



The LifeWatch ERIC Biodiversity
& Ecosystem eScience Conference
PLOVDIV, BULGARIA 7-10 JULY 2026

Gut microbiome Winter-Spring dynamics in protected wild herbivores in the Eastern Rhodopes

Bianka Marinova¹, Yana Evstatieva², Dilyana Nikolova², Georgi Pramatarov², Denis Borisov², Denitsa Teofanova¹

¹ Sofia University “St. Kliment Ohridski”, Faculty of Biology, Department of Biochemistry

² Sofia University “St. Kliment Ohridski”, Faculty of Biology, Department of Biotechnology

9 July 2026



Why Rewilding

- Supporting nature-driven processes;
- Bringing back wildlife and recovering of ecosystems;
- Ensuring wellbeing and future perspectives;
- Reintroduction of keystone species.



Why Rewilding Rhodopes

- Eastern Rhodopes have rich biodiversity;
- Home to several protected areas and nature reserves ;
- Keystone species such as European bison (*Bison bonasus*), red deer (*Cervus elaphus*), fallow deer (*Dama dama*) and Konik horses.

Bison bonasus



Image: Denitsa Teofanova

Konik horses



Image: Denitsa Teofanova

Cervus elaphus



Image: Denitsa Teofanova

Dama dama



Image: Stefan Stefanov

Goals with potential application

- Estimating their health status and investigating the influence of seasonal and species variations in diet;
- Determining bacteria in the feces of herbivores, which:
 - play a key role in maintaining ecosystem health;
 - have fiber-degrading activity and corresponding industrial application;
 - are potential probiotics for domestic animals.



Used methods

- Cultivation of microorganisms from suspensions of fecal samples on different selective media;
- MALDI-TOF identification of single colonies of microorganisms.



