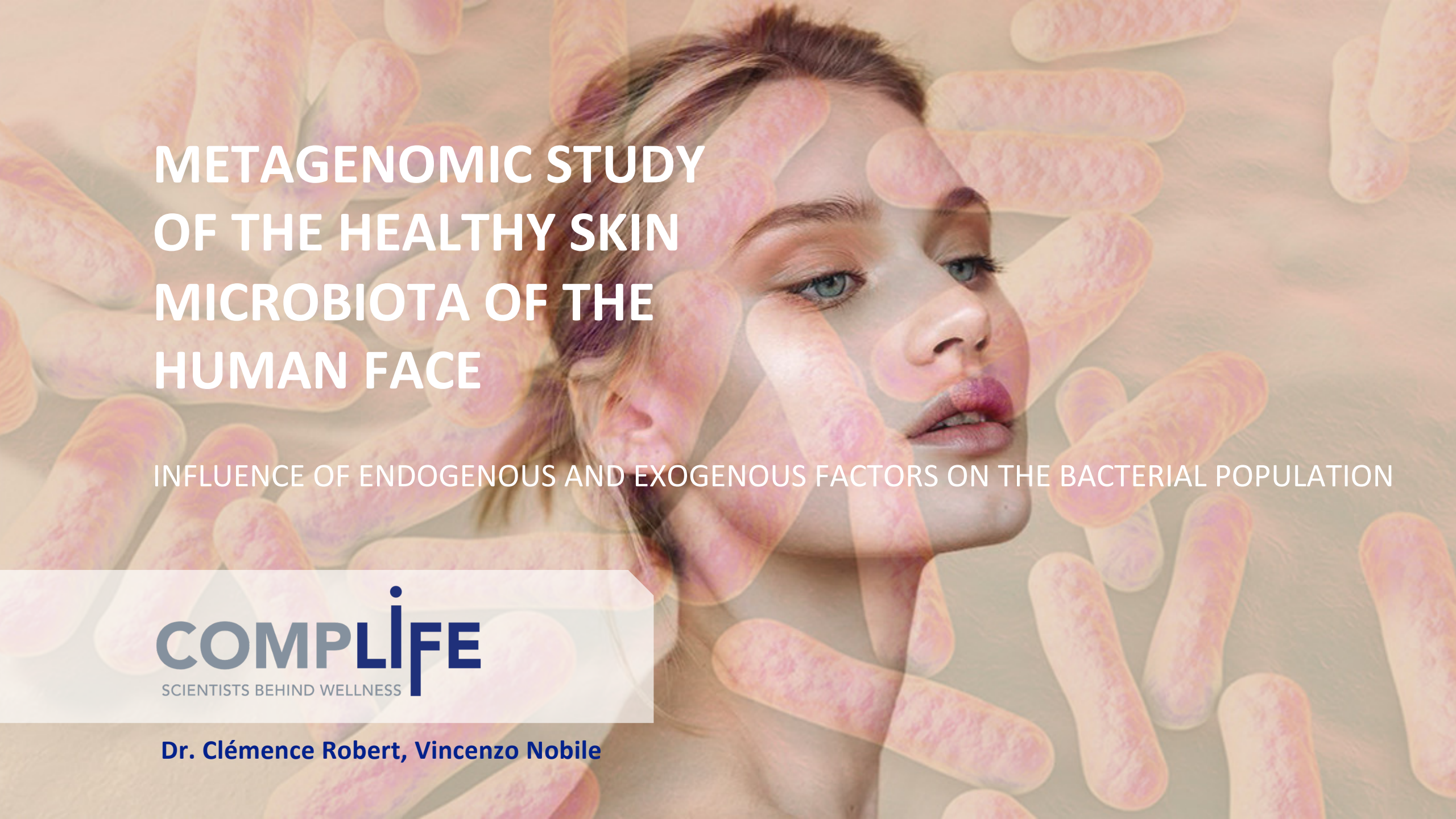


The background of the entire slide is a close-up of a woman's face, looking slightly upwards and to the right. Overlaid on this is a pattern of numerous pink and orange, elongated, rod-shaped bacteria, some with internal texture, scattered across the frame.

METAGENOMIC STUDY OF THE HEALTHY SKIN MICROBIOTA OF THE HUMAN FACE

INFLUENCE OF ENDOGENOUS AND EXOGENOUS FACTORS ON THE BACTERIAL POPULATION

COMPLiFE
SCIENTISTS BEHIND WELLNESS

Dr. Clémence Robert, Vincenzo Nobile

WHAT IS A MICROBIOTA

The microbiota is **the sum of all bacteria, fungi and arthropods that live in and on everything that composes an ecosystem** and disruption of these microscopic populations is linked to dysbiosis of a given environment.

THE HUMAN BEING ITSELF IS AN ECOSYSTEM, IN FACT THE ROLE OF THE HUMAN MICROBIOME IN HEALTH AND DISEASE HAS BEEN RECOGNISED IN RECENT YEARS.



FOCUS ON HUMAN FACE

The gut, oral and skin microbiota have been extensively studied over the last 10 years, but **cutaneous studies** mostly focused on diseased skin and population variations depending on the geographical niche.

And relatively **little information is available on the composition of the healthy microbiome of the human face**



FOCUS ON HUMAN FACE



COMPLIFE TEAMED UP WITH UPO TO CONDUCT A LARGE SCALE STUDY ON THE MICROBIOME OF THE HUMAN FACE AND COPAN PARTICIPATED TO THE PROJECT BY PROVIDING HIGH-QUALITY SAMPLING DEVICES.



COMPLIFE OFFERS SAFETY AND EFFICACY TESTING BOTH IN VIVO AND IN VITRO TO THE COSMETIC INDUSTRY AND EVERYDAY MORE OF OUR CLIENTS ARE INTERESTED IN DEVELOPING PRODUCTS THAT RESPECT AND/OR IMPROVES THE SKIN MICROBIOME.

