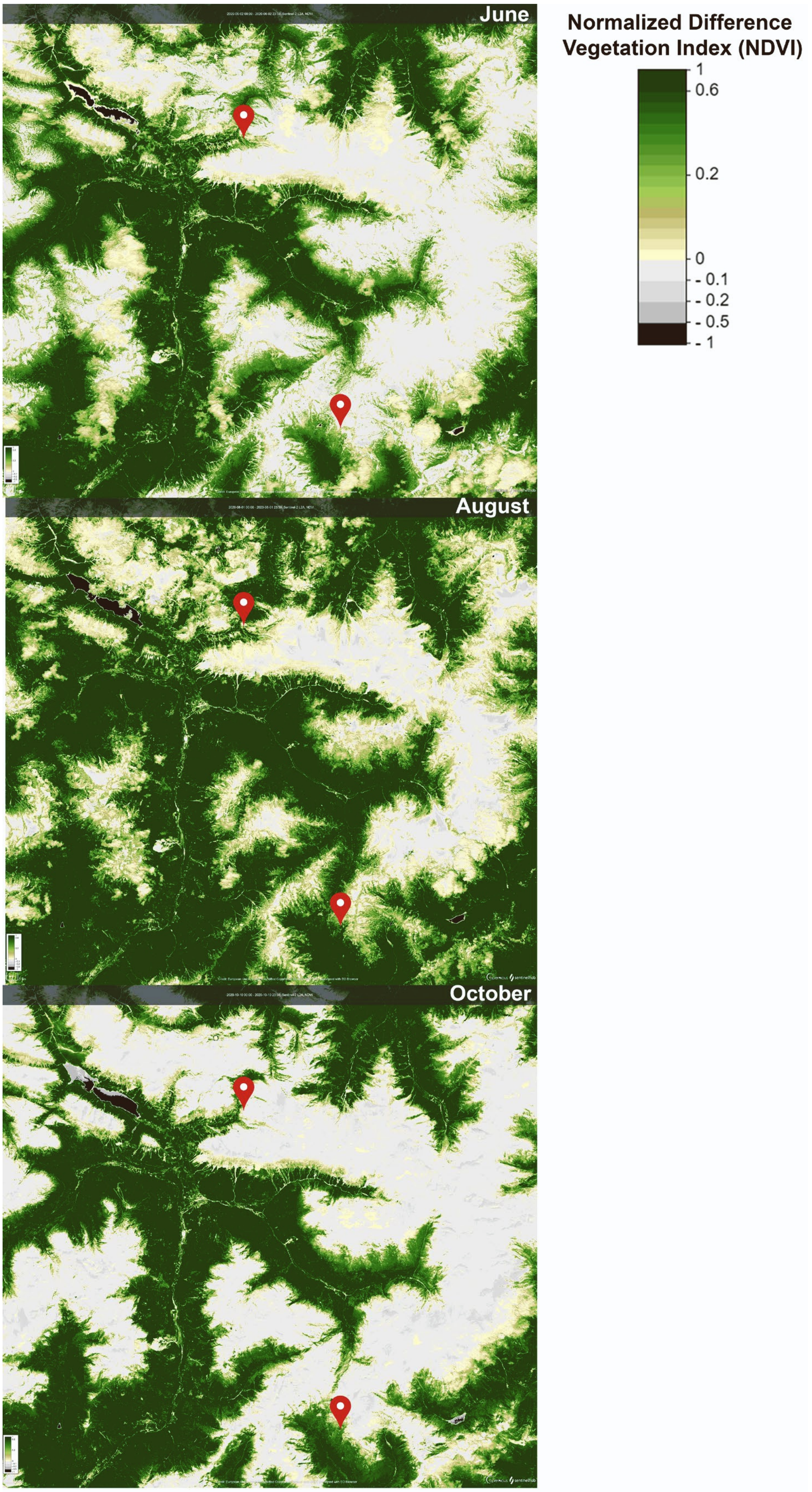
iScience, Volume *27*

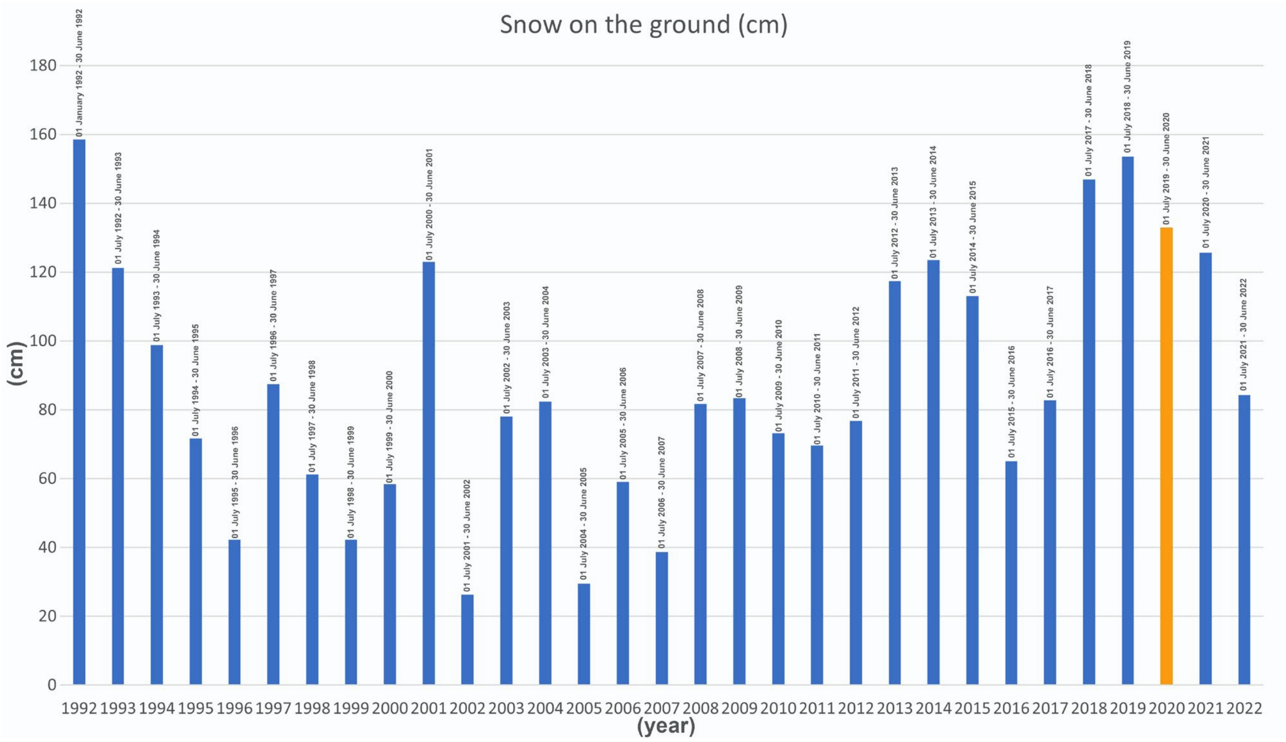
Supplemental information

The Alpine ibex (Capra ibex) gut microbiome, seasonal dynamics, and potential application in lignocellulose bioconversion

