

Bacterial communities associated with *Alexandrium*: study of their structure and composition by a metabarcoding approach

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Introduction

- **Phytoplankton-bacterial interactions** (exchange of metabolites and infochemicals) occur in the microenvironment surrounding algal cells known as the **phycosphere**.
- Marine dinoflagellates of the genus *Alexandrium* include a number of species that produce **saxitoxin** and its derivatives. Toxic blooms have negative impact on ecosystem and public health.
- Deciphering the **bacterial community (BC)** associated with *Alexandrium* will help to understand the mechanisms of *Alexandrium*-bacteria interactions that are essential to predicting dynamics of *Alexandrium* bloom.
- **Our aim was to determine and compare the structure and the composition of the BC associated with different species of *Alexandrium*.**

Experimental design

- 26 strains of *Alexandrium* (6 species) cultured under controlled conditions in triplicate.
- Sampling performed at the end of the exponential growth phase of *Alexandrium*.
- Characterization of the bacterial diversity using a 16S rRNA gene sequencing (Illumina MiSeq technology - Genotoul sequencing platform, INRA Toulouse, France).
- Bioinformatic pipeline DADA2¹ and R packages were used to process data. The taxonomy assignments of ASVs (Amplicon Sequence Variants) were obtained using SILVA 132².

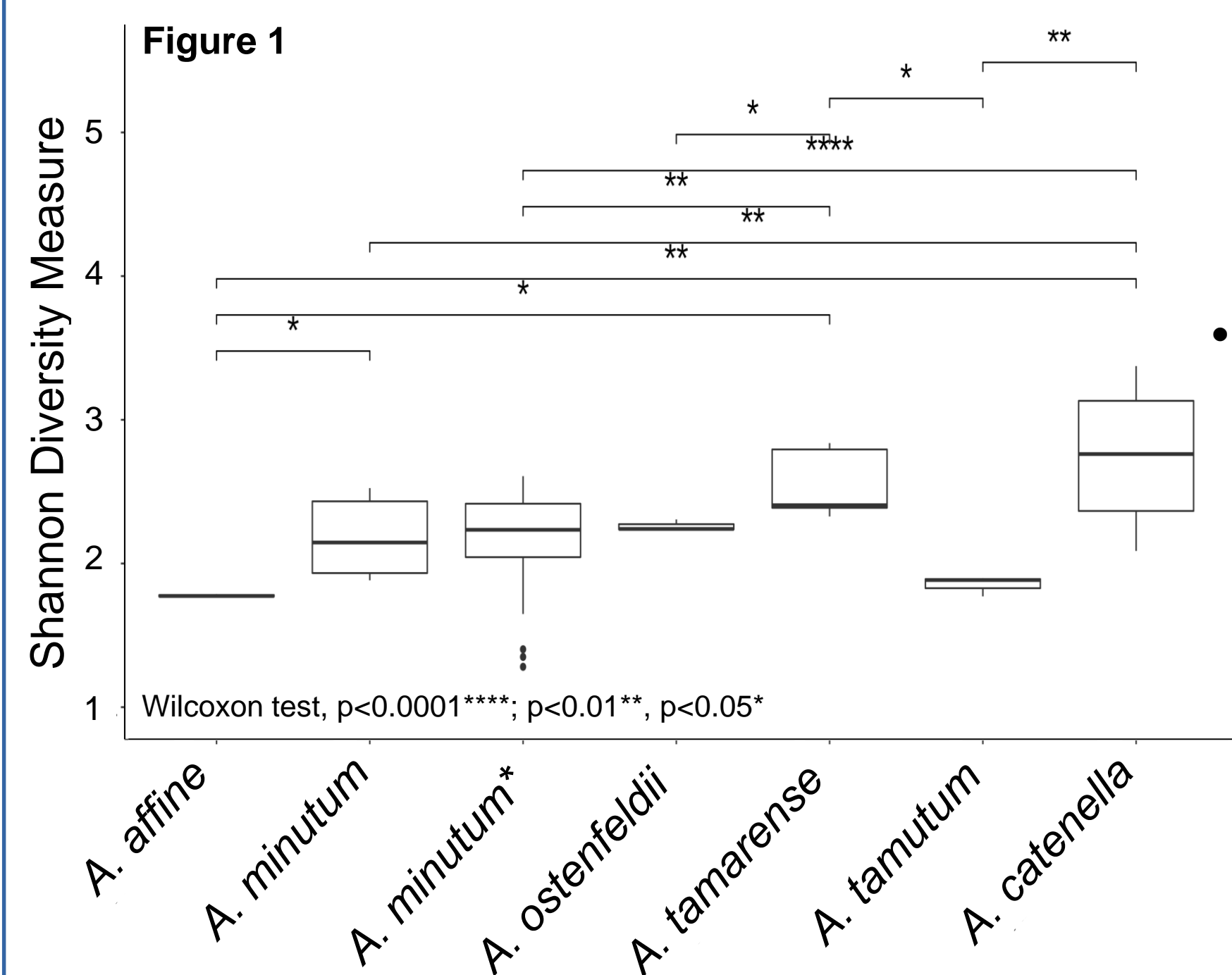
- Origins :**
- Northern Atlantic (NA)
 - Atlantic (A)
 - Mediterranean Sea (MS)
 - English Channel (EC)
 - Southern Pacific (SP)
 - Northern Pacific (NP)