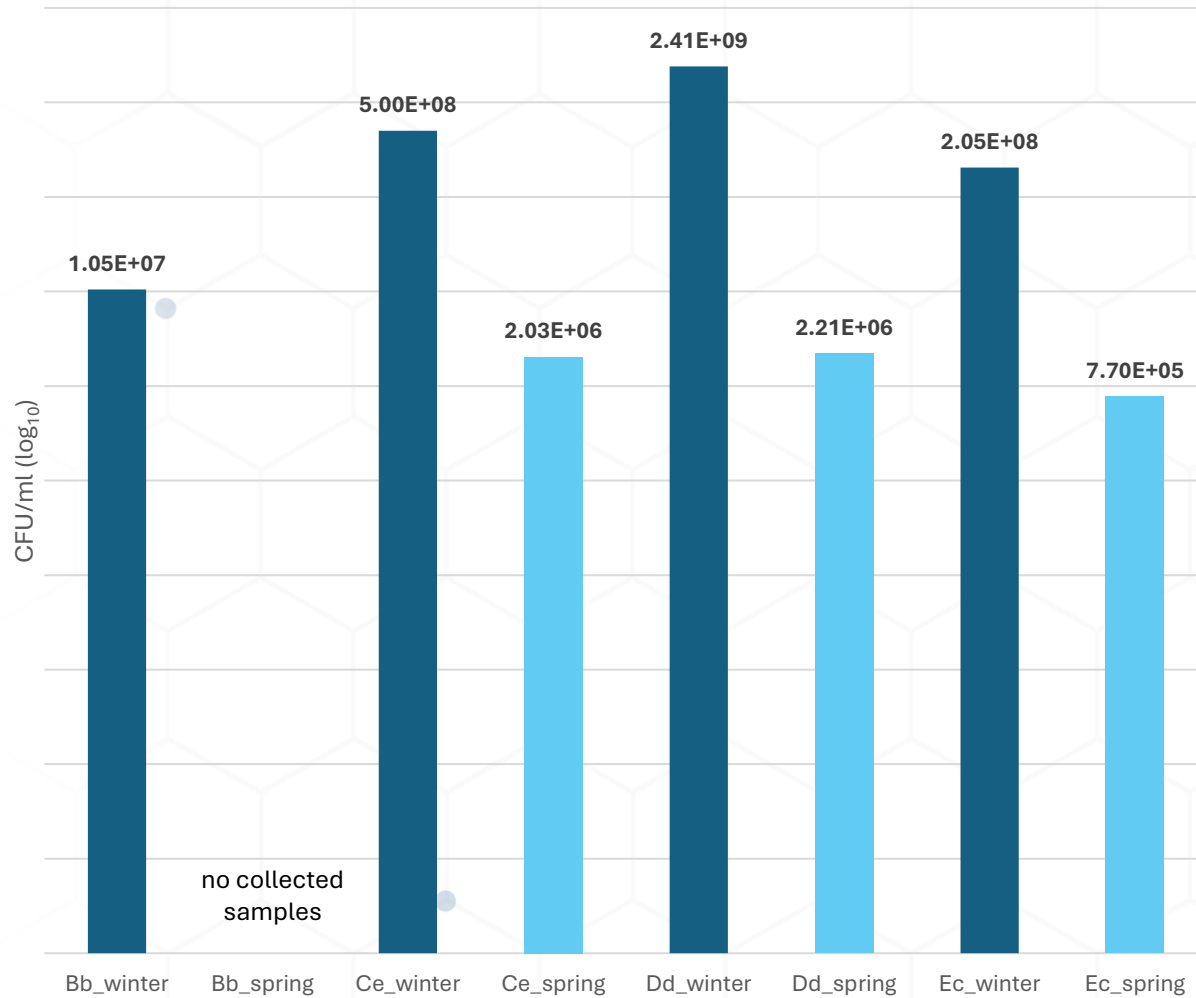
Sample 4

Sample Identifier: Ecll_7
 Sample Name: Y_G
 Sample Description: -
 Target Position: G6
 Sample Creation Date/Time: 5/21/2026 10:44 AM
 Sample Type / Prep. Protocol: Sample / eDT
 AutoXecute / ACQ Method: MBT_AutoX / D:\Methods\flexControlMethods\MBT_FC.par
 Preprocessing Method: Bruker Standard Pre-Processing Method 3/16/2021 3:48 PM
 Identification Method: Bruker Standard Identification Method 3/16/2021 3:48 PM
 Applied MSP Library(ies): BDAL / 12 / 11897
 SR_BBFV / 1 / 29
 Consistency category (based on two best matches): A

