

Compost-Bedded Pack Systems: the Importance of Tilling on Manure Microbial Populations

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Introduction

Free Stall Cubicle (**CUB**)



- Manure stored in an open-air pool.
- Predominant anaerobic conditions.

Compost-Bedded Pack (**CBP**)



- Daily composted manure used as bedding.
- Improvement in animal health & welfare.

Introduction

Free Stall Cubicle (**CUB**)



- Manure stored in an open-air pool.
- Predominant anaerobic conditions.

Compost-Bedded Pack (**CBP**)



- Daily tilling at 20-30 cm depth.
- ↑ bulk temperature,
- ↓ pack moisture & volume.

Introduction

Different manure management...

...different N losses

...different pollutant emissions

...different manure microbiota

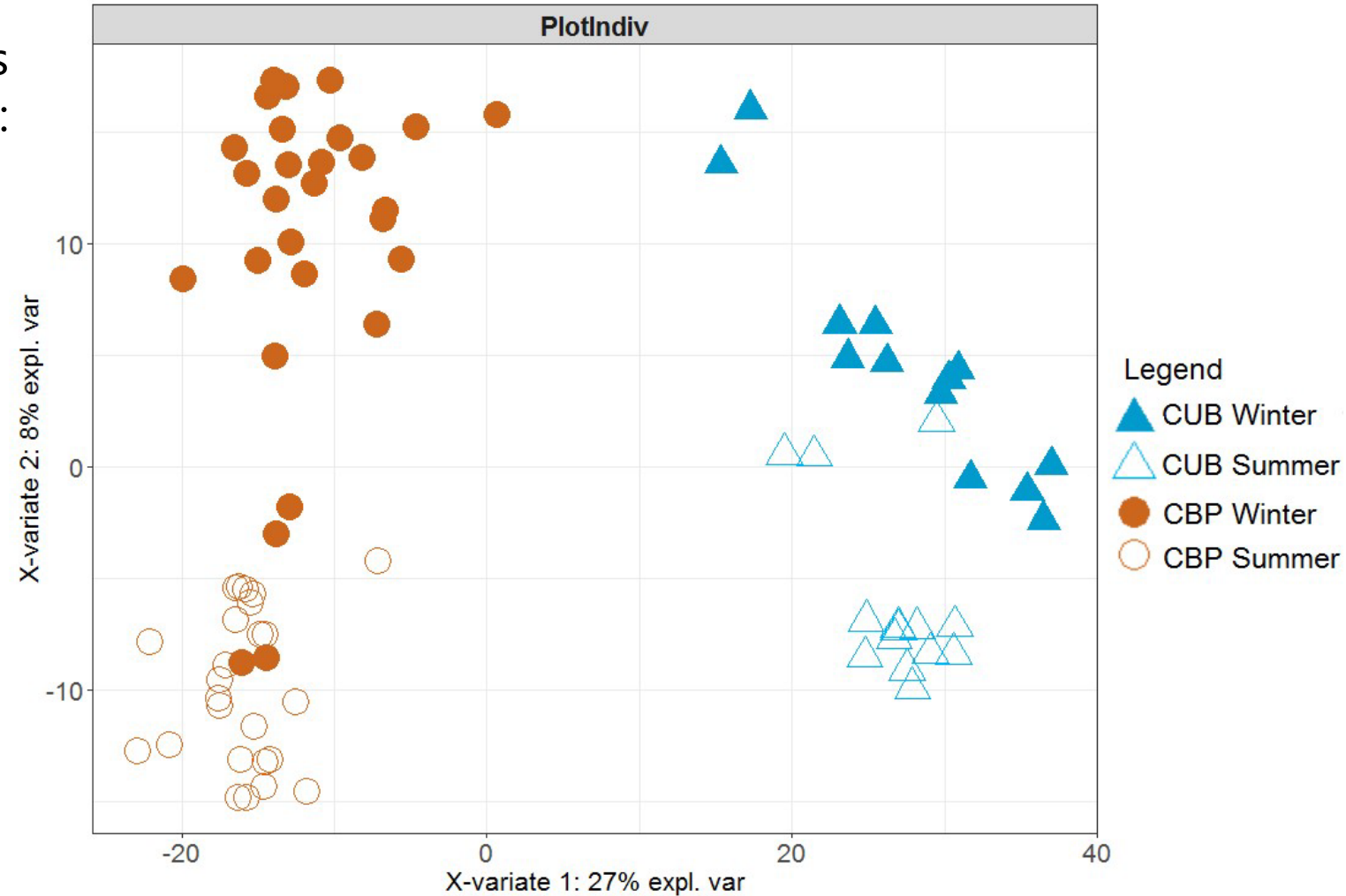


Introduction

Different manure microbiota: CUB vs. CBP (Fuertes et al., 2024)