COMPLIFE
SCIENTISTS BEHIND WELLNESS

UPO  UNIVERSITÀ DEL PIEMONTE ORIENTALE
CAAD CENTER FOR TRANSLATIONAL RESEARCH
ON AUTOIMMUNE AND ALLERGIC DISEASE

Copan
innovating together

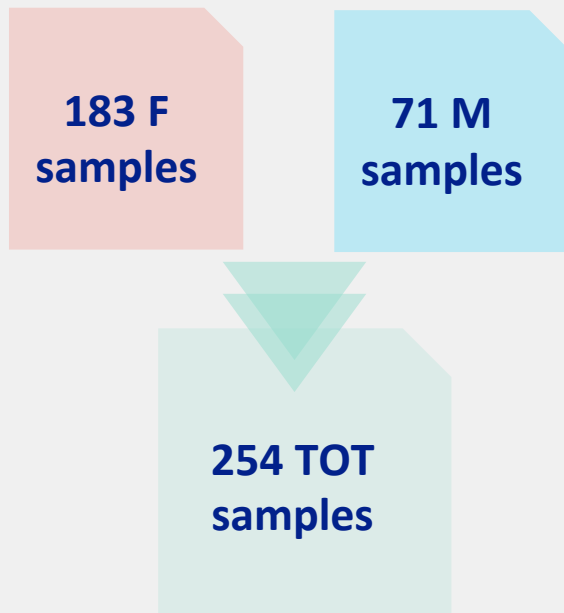


THIS PRESENTATION SHOWS THE **STUDY ABOUT THE VARIATIONS OF FACIAL MICROBIOME COMPOSITION** DEPENDING ON ENDOGENOUS AND EXOGENOUS FACTORS

THE BASAL MICROBIOME OF THE HUMAN FACE AND HOW IT IS IMPACTED BY EXTERNAL FACTORS

LARGE-SCALE STUDY OF THE HEALTHY CUTANEOUS MICROBIOME

- **254 samples total**
- 183 from **159 women** and 71 from **58 men**
- all samples were analysed by **16S rRNA gene sequencing**



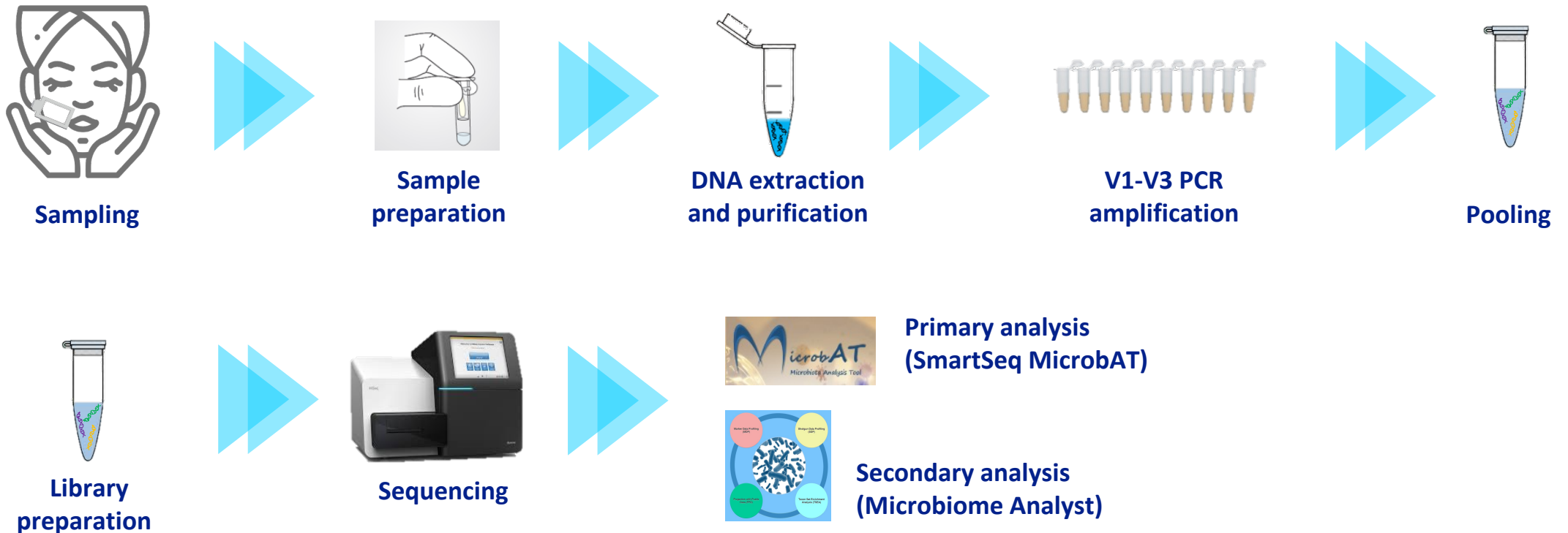
2 ENDOGENOUS AND 2 EXOGENOUS FACTORS

- 2 endogenous factors - **SEX AND AGE**
- 2 exogenous factors - **SEASON AND LIVING AREA**



ANALYSIS PIPELINE

After sample preparation, we **extracted and purified the bacterial DNA** and proceeded with the PCR amplification. Since we are working with skin microbiota, we chose to use **primers targeting the V1-V3 hypervariable regions of the 16S rRNA gene**. Then, we pooled our samples together, prepared the library and loaded our mix on the sequencer. The primary analysis was performed using the MicrobAT software while the secondary analysis relied on the Microbiome Analyst platform.