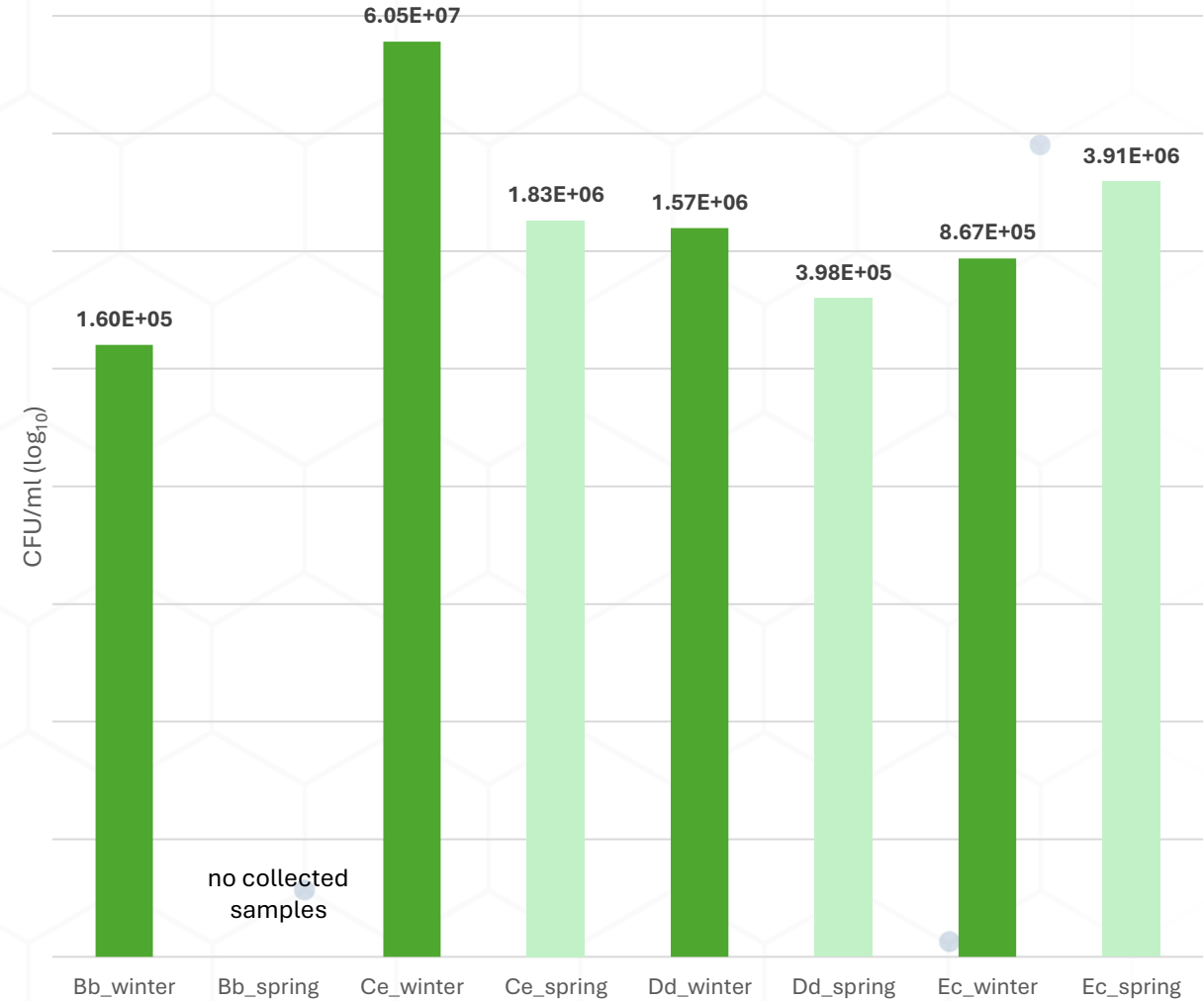
Rank	Matched Pattern	log(score) (Conf.)	NCBI Identifier
1	Streptococcus equi_ssp_zooepidemicus ATCC 43079T THL	2.23 (+++)	40041
2	Streptococcus equi_ssp_zooepidemicus DSM 20727T DSM	2.16 (+++)	40041
3	Streptococcus equi_ssp_zooepidemicus PAT5806_11 NVU	2.03 (+++)	40041
4	Streptococcus equi_ssp_zooepidemicus BKT28062_10 NVU	1.95 (+)	40041
5	Streptococcus equi_ssp_zooepidemicus BKT36582 NVU	1.94 (+)	40041
6	Streptococcus equi_ssp_zooepidemicus BKT17613_10 NVU	1.92 (+)	40041
7	Streptococcus equi_ssp_equi DSM 20561T DSM_2	1.88 (+)	148942
8	Streptococcus equi_ssp_zooepidemicus BKT7369_10 NVU	1.86 (+)	40041
9	Streptococcus equi_ssp_equi BKT48398_11 NVU	1.72	148942

The results so far

Bacteria



Yeast and mold



Microbiome diversity in *Bison bonasus*

Streptococcus infantarius

- Gram-positive bacteria;
- Found in traditionally fermented **diary products** (especially in Africa);
- Potential pathogen.

Limosilactobacillus reuteri

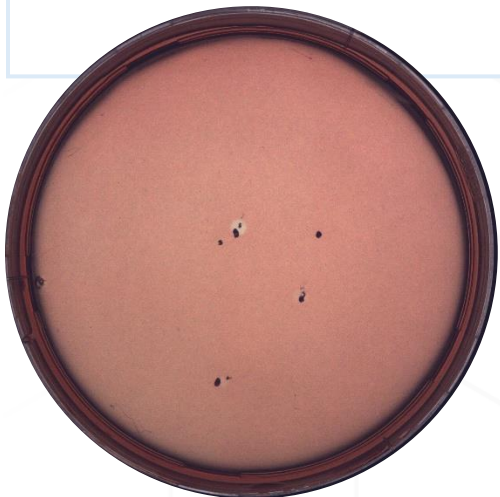
- One of the most important **probiotic bacteria**;
- Produces **reuterin** – inhibits growth of pathogenic bacteria.