Partial least squares-discriminant analysis
(PLS-DA):

- Samples clustered by system
(*Adonis test: $P < 0.001$*)



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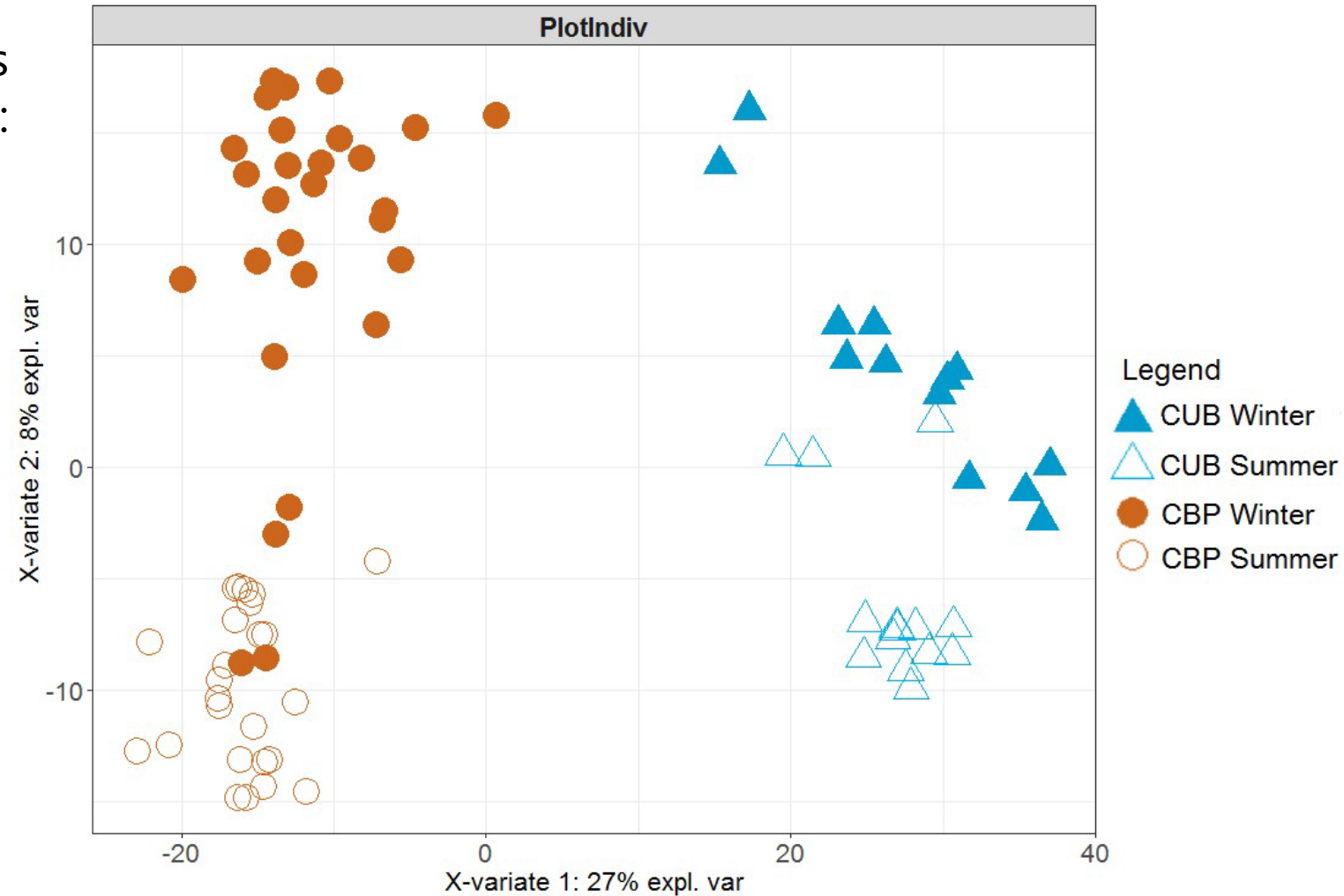
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CUB :

Methanobrevibacter
Methanosaeta
Methanosarcina
...

CBP :

Proteobacteria
Truepera
Pseudomonas
...



