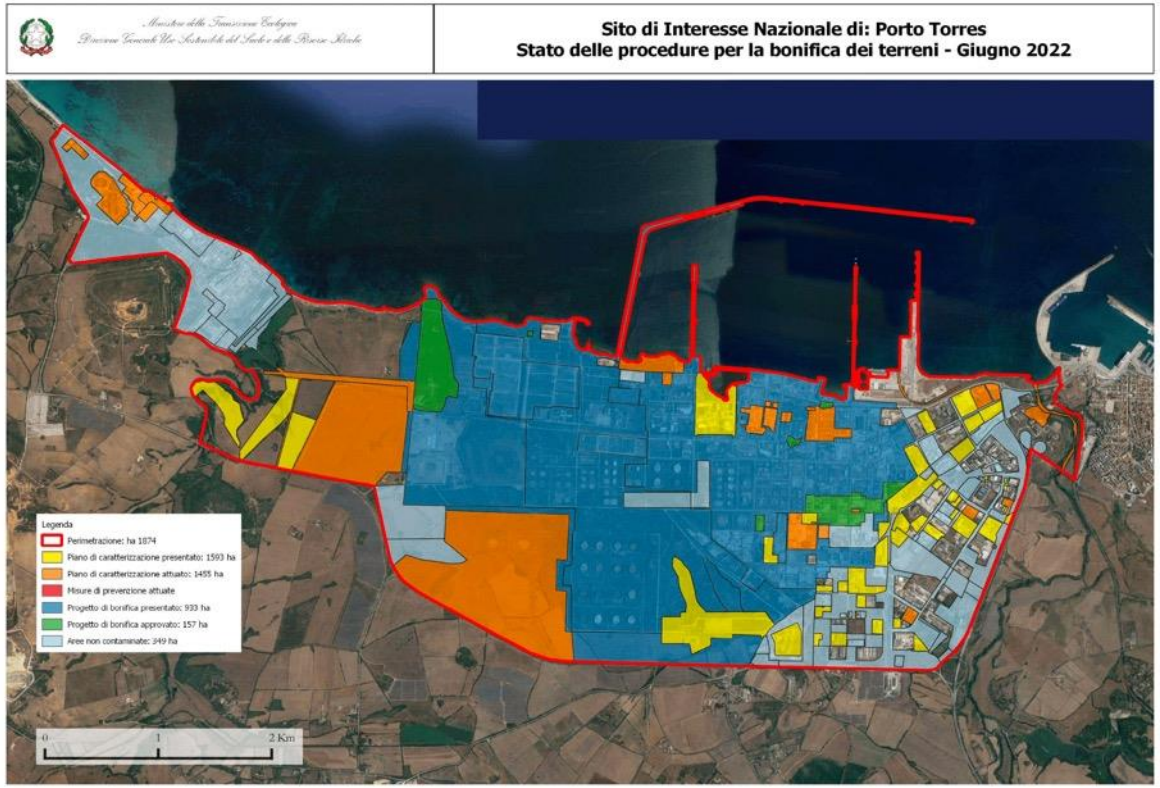
Sites of national Interest (SIN)



Porto Torres & Iglesias (Sardinia)



WP1 - Coastal dunes soil sample collection, community survey and contamination assessment

- Define the structure of the plant community along the coastal dune system
- Capture spatial variation in vegetation along environmental gradients
- Identify the area influenced by the presence of *Cakile maritima* (Sea rocket)

Abbreviation	Embryo dunes	Mobile dunes	Fixed dunes	Wooded dunes
EU habitat type name	Embryonic shifting dunes	Shifting dunes along the shoreline with *Ammophila arenaria* ('white dunes')	Coastal dunes with *Juniperus* spp.	Wooded dunes with *Pinus pinea* and/or *Pinus pinaster*
EU habitat code	2110	2120	2250*	2270*
Habitat description	Formation of the first sandy drift with *Elymus farctus*	Seaward and semi-permanent cordons of dune systems dominated by *Ammophila arenaria*	Fixed dunes with pioneer maquis dominated by *Juniperus oxycedrus* subsp. *macrocarpa* and *Juniperus communis*	Backdunes with forest dominated by *Pinus halepensis*, *P. pinea* and *P. pinaster*