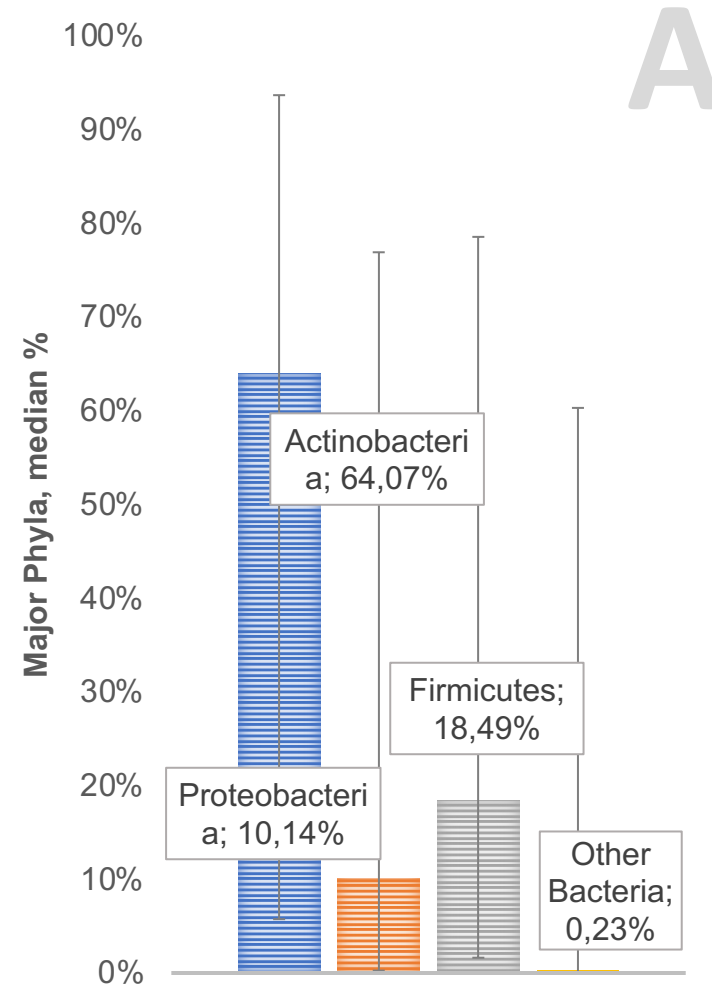
RESULTS

GENERAL CONSIDERATIONS

PRIMARY ANALYSIS AT THE PHYLUM LEVEL

Primary analysis at the Phylum level is in accordance with the literature on skin microbiota:

- 3 major phyla were identified (Actinobacteria, Proteobacteria and Firmicutes) with Actinobacteria accounting for more than 60% of all bacteria;
- very low percentage of other bacteria: a median percentage could be calculated only for Bacteroidetes and Cyanobacteria.



[1] E. A. Grice and J. A. Segre (2011) "The skin microbiome," *Nature Reviews Microbiology*, vol. 9, no. 4, pp. 244–253.

[2] Mukherjee, S. et al. (2016) "Sebum and Hydration Levels in Specific Regions of Human Face Significantly Predict the Nature and Diversity of Facial Skin Microbiome." *Sci. Rep.* 6, 36062.

RESULTS

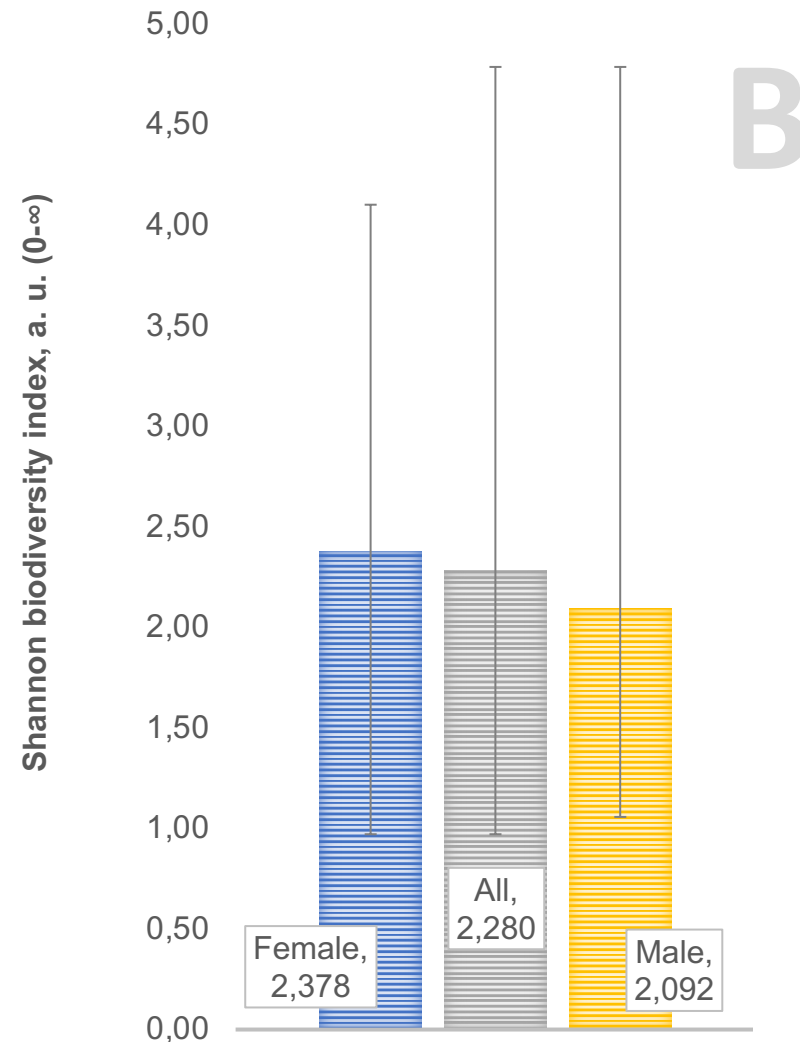
GENERAL CONSIDERATIONS

In terms of biodiversity, **women** within our cohort showed a higher bacterial diversity.

Which is in line with traditionally **lower sebum level and higher hydration levels**.

Frequent use of cosmetics could also play a role in modulating the microbiota, either by frequent depletion/colonisation of the skin or by microenvironment modifications.