Introduction

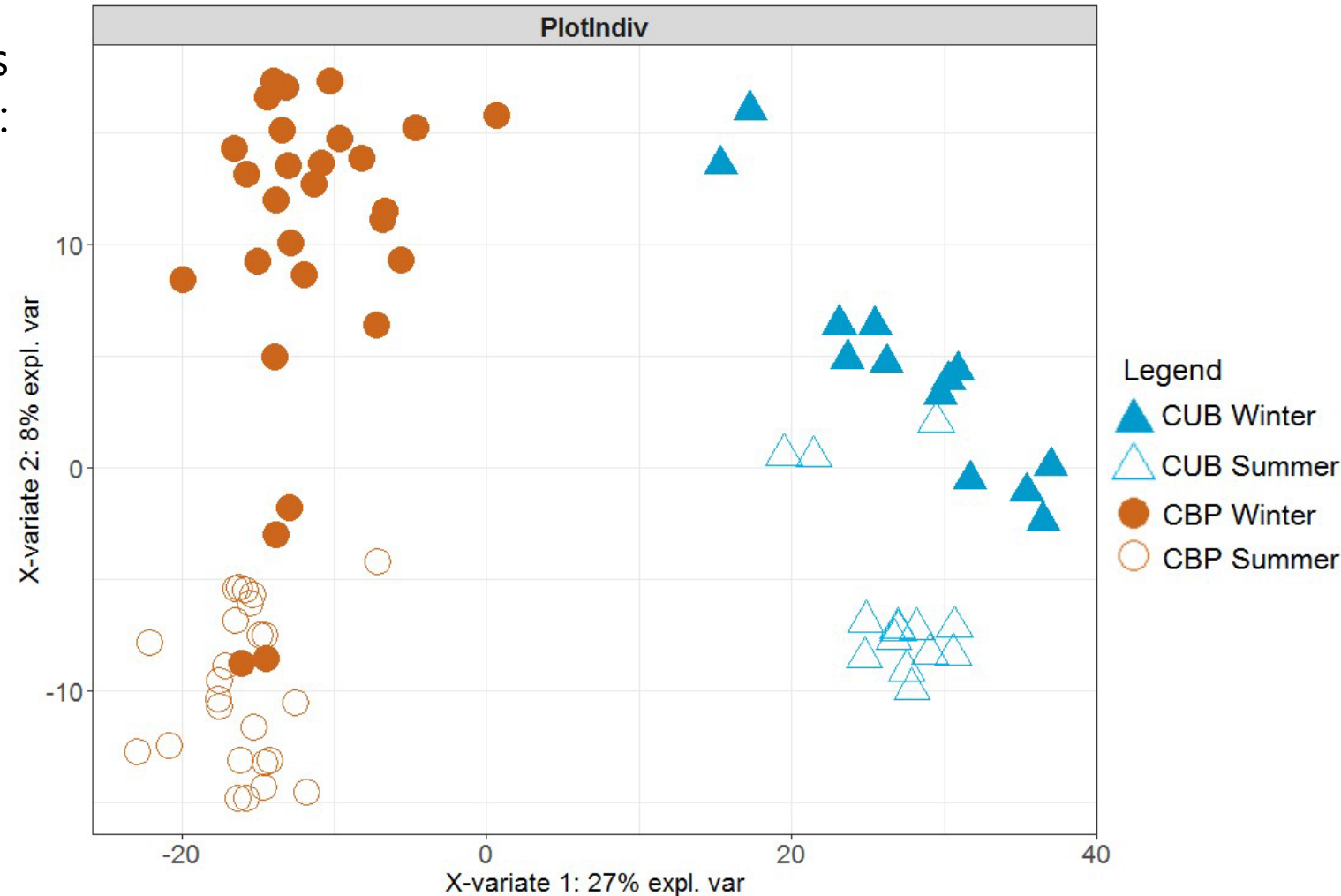
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CBP :

Fuertes et al., (2023) → **CH₄** emissions during the tilling period were 60 times higher than when the bed is not being composted.



Objectives



- Characterize microbial populations in CBP manure differentiating between two depths: superficial (0 cm) and deep (30 cm).
- Determine the seasonal effect (winter and summer) on the dynamics of those populations in each housing system.
- Check for possible relations between the relative abundance of certain microbial populations and the emission of pollutant gases.

Methodology

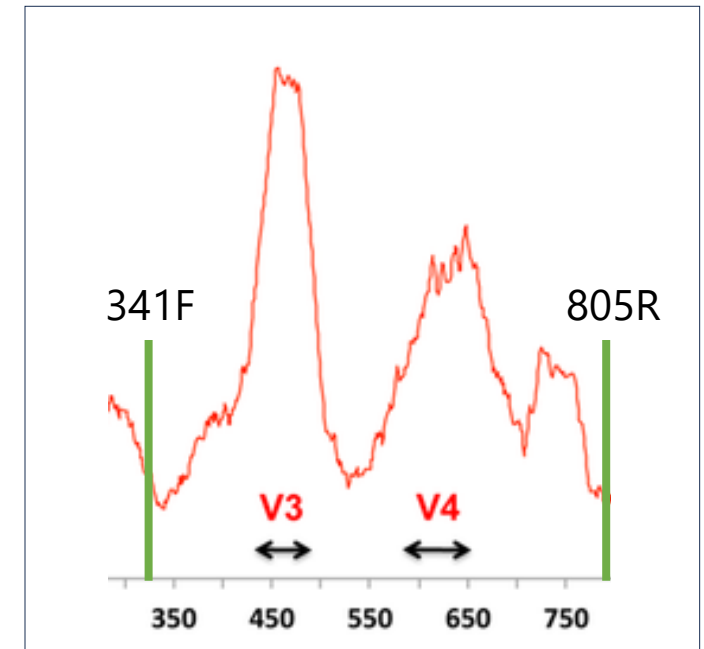
- 3 CBP farms → *manure samples (n = 60)* at 0 cm
>30 cm } 2 seasons: **Win & Sum**
6.2°C
36.4°C