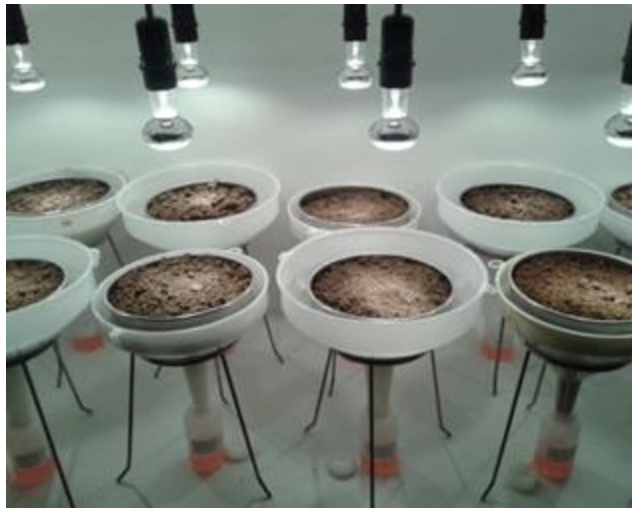
Method

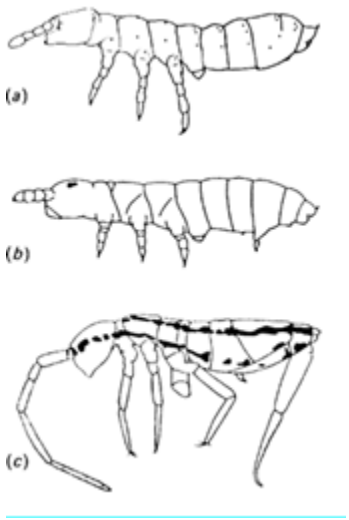
- A linear transect perpendicularly to the shoreline
- Along the transect, systematic or stratified plots of 2x2 m are placed at regular intervals (e.g., every 5 or 10 meters).
- Within each plot, all vascular plant species are identified: the abundance-dominance of each species is estimated

WP1 - Coastal dunes soil sample collection, community survey and contamination assessment

Objectives

- Analyze the soil microarthropod community in the project's sampling area using the QBS-ar protocol.
- Provide a basis for ecological monitoring and comparison across sites of the project.
- Address the limited knowledge of soil microarthropod communities in sandy environments, contributing to a better understanding of their diversity and ecological role.