Peribacillus simplex

- Gram-positive bacteria;
- **Plant growth promoting rhizobacteria**;
- Natural **pesticide**;
- Potential pathogen for people.

Mucor circinelloides

- Mold;
- **Model organism** for lipid production – rich of gamma-linolenic acid;
- Producer of enzymes, biodiesel, ethanol etc.



Sample identifier (Type)	Target Pos.	Organism (best match)	log(score) (Conf.)	Organism (second best match)	log(score) (Conf.)	Consistency
<u>Cel_4</u> (Filamentous Fungi)	<u>E8</u>	No organism identification possible	<u>1.54</u> (-)	No organism identification possible	<u>1.48</u> (-)	<u>(C)</u>
<u>Bbl_10</u> (Filamentous Fungi)	<u>E9</u>	Mucor circinelloides	<u>1.88</u> (+++)	Mucor circinelloides	<u>1.8</u> (+++)	<u>(A)</u>

Microbiome diversity in *Cervus elaphus*.

Pseudomonas taetrolens

- Gram-negative bacteria;
- Production of **lactobionic acid** – essential for pharmaceutical and food industry, cosmetics.

Flavobacterium piscis

- Gram-negative bacteria;
- Lives in **slowly running cold water**;
- Aerobic and adapted to low temperatures.

Aspergillus flavus_oryzae group

- Fungus community;
- *A. flavus* is a producer of **alphatoxins**, while *A. oryzae* can not produce toxins.

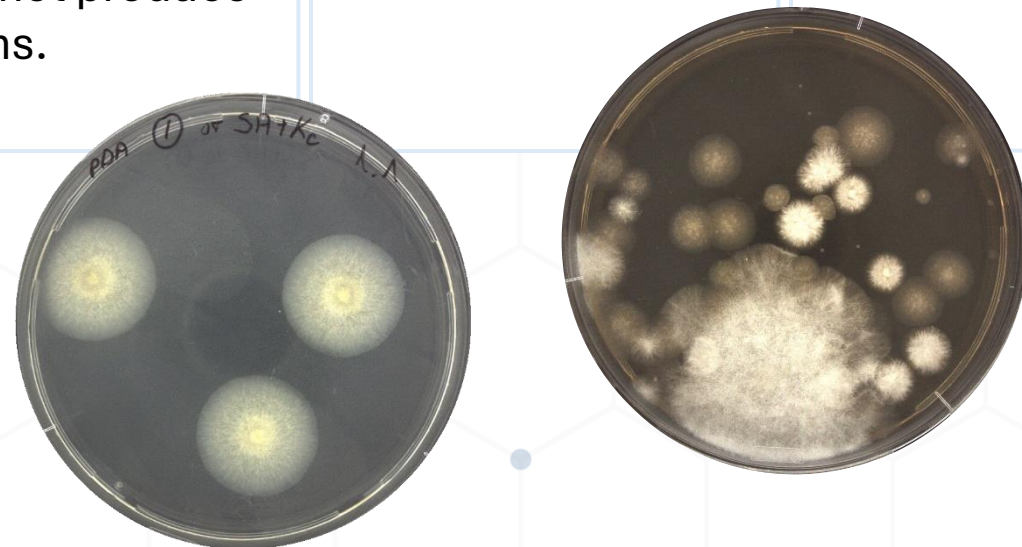
Streptococcus lutetiensis

- Represents risk of bacteremia and endocarditis;
- **Indicator of inflammatory processes.**

Stenotrophomonas rhizophila

- Gram-negative **rhizobacteria**;
- Easily adaptive – found in deserts, saline soil and seaside.

Streptococcus infantarius



Microbiome diversity in *Dama dama*

Bacillus mycoides

- Gram-positive **rhizobacteria** in soil;
- Helps in activation of plant immunity;
- Takes part in ammonification.