With higher standard deviation and higher maximum and minimum biodiversity, **men appear to have higher interpersonal variations**.



[1] E. A. Grice and J. A. Segre (2011) "The skin microbiome," *Nature Reviews Microbiology*, vol. 9, no. 4, pp. 244–253.

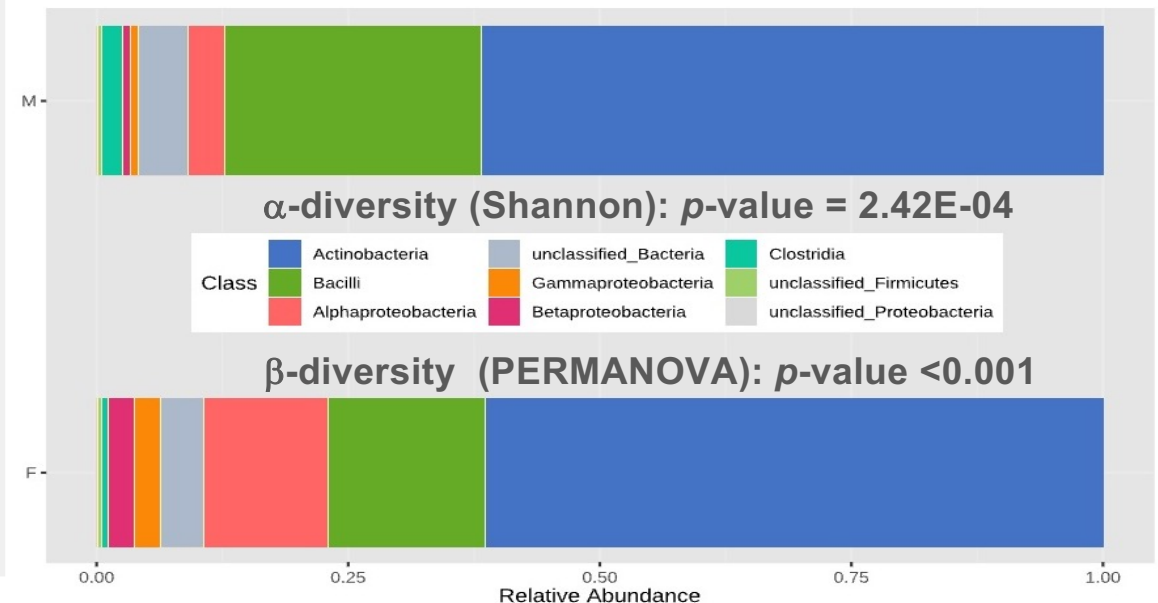
[2] Mukherjee, S. et al. (2016) "Sebum and Hydration Levels in Specific Regions of Human Face Significantly Predict the Nature and Diversity of Facial Skin Microbiome." *Sci. Rep.* 6, 36062.

RESULTS - MAJOR TAXA

SECONDARY ANALYSIS AT LOWER TAXONOMIC LEVELS

The **minor Phyla** are not relevant - p-values inferior to 0.05.

There are however clear differences in bacterial population between men and women in terms of both **taxa population** and **community structures**.



[1] E. A. Grice and J. A. Segre (2011) "The skin microbiome," *Nature Reviews Microbiology*, vol. 9, no. 4, pp. 244–253.

[2] Mukherjee, S. et al. (2016) "Sebum and Hydration Levels in Specific Regions of Human Face Significantly Predict the Nature and Diversity of Facial Skin Microbiome." *Sci. Rep.* 6, 36062.

RESULTS - MAJOR TAXA

SECONDARY ANALYSIS AT LOWER TAXONOMIC LEVELS

At the genus level, we focused on the 10 major genera:

- **3 ACTINOBACTERIA:**

Propionibacterium, Corynebacterium and Microbacterium;

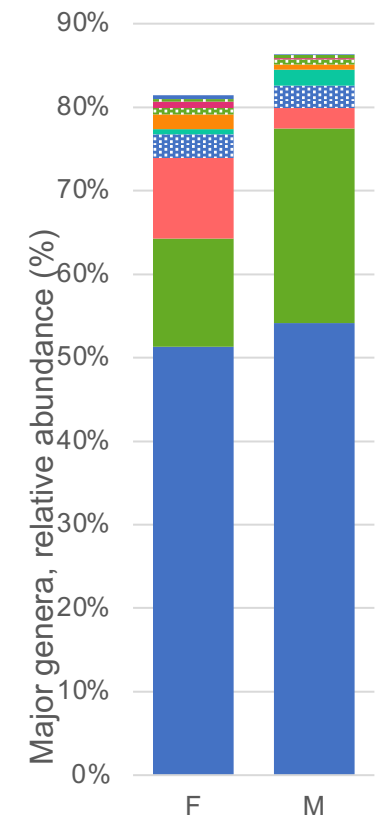
- **4 FIRMICUTES:**

the 3 bacilli Staphylococcus, Streptococcus and Anaerobacillus and the clostridia Anaerococcus;

- **3 PROTEOBACTERIA:**

- the Alphaproteobacteria Sphingomonas,
- the Gammaproteobacteria Pseudomonas
- and the Betaproteobacteria Pelomonas.

	F	M
Propionibacterium	51.30%	54.11%
Staphylococcus	12.99%	23.30%
Sphingomonas	9.61%	2.52%
Corynebacterium	2.88%	2.63%
Anaerococcus	0.57%	1.92%
Pseudomonas	1.75%	0.65%
Streptococcus	0.80%	0.59%
Pelomonas	0.78%	0.18%
Anaerobacillus	0.30%	0.36%
Microbacterium	0.44%	0.10%



[1] E. A. Grice and J. A. Segre (2011) "The skin microbiome," Nature Reviews Microbiology, vol. 9, no. 4, pp. 244–253.