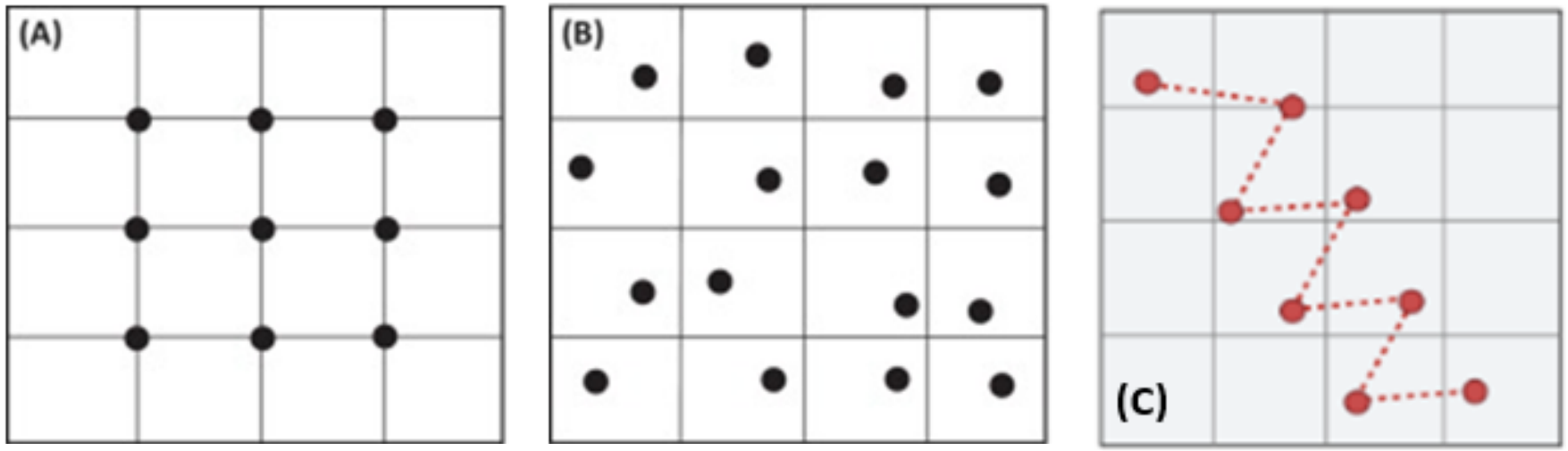




Method

- Soil cores (10×10×10 cm) were collected from multiple dune sites.
- Arthropods were extracted using Berlese-Tullgren funnels (micro-mesofauna).
- Each taxon (biological form) was assigned an Eco-Morphological Index (EMI) score based on adaptation to soil life.
- QBS-ar index = sum of EMI scores of all taxa found.

WP1 - Coastal dunes soil sample collection, community survey and contamination assessment



SOIL PARAMETERS: Heavy metals (Pb, Cd, As, Zn, Cr, etc. based on the characterisation already present in the literature for each selected SIN site), macronutrients, salinity, pH, EC (electrical conductivity), N (nitrogen) and C (carbon) content.

WP1 - Coastal dunes soil sample collection, community survey and contamination assessment

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WP2 - Marine Sample Collection, Macrobenthos community survey and granulometry

The Marine Strategy Framework Directive employs indicators based on macrobenthos parameters (e.g., density, biomass, diversity) to measure the 'health' of marine ecosystems

Coastal marine sediments near industrial areas often accumulate heavy metals and pollutants. Macrobenthic communities, due to their limited mobility and ecological role, serve as indicators of environmental quality and contamination.

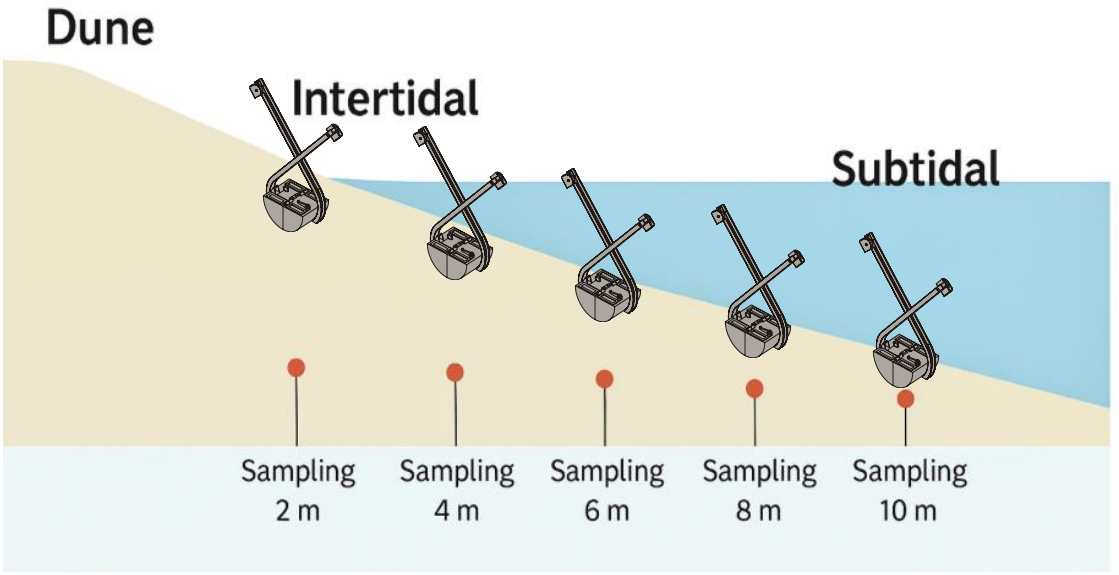
WP2 - Marine Sample Collection, Macrobenthos community survey and granulometry

Aim: To assess the ecological status and contamination levels of coastal marine environments by analysing sediment characteristics and macrobenthic community structure along impact gradients.