Homogeneous in terms of:
Location
Diet composition
Productive management

- Microbial Analysis: **High-Throughput Sequencing**

16S rRNA gene

- Primers 341F and 805R → V3-V4 regions
- Illumina MiSeq 2×300 platform
- Bioinformatic analysis: R software 4.3.2.



Results & Discussion

Manure Chemical Characteristics

Parameter	0 cm		30 cm		SEM	P-value		
	Sum	Win	Sum	Win		D*	S	D x S
DM (%FM)	33.7	29.9	44.7	44.9	1.25	0.001	0.45	0.46
VS (%DM)	82.3	80.4	77.4	78.5	0.59	0.01	0.12	0.89
C:N	10.9	13.4	11.5	14.3	0.64	0.43	0.03	0.75
pH	8.4	8.3	7.9	7.8	0.07	0.001	0.39	0.89

*D: deep; S: season

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- Higher DM at 30 cm depth.

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- Higher DM at 30 cm depth.
- Higher VS at 0 cm depth.

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- Higher DM at 30 cm depth.
- Higher VS at 0 cm depth.
- Higher C:N during Winter.

Results & Discussion

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*D: deep; S: season

- Higher DM at 30 cm depth.
- Higher VS at 0 cm depth.
- Higher C:N during Winter.
- Higher surface pH.

Results & Discussion

Alpha biodiversity (*Vegan package*):

Alpha index	0 cm		30 cm		SEM	P-value		
	Sum	Win	Sum	Win		D*	S	D x S
Shannon	4.54	4.51	4.54	4.48	0.021	0.84	0.25	0.76
Simpson	0.981	0.979	0.983	0.980	0.0006	0.14	0.04	0.90
Richness	212.3	221.5	196.7	197.8	3.48	<0.001	0.46	0.56
Evenness	0.849	0.835	0.863	0.850	0.0019	<0.001	0.001	0.94

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*D: deep; S: season

- Higher Simpson & Evenness in Summer.

Results & Discussion

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- Higher Simpson & Evenness in Summer.
- Higher Richness at 0 cm.

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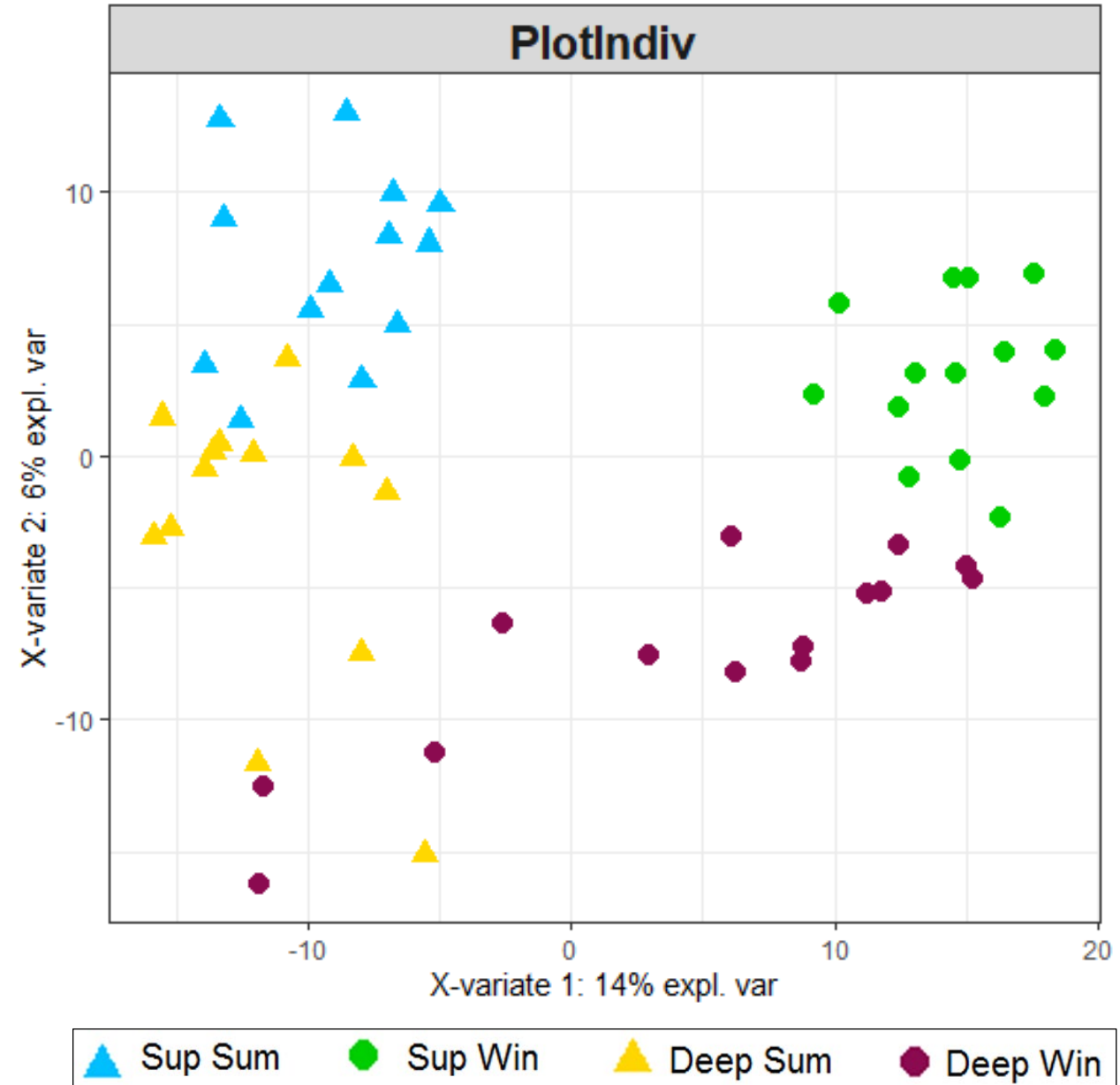
- Higher Simpson & Evenness in Summer.
- Higher Richness at 0 cm.
- Higher Evenness at 30 cm → *more balanced relative abundances*.