[2] Mukherjee, S. et al. (2016) "Sebum and Hydration Levels in Specific Regions of Human Face Significantly Predict the Nature and Diversity of Facial Skin Microbiome." Sci. Rep. 6, 36062.

RESULTS: BIODIVERSITY VARIANTS

The statistical differences between men and women, in terms of both taxa population and community structure, prompted us to analyse the **influence of the other factors separately** for men and women. This should allow us to evaluate if the same factor effect the same variations in both sexes.



SEX

- a- and b- diversity: **p-value <0.05**
- Similar to other body niches [1].



SEASON

- In women: changes in both taxa distribution and community structure;
- In men: visual variation but no statistical difference.



AGE

- In women: changes in both taxa distribution and community structure;
- In men: microbiome composition unchanged upon ageing.



LIVING AREA

- Statistical difference for both sexes and both diversity indexes [2];
- Same trend for both sexes.

[1] Fierer, N. et al. (2008) "The influence of sex, handedness, and washing on the diversity of hand surface bacteria", PNAS 46, 105, 17994-99

[2] Ying S, et al. (2015) The Influence of Age and Gender on Skin-Associated Microbial Communities in Urban and Rural Human Populations. PLoS ONE 10(10): e0141842.



RESULTS: PHYLUM ACTINOBACTERIA

ACTINOBACTERIA: **PROPIONIBACTERIUM, CORYNEBACTERIUM AND MICROBACTERIUM**

PROPIONIBACTERIUM - dominates the population of sebaceous sites like the face.

No statistical difference upon **ageing** for any gender, but we can still observe opposite trends in men and women. Depending on **seasonality**, the variation is statistically relevant only in women, but both sexes exhibit similar trends. Depending on the **living area** however, both gender exhibit significant variations but the data does not allow us to conclude on an eventual trend similarity.

CORYNEBACTERIUM

Statistically **relevant** variation **only for women**, depending on all three factors.

Thrives in moist areas: it is higher in older women and also higher in the city and metropolitan area rather than in a more rural context.

MICROBACTERIUM

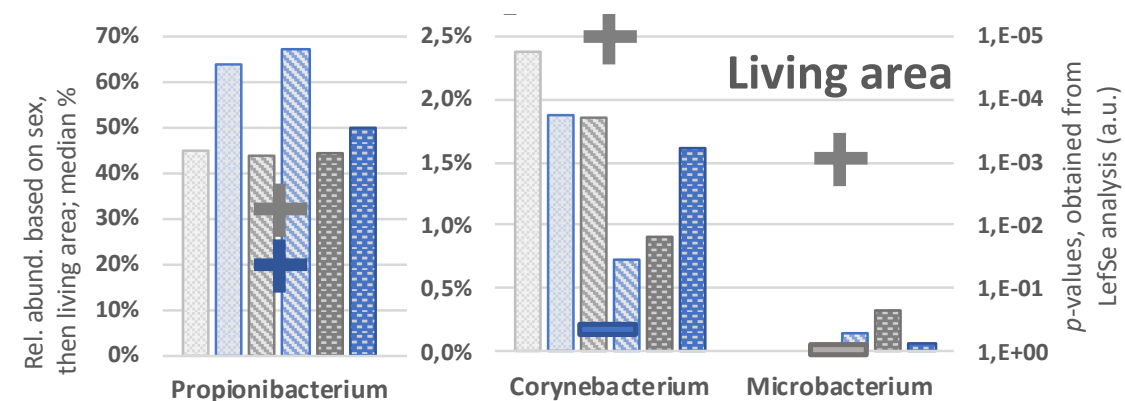
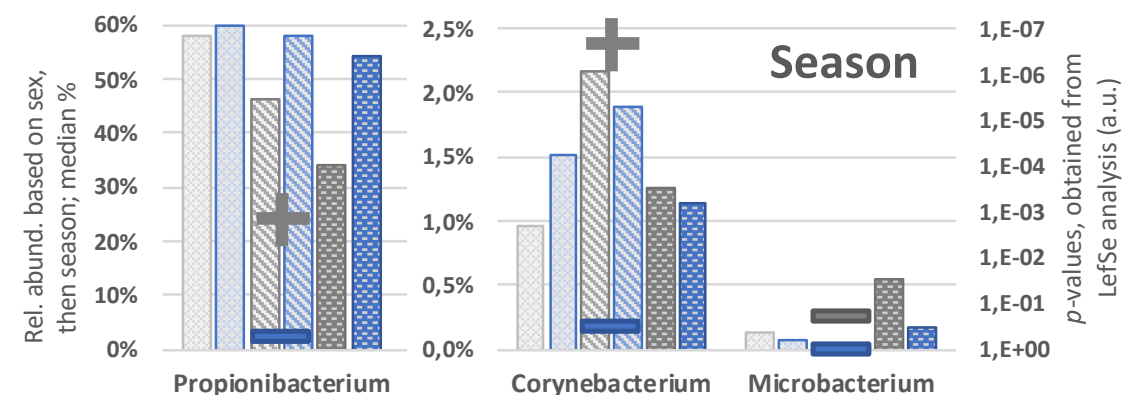
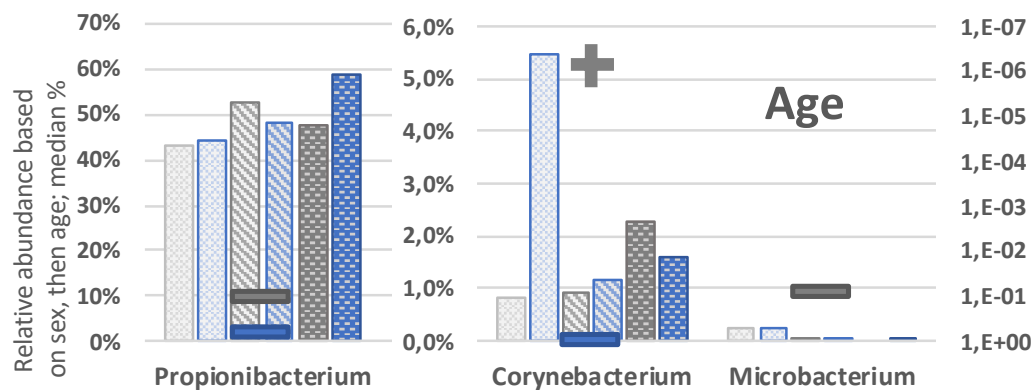
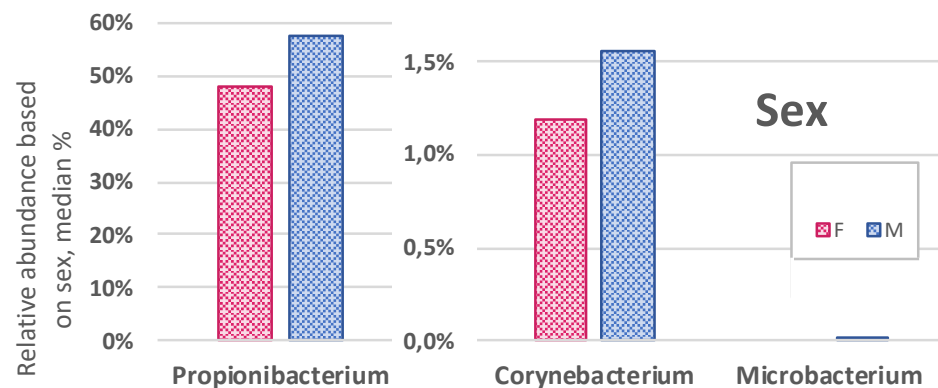
Changes are statistically different between men and women.