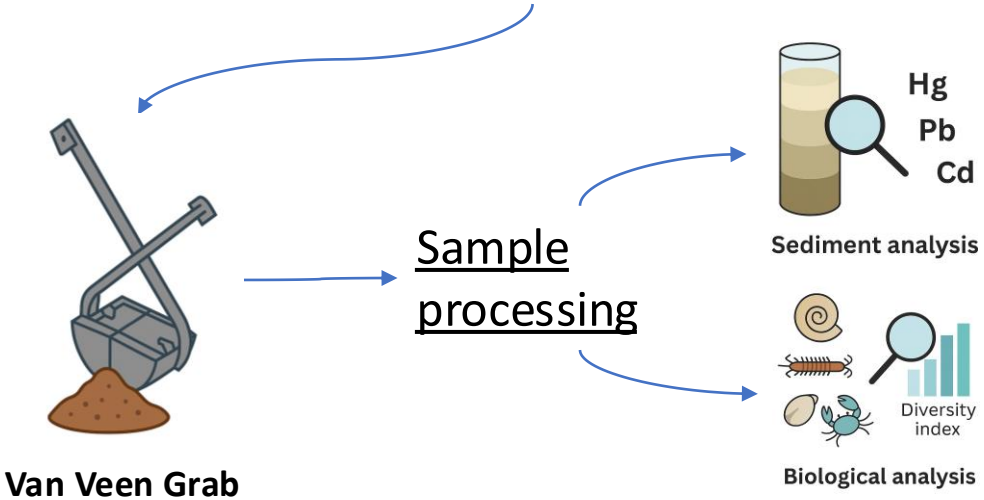
Methods : Sampling along transects perpendicular to the shoreline (depths: 2, 4, 6, 8, 10 m)

Sediment analysis: granulometry and heavy metals.

Biological analysis: species identification, diversity indices (Shannon, Simpson, Margalef, Pielou).



Field sampling: Transects perpendicular to the shoreline with sampling stations at 2 to 10 m depth using a Van Veen grab.

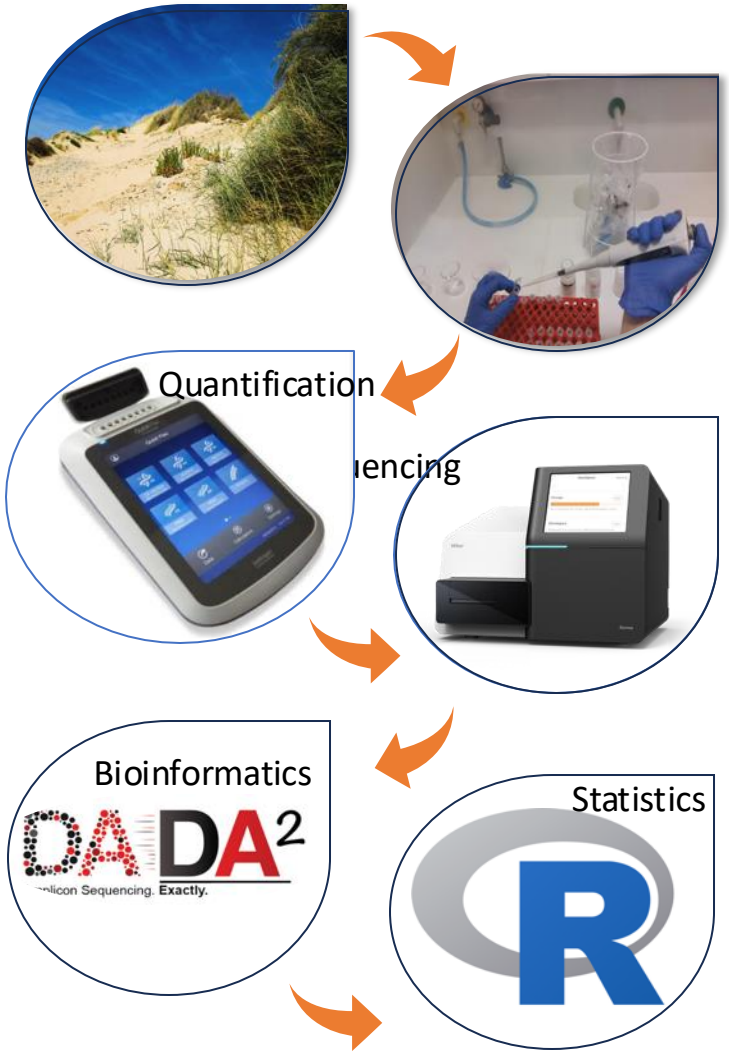


WP 3 – Characterization of bacterial communities in soil and marine sediments

Background: Soil and sediment microbes are known to be highly susceptible to different types of pollutants such as heavy metals. These contaminants can alter both composition and functioning of bacteria by selecting more chemical-tolerant taxa with specialised degradation abilities, thereby decreasing the overall microbial diversity.