Results & Discussion

Beta biodiversity (*MixOmix* package):

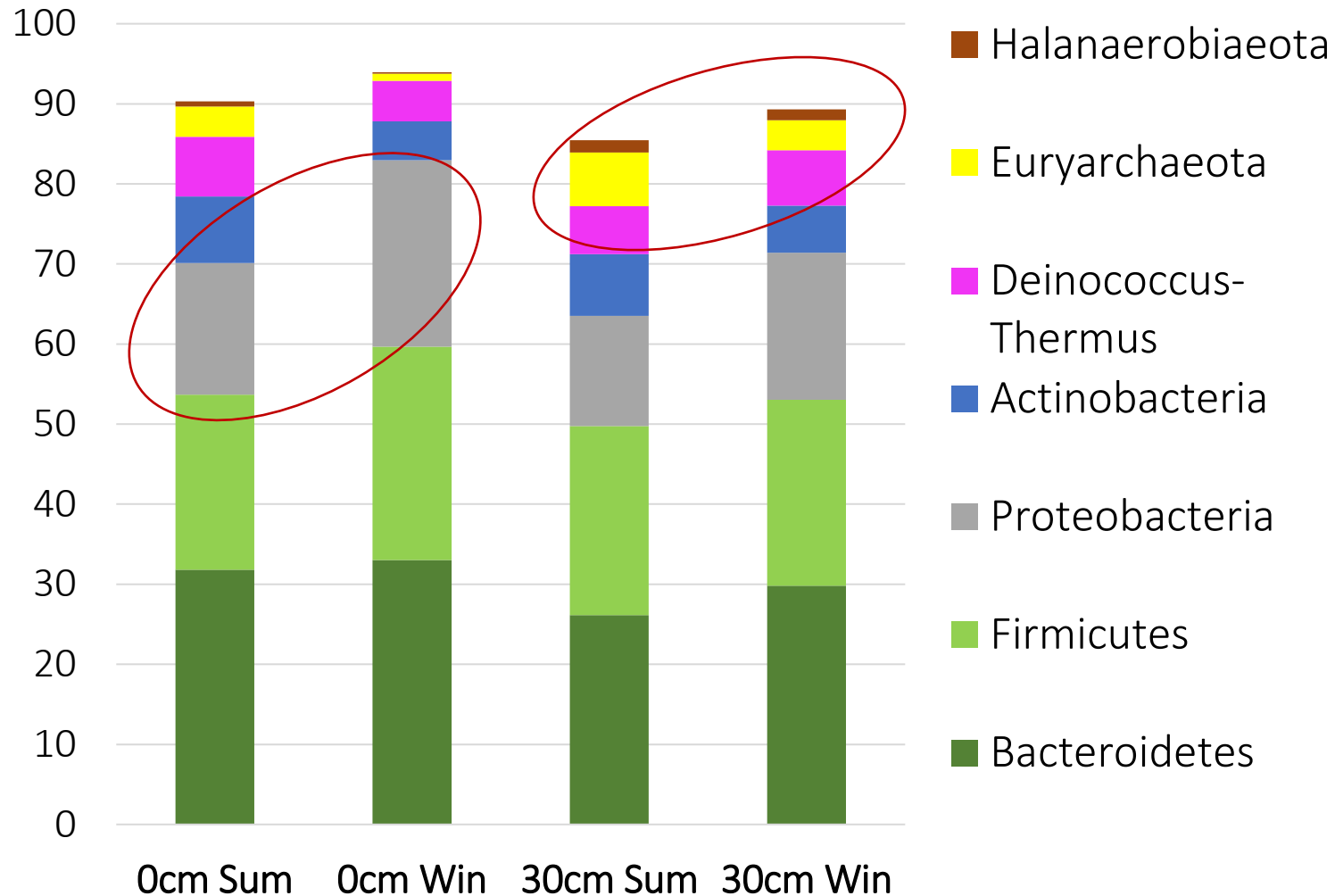
Partial least squares-discriminant analysis
(**PLS-DA**):