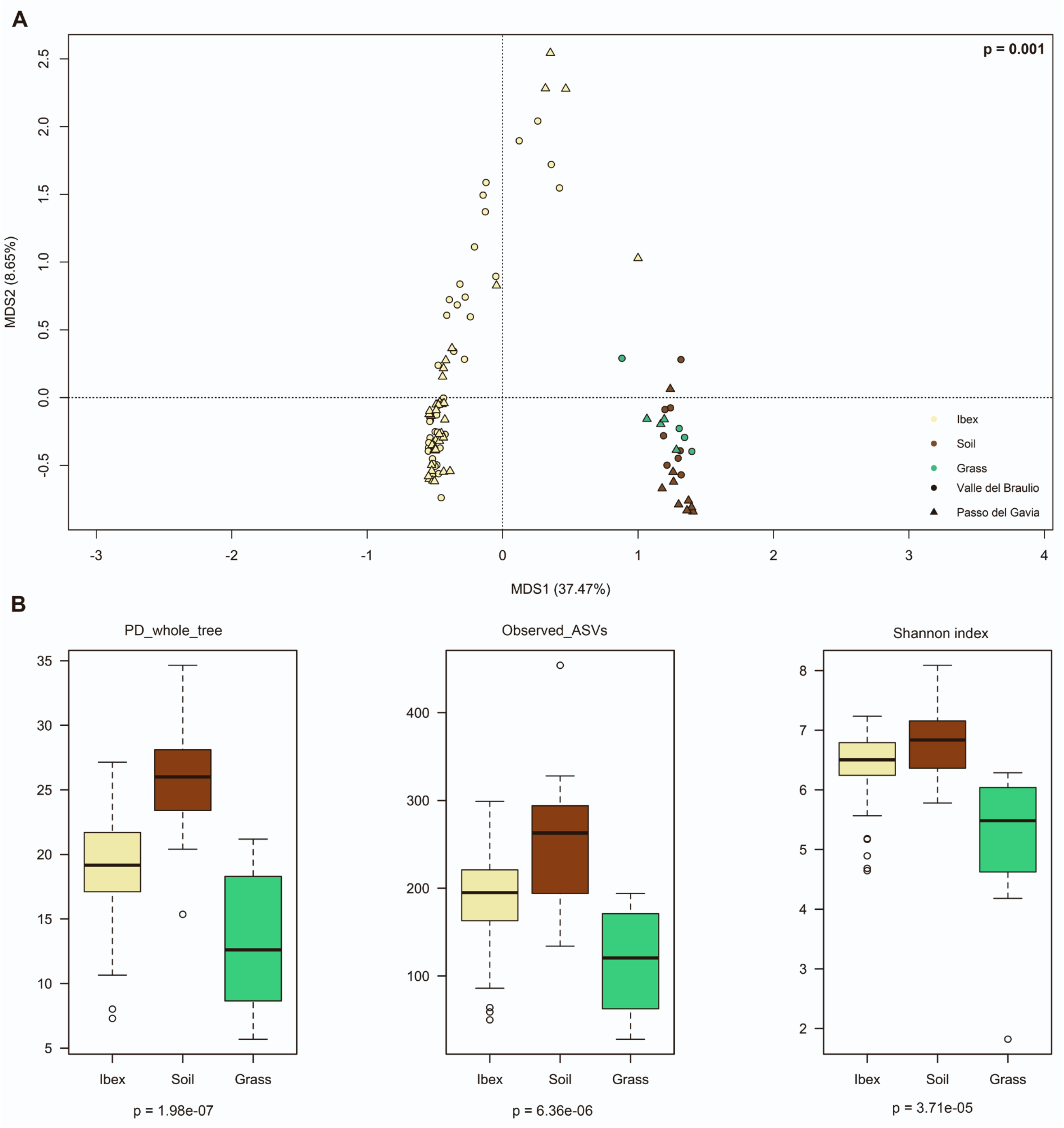
Enrico Nanetti, Daniel Scicchitano, Giorgia Palladino, Nicolò Interino, Luca Corlatti, Luca Pedrotti, Federica Zanetti, Elena Pagani, Erika Esposito, Alice Brambilla, Stefano Grignolio, Ilaria Marotti, Silvia Turroni, Jessica Fiori, Simone Rampelli, and Marco Candela