Species	Strain code	Origin	Isolation date	Toxin prod.	L1 culture medium
<i>A. catenella</i> (group IV)	Ac1	SP	1996	toxic	Mediterranean Sea water (S=38)
	Ac2	MS		toxic	
	Ac3	MS		toxic	
	Ac4	MS		toxic	
	Ac5	MS	2015	toxic	
	Ac6	MS	2007	toxic	
	Ac13	MS	2007	toxic	
<i>A. minutum</i>	Am2*	NA	2013	toxic	English Channel water (S=35)
	Am6*	NA	2012	toxic	
	Am8*	NA	2011	toxic	
	Am9	EC		non toxic	
	Am10*	EC	2010	toxic	
	Am11*	EC	1989	toxic	
	Am12	EC	1999	toxic	
	Am14*	EC	2010	toxic	
	Am15*	EC	2011	toxic	
	Am17*	EC	2011	toxic	
<i>A. affine</i>	Af1	NA	2017	non toxic	
	At1	NP	1983	toxic	
	At2	MS	2007	non toxic	
<i>A. tamarensis</i>	At3	A	1991	non toxic	
	Ao1	NA	2008	toxic	
<i>A. ostenfeldii</i>	Att1	NA	2010	non toxic	

* : antibiotic-treated strains (once in 2014)