- Samples clustered by season & depth
(*Adonis* test: $P < \mathbf{0.001}$).
- More remarkable separation between seasons at surface layer (**0 cm, *Sup***) than in the deeper layer (**30 cm, *Deep***).



Results & Discussion

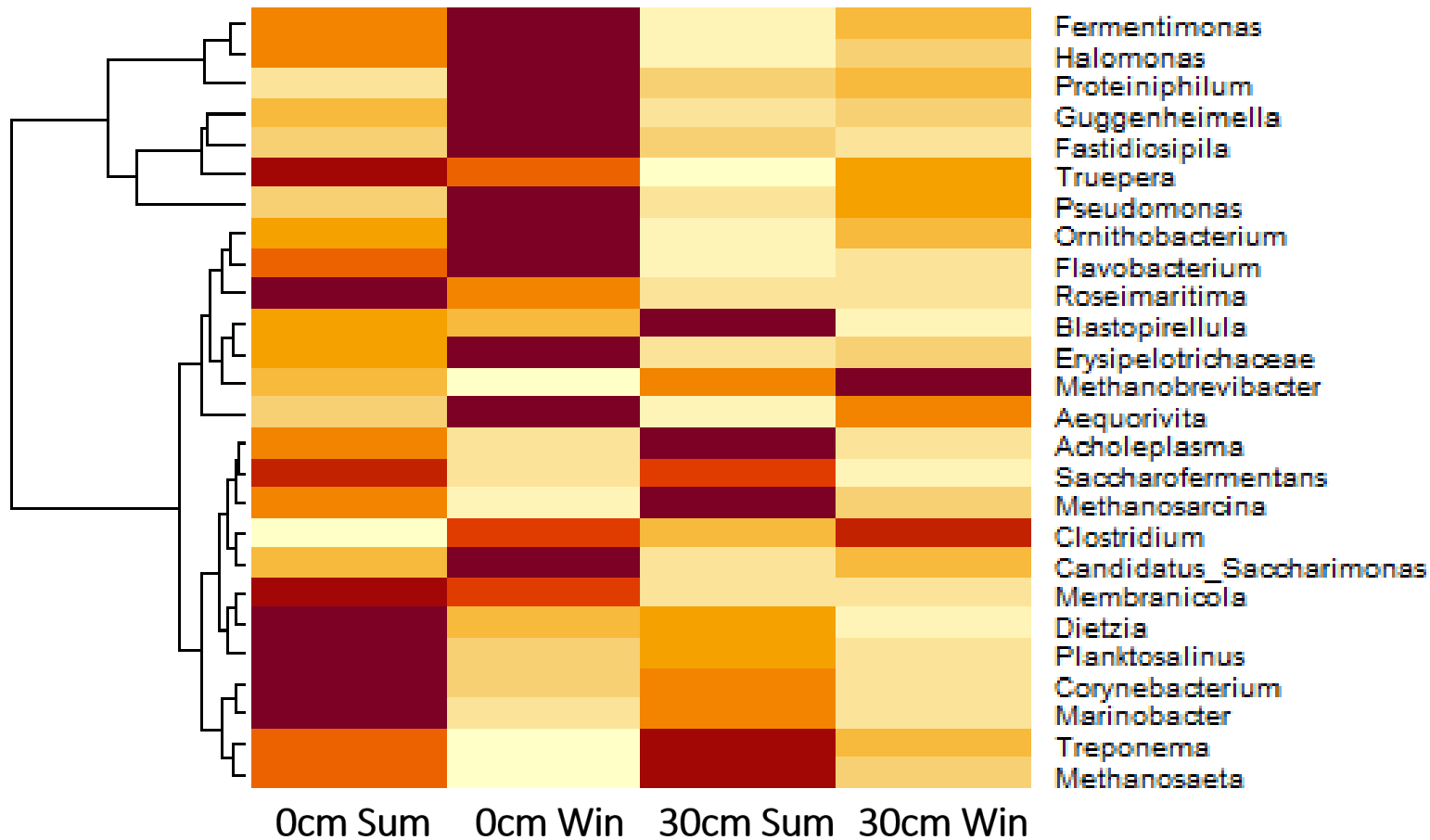
Most abundant **phyla**:



- Bacteroidetes & Firmicutes: most abundant genera in gut microbial environments.
- Proteobacteria: main AOB, higher in **0 cm** samples ($P < 0,01$).
- Euryarchaeota: methanogenic archaea, 3 times higher in **30cm** samples ($P < 0,01$).

Results & Discussion

Most abundant **genera**:



CH₄ - related:

*Methanobrevibacter, Methanosaeta
& Methanosarcina:*

higher relative abundance at
30 cm depth ($P < 0.05$)

NH₃, N₂O - related:

*Corynebacterium, Pseudomonas,
Halomonas, Truepera :*

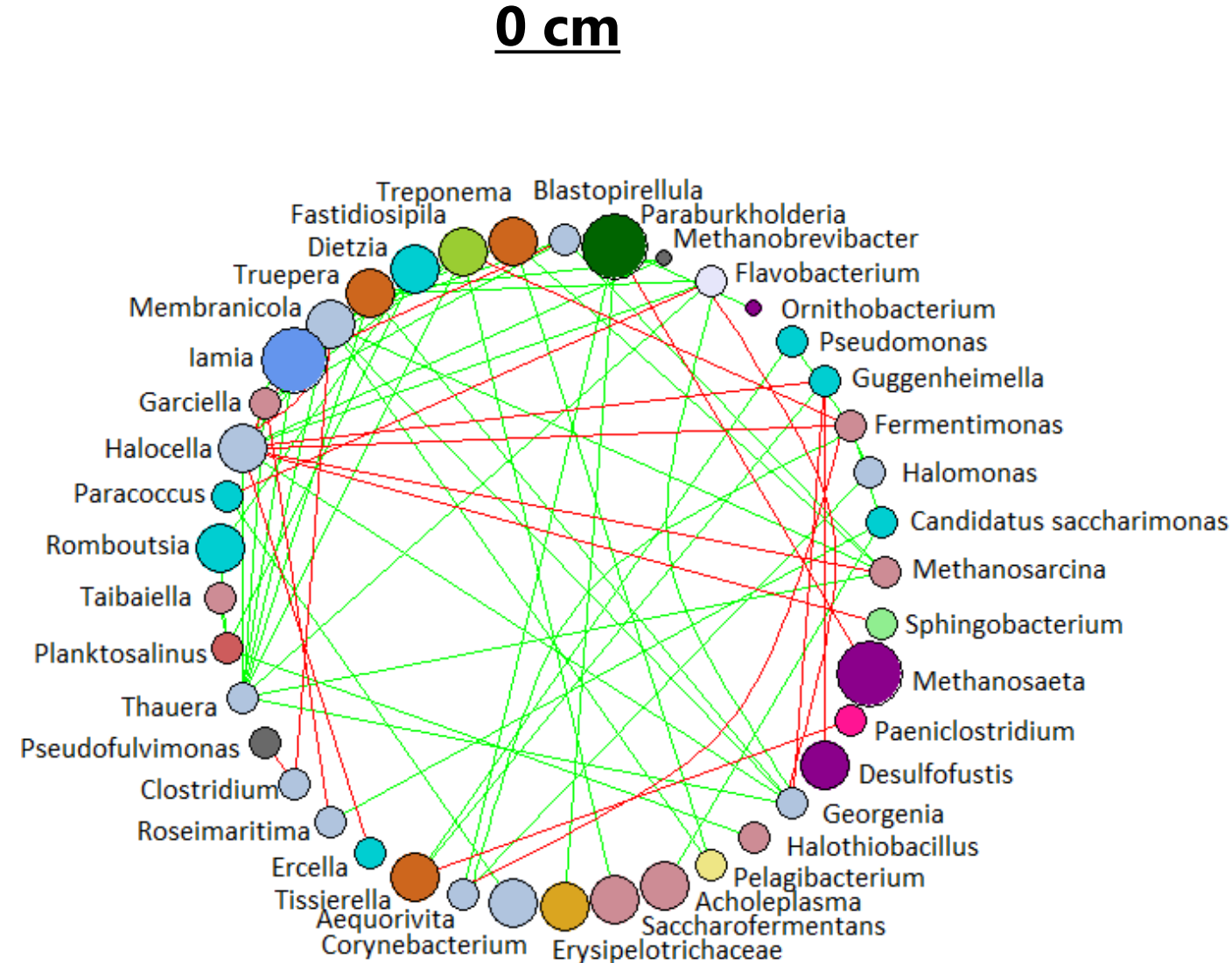
higher relative abundance at
0 cm depth ($P < 0.05$)