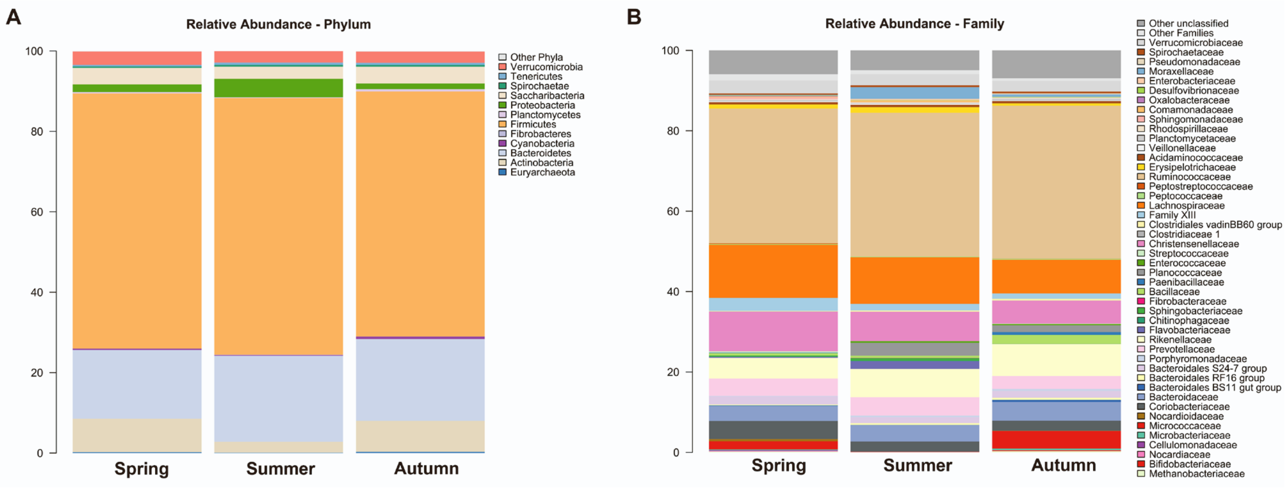
Supplementary Figure 1 - Sampling sites. Map of the sampling sites at Stelvio National Park in Lombardia (Italy), showing the centroid of the sampling spots at “Passo del Gavia” and “Valle del Braulio”. Sampling coordinates are marked in red. Satellite images from Sentinel-2 were retrieved for the three timepoints (i.e., June, August and October) (https://apps.sentinel-hub.com/eo-browser/). The days chosen for the three sampling periods were June 2nd, August 1st and October 10th, 2020, in order to have pictures with cloud cover < 20%. For each timepoint, the Normalized Difference Vegetation Index, NDVI, is shown (see color bar on the right).