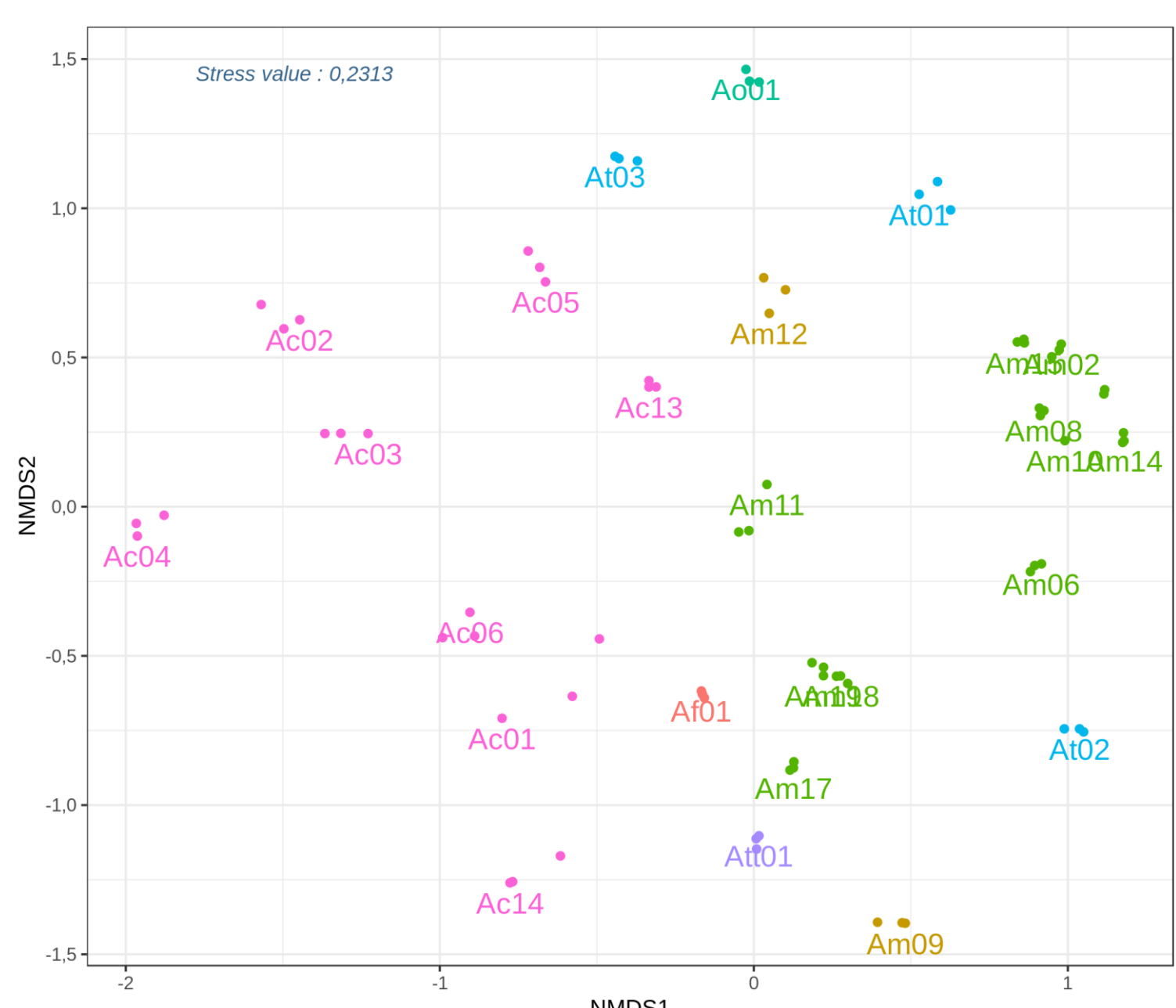
Results

1. Diversity of *Alexandrium* microbiomes



- The *A. catenella* microbiome showed a higher diversity compared to the *A. minutum*, *A. tamutum* and *A. affine* diversity.

2. Similarity of microbiome composition



- Among the different characteristics of *Alexandrium* strains, the **species** and **culture conditions** (salinity and antibiotic treatment) had important impacts on the composition of the microbiomes.

(PERMANOVA analysis, species: $R^2=0.31$, $p=0.001$; salinity: $R^2=0.14$, $p=0.001$; antibiotic treat.: $R^2=0.06$, $p=0.001$)

Figure 2. Non-metric dimensional scaling (NMDS) ordination based on the Bray-Curtis dissimilarity for 16S rRNA gene libraries between the different *Alexandrium* strains.