Pantoea agglomerans

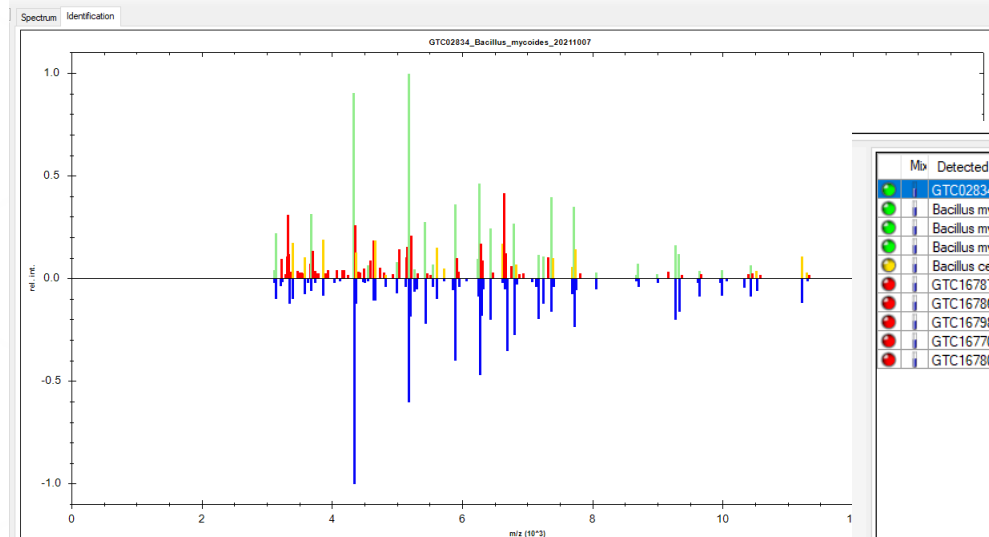
- Gram-negative bacteria;
- **Biopesticide** – against *Erwinia amylovora*;
- Essential for agriculture.

Arthrobacter citreus

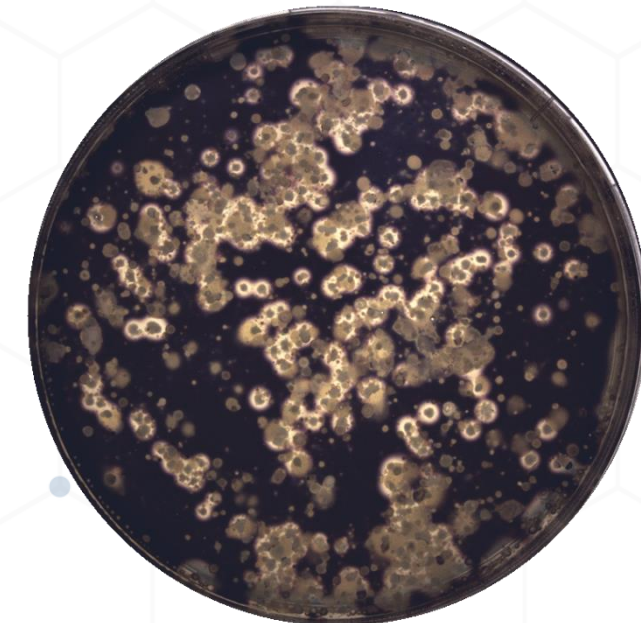
- Gram-positive bacteria;
- Found in soil.

Bacillus pumilus

- Nitrogen-fixator;
- Antimicrobial activity – **biofungicide** (in Bulgaria strain QST 2808 is used);
- **Probiotic** properties.



Mix	Detected Species	Log(Score)
✓	GTC02834 <i>Bacillus mycoides</i> 20211007	2.240
✓	<i>Bacillus mycoides</i> DSM 11821T DSM	2.210
✓	<i>Bacillus mycoides</i> DSM 11821T DSM 2	2.100
✓	<i>Bacillus mycoides</i> DSM 2048T DSM	2.010
✓	<i>Bacillus cereus</i> DSM 31T DSM	1.830
✓	GTC16787 <i>Bacillus cereus</i> 20211015	1.690
✓	GTC16786 <i>Bacillus cereus</i> 20211015	1.680
✓	GTC16798 <i>Bacillus cereus</i> 20211018	1.670
✓	GTC16770 <i>Bacillus cereus</i> 20211015	1.670
✓	GTC16780 <i>Bacillus cereus</i> 20211011	1.630



Microbiome diversity in *Dama dama*

Acinetobacter guillouiae

- Gram-negative bacteria;
- Found in traditionally fermented **yogurt**, especially in the **Rhodopes**.