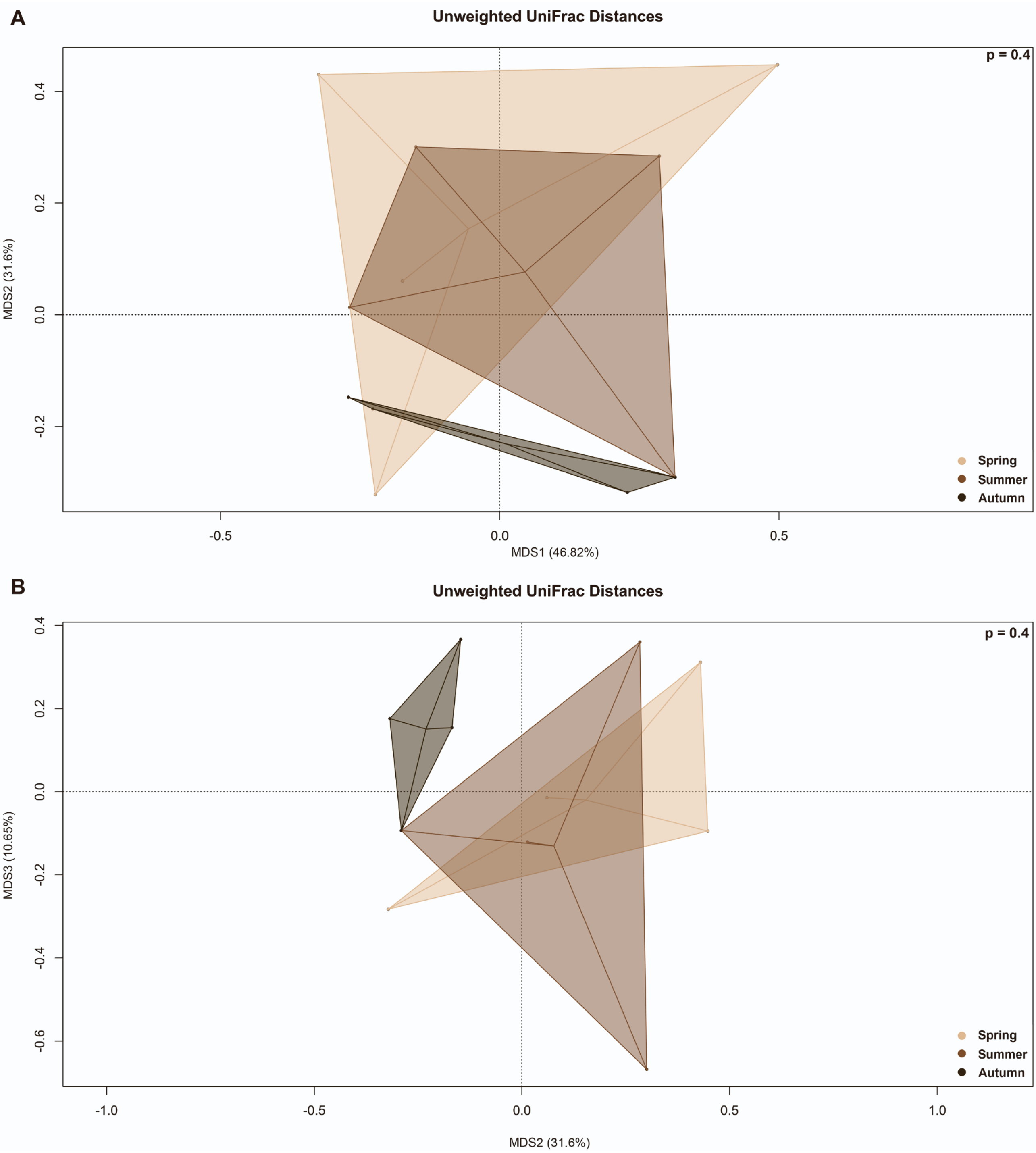
Supplementary Figure 2 - Snow on the ground (cm) from 1992 to 2020. The mean value of snow on the ground (cm) from July 1st to June 30th of the next year (when possible) is shown. The bar regarding the period of our study is highlighted in orange.



Supplementary Figure 3 - Comparison between the *C. ibex* gut microbiome and environmental microbiomes. (A) Principal Coordinates Analysis (PCoA) based on Bray-Curtis distances between the microbiome profiles of Alpine ibex gut (yellow), soil (brown) and grass (green) across the two sampling sites, i.e., “Passo del Gavia” (triangles) and “Valle del Braulio” (circles) (permutation test with pseudo-F ratio, p = 0.001). The first and second principal components (MDS1 and MDS2) are plotted and the percentage of variance in the dataset explained by each axis is shown. **(B)** Boxplots showing the alpha-diversity distributions of the *C. ibex* gut, soil and grass microbiomes, based on the Faith’s Phylogenetic Diversity (PD whole tree), the number of observed ASVs and the Shannon index (Kruskal-Wallis test, p ≤ 0.001).



Supplementary Figure 4 - Taxonomic composition of the *C. ibex* gut microbiome. Barplots summarizing the phylum- **(A)** and family-level **(B)** bacterial composition of *C. ibex* feces in spring, summer and autumn. Only phyla and families with relative abundance > 0.5% in at least 2 samples are shown.