Results

3. Characterization of *Alexandrium* microbiomes

At the Order level

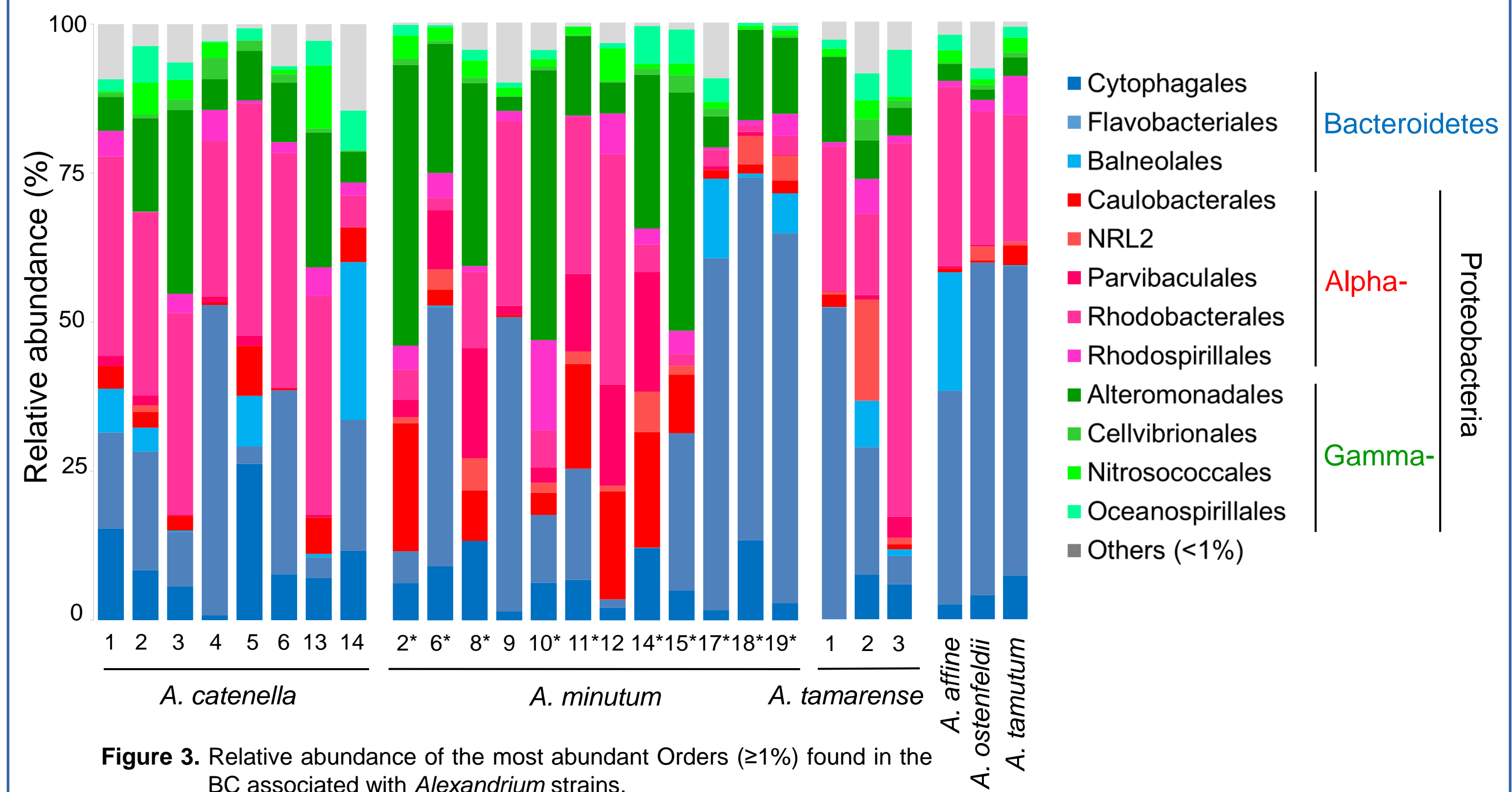


Figure 3. Relative abundance of the most abundant Orders ($\geq 1\%$) found in the BC associated with *Alexandrium* strains.

- The composition of microbiomes of *Alexandrium* isolates was characterized by the dominance of: **Bacteroidetes (38%)**, **α -Proteobacteria (36%)**, and **γ -Proteobacteria (22%)**.
- Among the top 12 Orders, (96% of the total sequences) **Flavobacteriales (27%)**, **Rhodobacteriales (21%)** and **Alteromonadales (16%)** were the dominant contributors.