Erwinia persicina

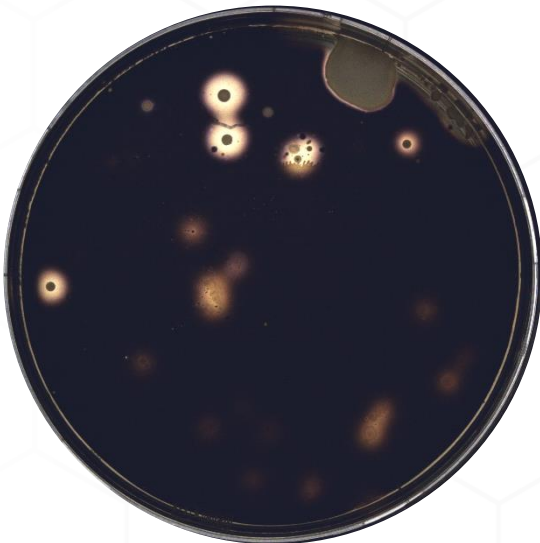
- Gram-negative bacteria;
- **Phytopathogen**;
- Causes “soft rot”.

Pseudomonas tolaasii

- Gram-negative bacteria;
- Well-known **pathogen** over cultivated mushrooms – causes “Bacterial Blotch”.

Bacillus mycoides

- Gram-positive bacteria in soil;
- Supports **plant development** and nitrogen feeding.



Sample identifier (Type)	Target Pos.	Organism (best match)	log(score) (Conf.)	Organism (second best match)	log(score) (Conf.)	Consistency
Dd II 6 1 (Sample)	F12	Acinetobacter guillouiae	2.09 (+++)	Acinetobacter guillouiae	2.07 (+++)	(A)
Dd II 6 2 (Sample)	G1	No organism identification possible	1.62 (-)	No organism identification possible	1.62 (-)	(C)

Microbiome diversity in Konik horses

Staphylococcus warneri

- Gram-positive bacteria;
- Found in human and animal **skin and mucosa**;
- Low pathogenic risk.

Ligilactobacillus agilis

- Gram-positive bacteria;
- Unique possibility of movement;
- Found in rumen of herbivores;
- **Reduces inflammation** and represents a **possible additive** for forage.

Ligilactobacillus equi

- Gram-positive bacteria exclusively found in **horses**;
- Maintains the **balance** in horses' **microflora**.

Enterococcus casseliflavus

- Gram-positive bacteria;
- Found in soil, water, some foods (olives), over plants and in human and animal digestive system;
- Produces of **antimicrobe peptides** and it is used as starter for olive fermentation.



Conclusions

- All analyzed animal samples showed the presence of micromycete species as well as *Escherichia coli*.
- The concentration of microorganisms differed between the analyzed samples from the two seasons – the total microbial count in winter samples was 10^8 CFU/mL, whereas in spring samples it was 10^6 CFU/mL.
- The decrease in microbial concentration is most likely attributable to changes in the animals' diet associated with the availability of fresh green forage.



The LifeWatch ERIC Biodiversity
& Ecosystem eScience Conference
PLOVDIV, BULGARIA 7-10 JULY 2026

THANK YOU!

• *Questions?*
bimarinova@uni-sofia.bg

Special thanks to our partners from
Rewilding Rhodopes Foundation -
Desislava Kostadinova and Hristo Hristov

This work was supported by the National Science Fund of the Bulgarian,
MES project КП-06-H91/9, 2025 and the Centre of Competence
“Sustainable Utilization of Bio-resources and Waste of Medicinal and
Aromatic Plants for Innovative Bioactive Products” (BIORESOURCES BG),
project BG16RFPR002-1.014-0001, PRIDST, 2021–2027.