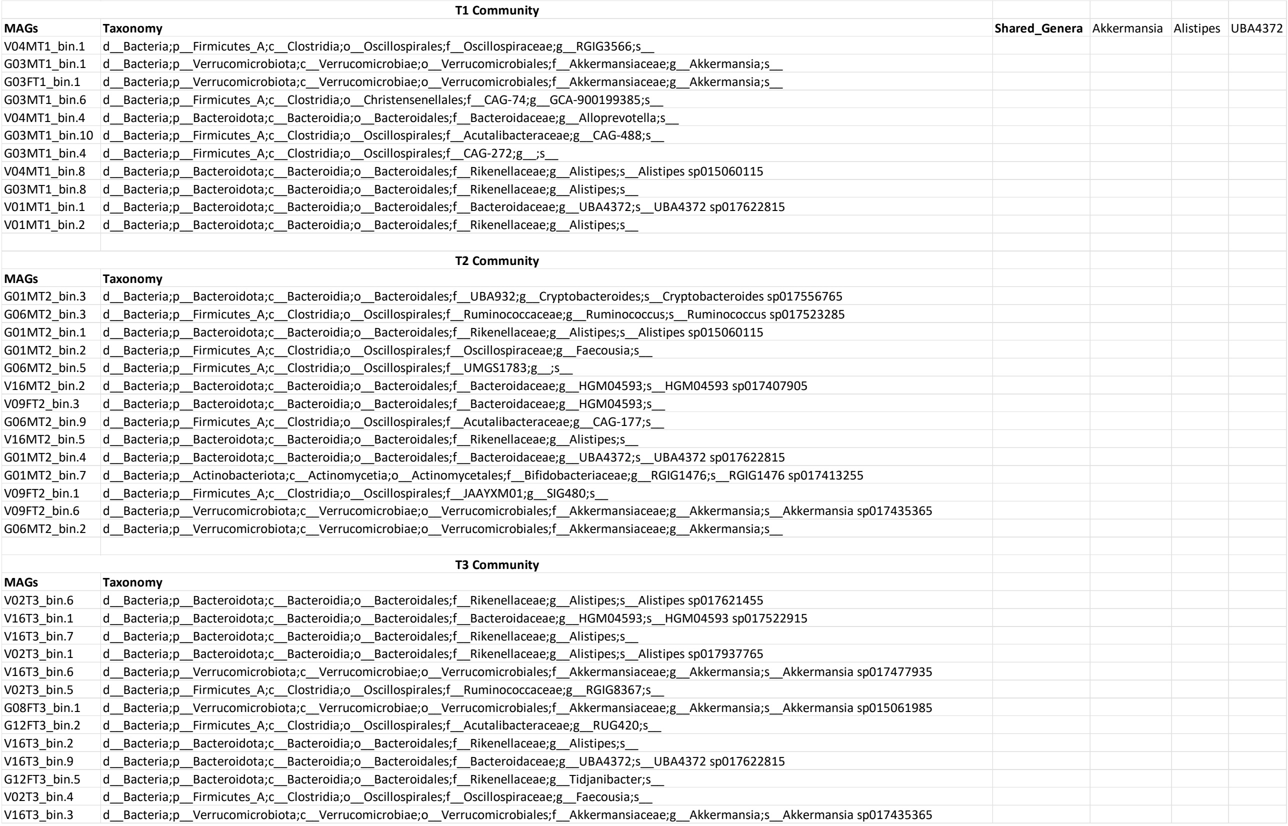


Supplementary Figure 5 - Seasonal variation of SGB communities in the Alpine ibex gut microbiome. Principal Coordinates Analyses (PCoAs) based on unweighted UniFrac distances between Alpine ibex SGB gut microbial communities across seasons, i.e., spring (June, light brown), summer (August, brown) and autumn (October, dark brown) (permutation test with pseudo-F ratio, p = 0.4). The first and second principal components (MDS1 and MDS2) are plotted in **(A)** while the second and third principal components (MDS2 and MDS3) are plotted in **(B)** and the percentage of variance in the dataset explained by each axis is shown. Squared areas were drawn to enclose all samples.