Age and season are not correlated to population fluctuation, but **women** express growing numbers the more they move away from urban settings.

RESULTS:

PHYLUM ACTINOBACTERIA

F	M	Age (B)	Season (C)	Living Area (D)
		<25	Winter	Milan
		25-45%	Spring	Po River plains
		>45	Summer	Alps' footing
+	+	p -value <0.05		
+	+	p -value >0.05		



p -values, obtained from
LefSe analysis (a.u.)

p -values, obtained from
LefSe analysis (a.u.)

p -values, obtained from
LefSe analysis (a.u.)



RESULTS: PHYLUM FIRMICUTES

FIRMICUTES: STAPHYLOCOCCUS, STREPTOCOCCUS AND ANAEROBACILLUS AND THE CLOSTRIDIA ANAEROCOCCUS

STAPHYLOCOCCUS

Traditionally present in skin microbiota, although more present in moist skin sites.

The proportion of Staphylococci is **statistically different between both sexes**, but other factors do not induce fluctuations in men while they do in women. Interestingly, **for age and season** men and women display **opposite trends**, while it is similar in both sexes based on **living area**.

ANAEROCOCCUS + ANAEROBACILLUS

Anaerobic microorganisms such as Anaerobacillus and Anaerococcus, as it is expected on face because of the hair follicles and sebaceous glands, are forming an anoxic environment.

Anaerococcus does not undergo significant variations based on other factors than sex, suggesting its population is subject-related.

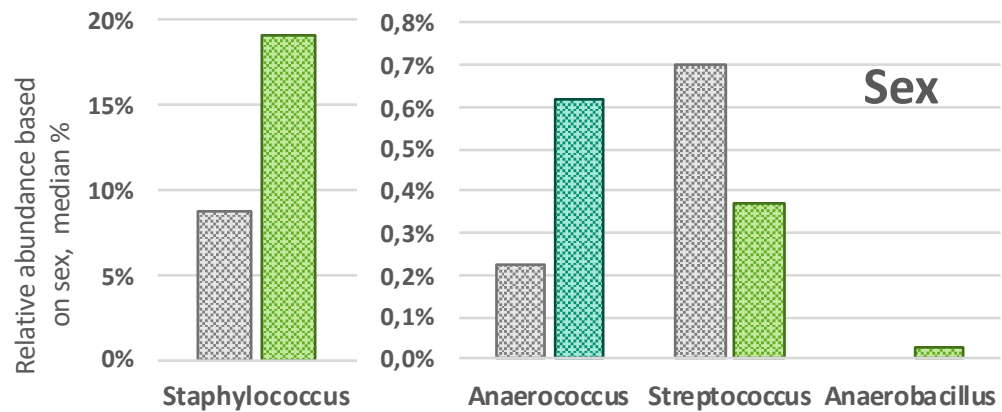
Anaerobacillus has a similar representation in both sexes, but the **other factors influence its population only in women and not in men**.

STREPTOCOCCUS displayed significant **differences** for both sexes **but only based on living area**.

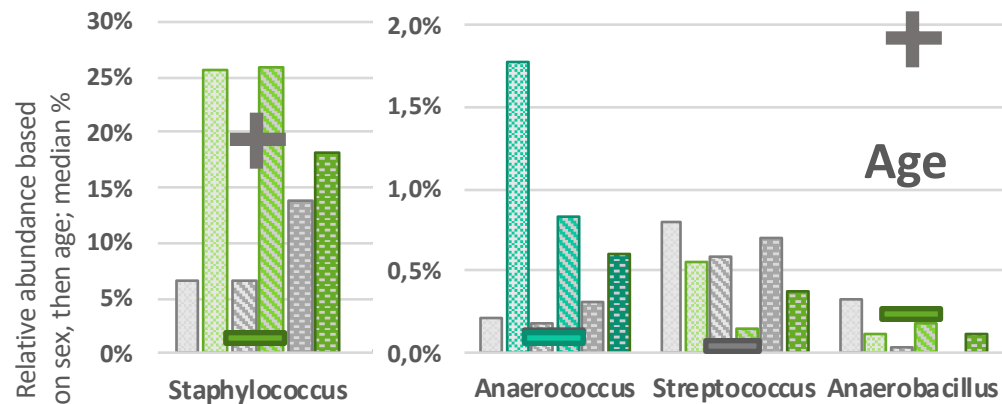
RESULTS:

PHYLUM FIRMICUTES

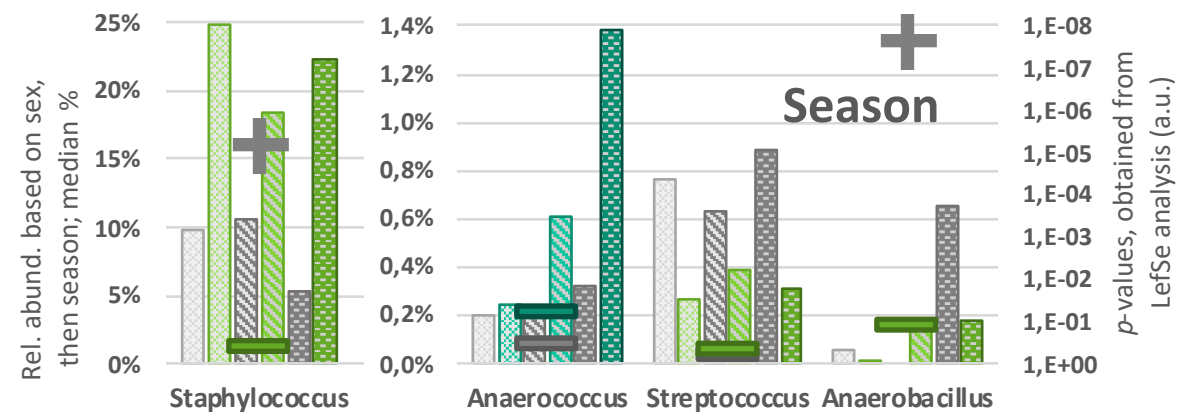
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		25-45%	Spring	Po River plains
		>45	Summer	Alps' footing
+	+	+	p-value <0.05	
-	-	-	p-value >0.05	



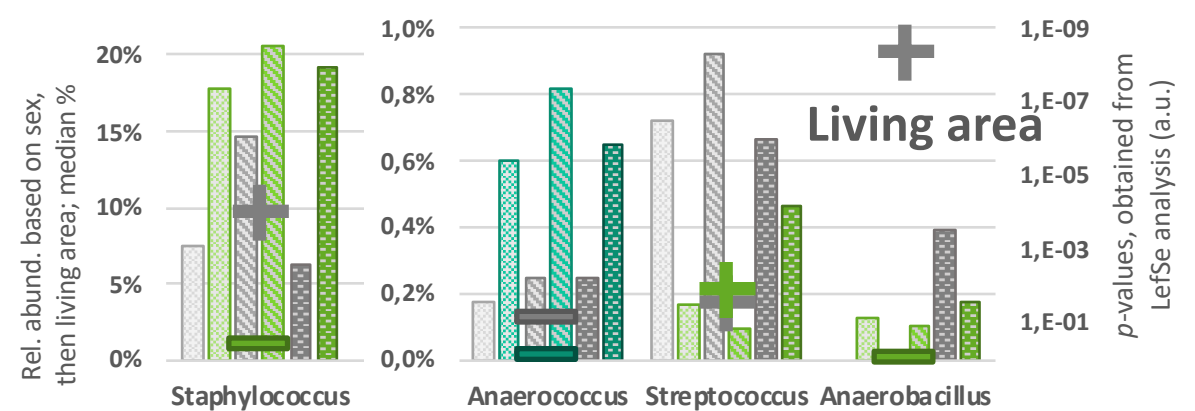
p-values, obtained from
LefSe analysis (a.u.)



p-values, obtained from
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LefSe analysis (a.u.)



p-values, obtained from
LefSe analysis (a.u.)



RESULTS: PHYLUM PROTEOBACTERIA

PROTEOBACTERIA: ALPHAPROTEOBACTERIA SPHINGOMONAS, GAMMAPROTEOBACTERIA PSEUDOMONAS AND BETAPROTEOBACTERIA PELOMONAS.

Proteobacteria initially not considered major inhabitants, mostly because, until recently, they could not be identified easily. Usually more representative of dry and moist areas, but our **results show a non-negligible proportions of Alpha, Beta and Gammaproteobacteria.**

Independently of class, proportions of **Proteobacteria is significantly higher in women**, with Sphingomonas representing the larger population. Its proportion is **influenced by age only in women**, while **seasonality and living area** influence **both men and women**.

Pseudomonas does not show variations in men based on any factors, but in women it is impacted by all.

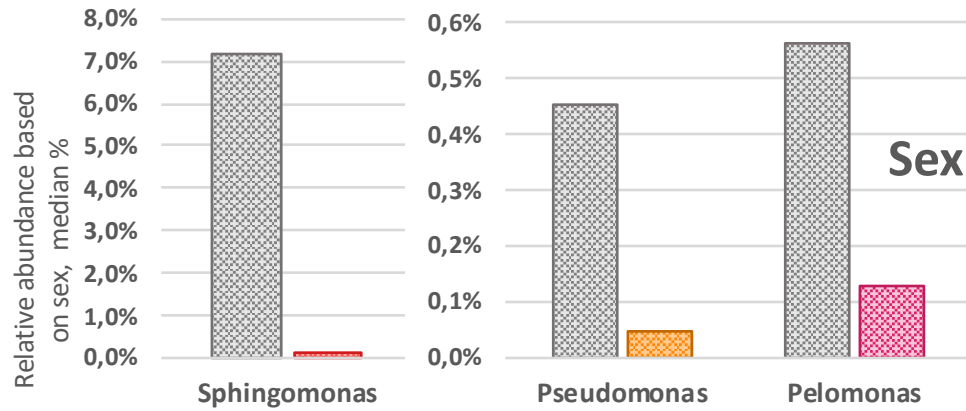
Pelomonas on the other hand, influences the living area in both sexes while seasonality and ageing impacts only women.