Aim: Investigate the patterns underlying taxonomic changes within the microbial communities in contaminated versus not contaminated soils and sediments to find out indicators that may provide key elements to predict the coastal ecosystem's response to environmental stressors.

Methods: e-DNA, 16S metabarcoding



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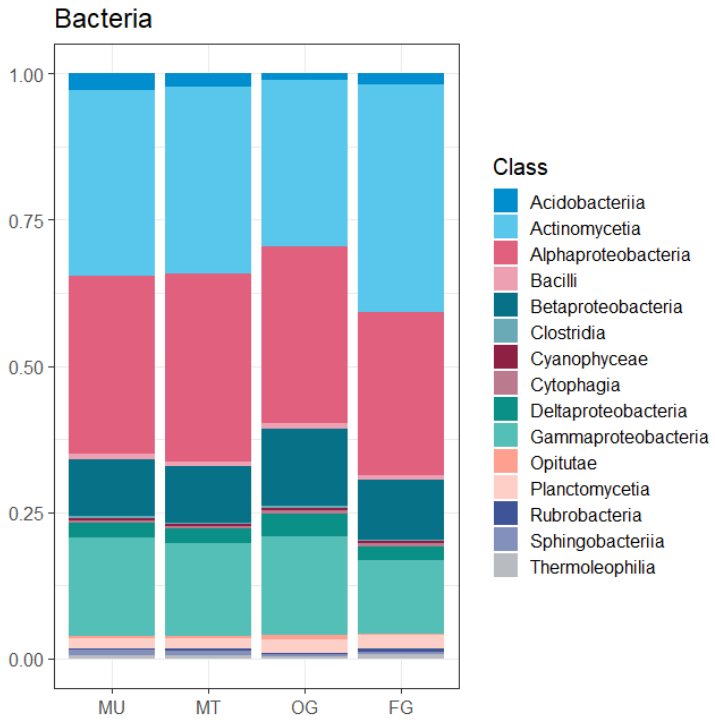
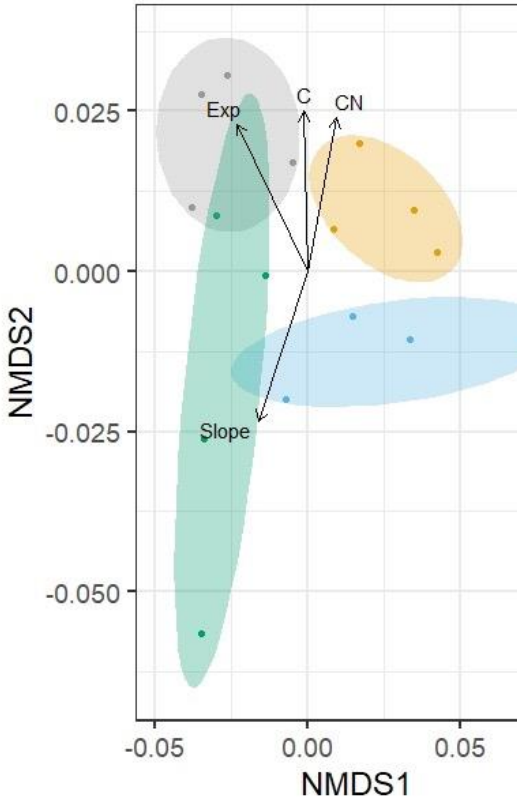
WP 3 – Characterization of bacterial communities in soil and marine sediments