At the ASV level

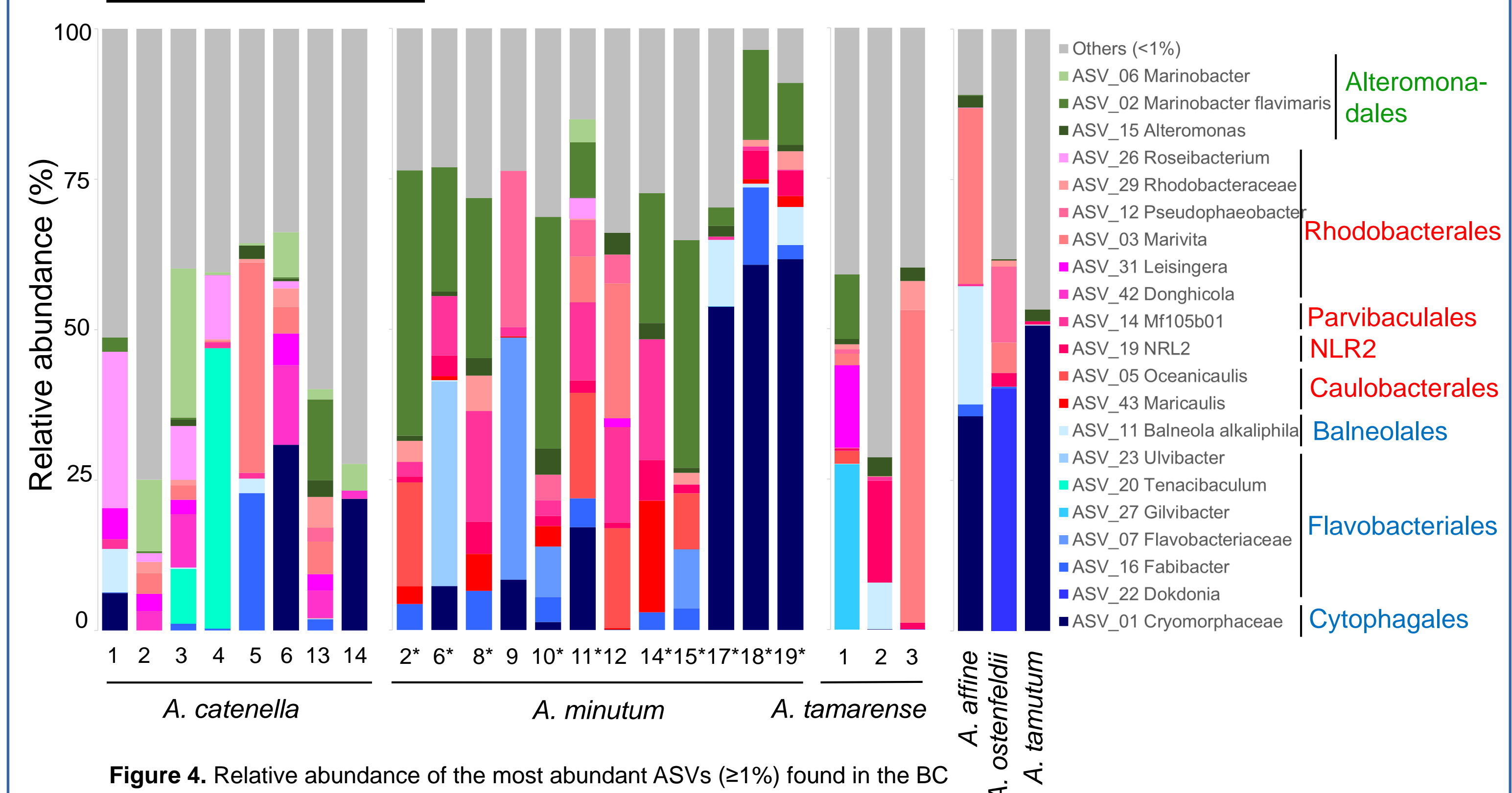


Figure 4. Relative abundance of the most abundant ASVs ($\geq 1\%$) found in the BC associated with *Alexandrium* strains.

- The 21 most abundant ASVs represented 64% of the total sequences with **Cryomorphaceae (14%)**, **Marivita (6%)** and **Marinobacter (10%)** the major contributors.
- Even if the global structure of BCs was similar, some ASVs were preferentially detected within one species (*i.e.* *Marinobacter* in *A. minutum*) or within one strain.
- **ASV_15 *Alteromonas*** and **ASV_89 *Litorivivens*** were present in 100% of samples and may be considered the core microbiome of *Alexandrium*.

Conclusions

- **BCs associated with *Alexandrium* were species-specific** and an intraspecific variability was highlighted even among antibiotic-treated strains.
- Salinity seems to influence the BC diversity associated with *Alexandrium*, the mechanisms of this selection remain to be explored.
- **The microbiomes were highly similar to what has previously been reported from cultures of *Alexandrium* species^{3,4,5}.**
- **The BC was dominated by groups that are abundant in coastal waters and in phytoplankton blooms and that are most likely adapted to degrade phytoplankton-derived organic matter^{6,7}.**
- The identification of ***Alexandrium* core microbiome** is highly relevant to resolve the metabolic interactions but need further investigations, such as the comparison with *in situ* *Alexandrium* microbiome dynamics.

Acknowledgments

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References

- ¹Callahan et al., 2016. Nat Methods 13: 581-583. ²Quast et al., 2013. Nucl Acids Res 41: D590-D596. ³Sorenson et al., 2019. Env Mic Rep 11(3), 425-433. ⁴Jasti et al., 2005. AEM 71: 3483-3494. ⁵Sala et al., 2005. FEMS Microb Ecol 54: 257-267. ⁶Teeling et al., 2012. Science 336: 608-611. ⁷Buchan et al., 2014. Nat Rev Microbiol 12: 686-698.