Results & Discussion

Network analysis:

(SpiecEasi package)

Parameter	0 cm
Edges	68
Average Node Degree	3.31
Average Betweenness	0.94



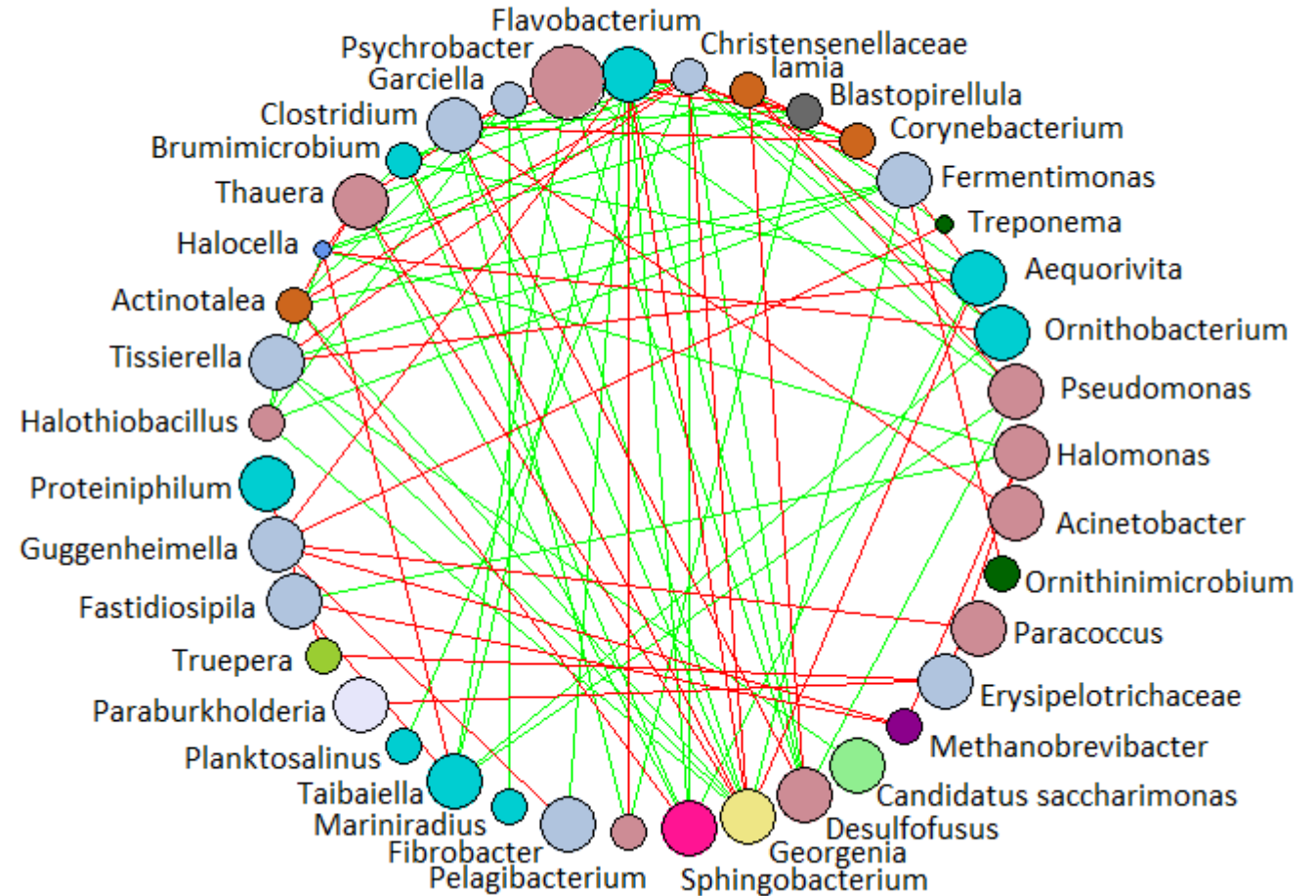
Results & Discussion

Network analysis:

(SpiecEasi package)

Parameter	0 cm	30 cm
Edges	68	90
Average Node Degree	3.31	4.61
Average Betweenness	0.94	0.81

30 cm



Conclusions

- Manure tilling performed in compost-bedded pack housing systems generates different pack layers, resulting in different microbial populations. Understanding this can allow for targeted management practices.
- Methanogenic archaea were found more abundant in deeper layers (30 cm), while N-transforming bacteria presence was higher at a surface level (0 cm).
- Environmental temperature had a significant effect on microbial populations differentiation between cold and warm periods at both depths under study.

Thank you for your attention!

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