Interestingly, each genus displays a similar trend in men and women for each factor.

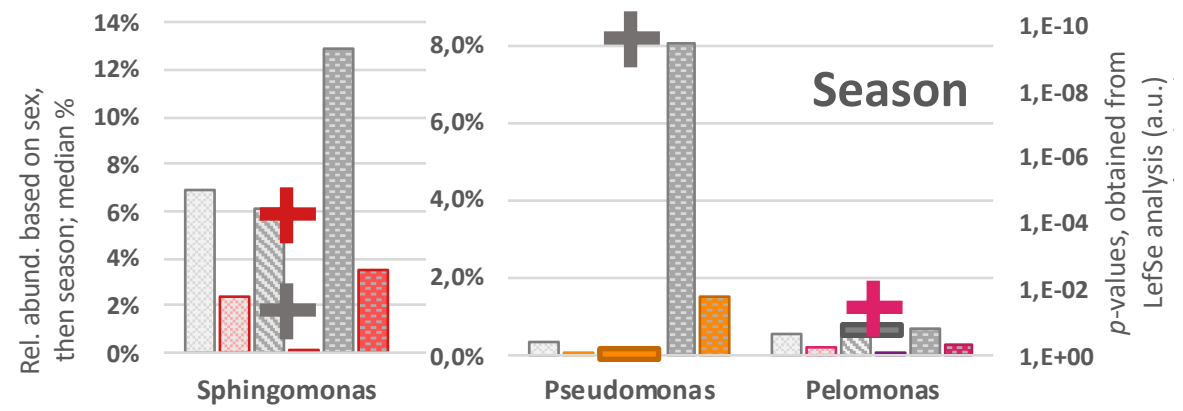
RESULTS:

PHYLUM PROTEOBACTERIA

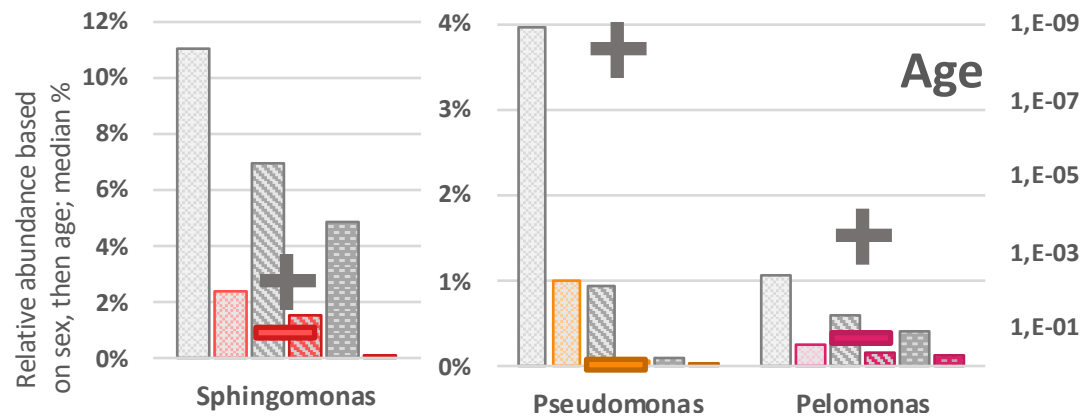
F	M	Age (B)	Season (C)	Living Area (D)
		<25	Winter	Milan
		25-45%	Spring	Po River plains
		>45	Summer	Alps' footing
+	+	+	+	p -value <0.05
-	-	-	-	p -value >0.05



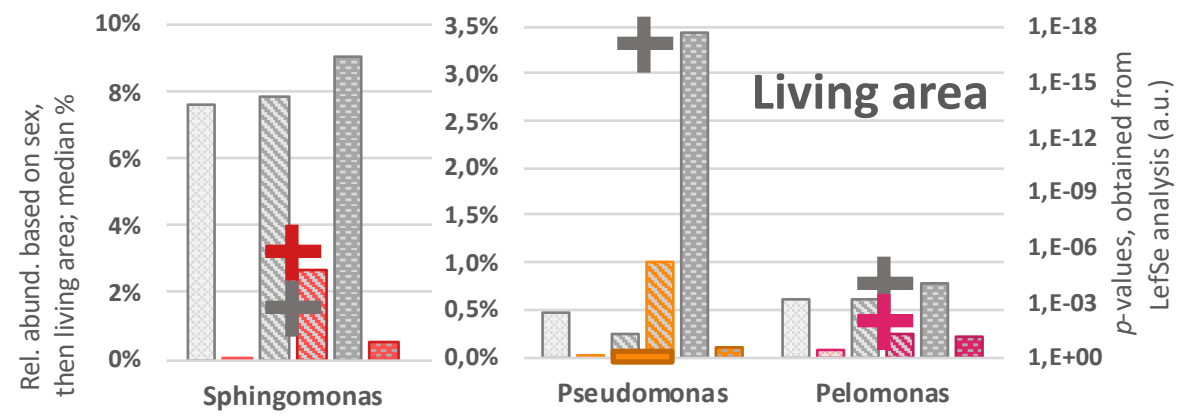
p -values, obtained from LefSe analysis (a.u.)



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p -values, obtained from LefSe analysis (a.u.)



p -values, obtained from LefSe analysis (a.u.)

CONCLUSIONS

- 1 Use of eNAt® 2mL and Smart eNAt® kit
All samples gave good results, quantitatively and qualitatively
- 2 Cheeks host more Proteobacteria and Firmicutes than neighbouring sites (alar crease, nares, glabella) [1]. **Whole-face studies could elucidate** the local variations.
- 3 **Men have lower median biodiversity** and less statistically relevant variations than women:
 - a. More frequent use of cosmetics in women;
 - b. Translation of a higher interpersonal variability in men.
- 4 The general observation is that the **major taxa are not necessarily impacted by endogenous and exogenous factors**. Suggesting that factor-induced microbiome **variations** could be **driven by minor taxa populations**.

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