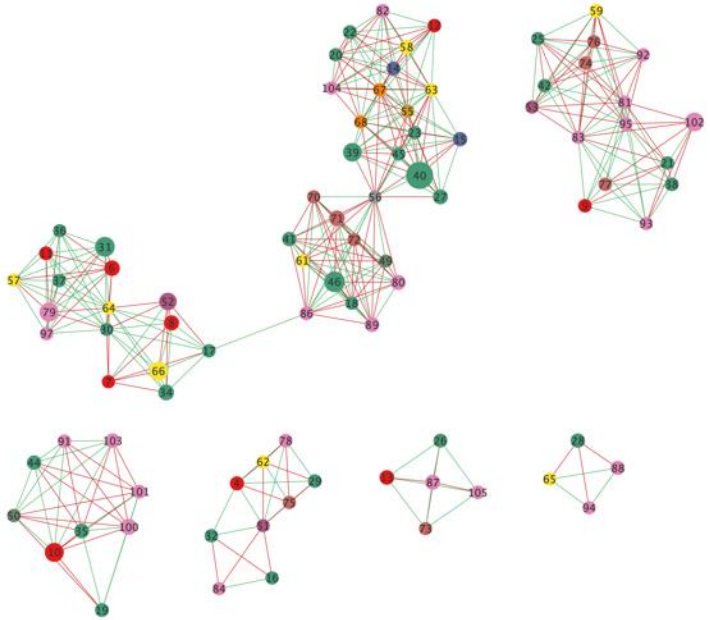
Alpha diversity



Beta diversity



Co-occurrences



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WP4 - Population genomics and differential expression

Cakile maritima
(Ravestrello)

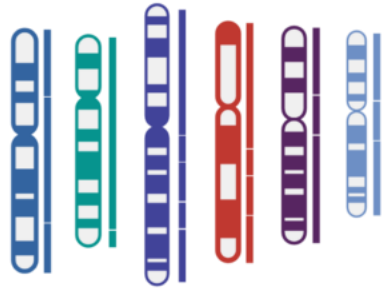
WGS + Long Reads

Contigs

Hi-C

Scaffolding

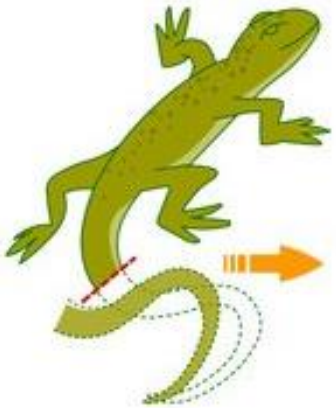
Genome Assembly



Chromosome level assembly

WP4 - Population genomics and differential expression

Sea rocket Root



Lizard tail

DNA/RNA extraction & Quality control

Library preparation, amplification & validation

Validation of candidate biomarkers

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Expected outputs

- **Assess impact of heavy metal contamination at ecosystem level.**
  - Particularly we expect to provide relevant data on the impact of stress due to heavy metal contamination at molecular level along different levels of the trophic chain in coastal dune ecosystems. The combination of DNA/RNA analysis among different sites will allow to identify DNA mutation and alteration of gene expression linked to different contaminants.
- **Develop new biomarkers**
  - using metabarcoding and transcriptomic data, we could identify microbial community alteration and plant/animal gene expression up- or down-regulated. Because gene expression fast responds to the environment it could allow us to much better track population stress.
- **Integrate knowledge on gene expression under natural conditions**
  - understanding molecular pathways involved in response to the environment, generating and testing hypotheses about the mechanisms and evolution of these responses to the environment, and applying this knowledge to monitoring, management and conservation of ecosystems
- **de novo assembly of a reference genome**
  - Although we could work also in absence of a reference genome, the project will benefit in producing one. This genome will be deposited in an international database (EMBL, Genbank) and will be very useful for further research at international level.

Timeline

Field work
Lab work

	Year 1				Year 2			
	I	II	II	IV	I	II	III	IV
WP1	1.1							
			1.2					
			1.3					
WP2	2.1							
			2.2					
			2.3					
WP3			3.1					
				3.2				
WP4		4.1						
			4.2					
				4.3				
					4.4			
WP5	5.1							
				5.2				
				5.3				
							5.4	
								5.5

Field work in early Autumn 2025 (october) and spring 2026

Genome assembly of *Cakile maritima* instead of *Pancratium maritimum*

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Team

Sicuriello, De Cinti

phytosociological surveys and analysis of indexes of stress of vegetation based on nutrient and chemical content.

Brunetti, Marzi, Antenozio

gene expression in plants, molecular mechanisms of tolerance and accumulation of heavy metals.

Profeta, Giacobbe

study of sublittoral coastal marine environment by analysisng sediments and macrozoobenthos community.

Pioli

metabarcoding analysis of microbial communities in different context and a strong bionformatic background.

Colangelo, Franchini, Gramolini

genomics and transcriptomics in non-model organisms and bioinformatic analysis.

Grazie per l’attenzione arrivederci!

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