Supplementary Table S1. Sampling details of the present study; number and type of samples collected.

SITE	Number of Samples collected								
	June 2020			August 2020			October 2020		
	Alpine ibex	Soil	Grass	Alpine ibex	Soil	Grass	Alpine ibex	Soil	Grass
Passo del Gavia	14	2	0	9	2	1	15	5	3
Valle del Braulio	12	1	0	17	2	1	19	5	3

Supplementary Table S2. SGBs within the bacterial communities in the 3 seasons interacting for the degradation of plant cell wall biopolymers. The right side highlights the bacterial genera shared between seasons.



	T1 Community				
MAGs	**Taxonomy**	**Shared_Genera**	Akkermansia	Alistipes	UBA4372
V04MT1_bin.1	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Oscillospiraceae;g__RGIG3566;s__				
G03MT1_bin.1	d__Bacteria;p__Verrucomicrobiota;c__Verrucomicrobiae;o__Verrucomicrobiales;f__Akkermansiaceae;g__Akkermansia;s__				
G03FT1_bin.1	d__Bacteria;p__Verrucomicrobiota;c__Verrucomicrobiae;o__Verrucomicrobiales;f__Akkermansiaceae;g__Akkermansia;s__				
G03MT1_bin.6	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Christensenellales;f__CAG-74;g__GCA-900199385;s__				
V04MT1_bin.4	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Alloprevotella;s__				
G03MT1_bin.10	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g__CAG-488;s__				
G03MT1_bin.4	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__CAG-272;g__;s__				
V04MT1_bin.8	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Alistipes;s__Alistipes sp015060115				
G03MT1_bin.8	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Alistipes;s__				
V01MT1_bin.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__UBA4372;s__UBA4372 sp017622815				
V01MT1_bin.2	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Alistipes;s__				
	T2 Community				
MAGs	**Taxonomy**				
G01MT2_bin.3	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__UBA932;g__Cryptobacteroides;s__Cryptobacteroides sp017556765				
G06MT2_bin.3	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae;g__Ruminococcus;s__Ruminococcus sp017523285				
G01MT2_bin.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Alistipes;s__Alistipes sp015060115				
G01MT2_bin.2	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Oscillospiraceae;g__Faecousia;s__				
G06MT2_bin.5	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__UMGS1783;g__;s__				
V16MT2_bin.2	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__HGM04593;s__HGM04593 sp017407905				
V09FT2_bin.3	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__HGM04593;s__				
G06MT2_bin.9	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g__CAG-177;s__				
V16MT2_bin.5	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Alistipes;s__				
G01MT2_bin.4	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__UBA4372;s__UBA4372 sp017622815				
G01MT2_bin.7	d__Bacteria;p__Actinobacteriota;c__Actinomycetia;o__Actinomycetales;f__Bifidobacteriaceae;g__RGIG1476;s__RGIG1476 sp017413255				
V09FT2_bin.1	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__JAAYXM01;g__SIG480;s__				
V09FT2_bin.6	d__Bacteria;p__Verrucomicrobiota;c__Verrucomicrobiae;o__Verrucomicrobiales;f__Akkermansiaceae;g__Akkermansia;s__Akkermansia sp017435365				
G06MT2_bin.2	d__Bacteria;p__Verrucomicrobiota;c__Verrucomicrobiae;o__Verrucomicrobiales;f__Akkermansiaceae;g__Akkermansia;s__				
	T3 Community				
MAGs	**Taxonomy**				
V02T3_bin.6	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Alistipes;s__Alistipes sp017621455				
V16T3_bin.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__HGM04593;s__HGM04593 sp017522915				
V16T3_bin.7	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Alistipes;s__				
V02T3_bin.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Alistipes;s__Alistipes sp017937765				
V16T3_bin.6	d__Bacteria;p__Verrucomicrobiota;c__Verrucomicrobiae;o__Verrucomicrobiales;f__Akkermansiaceae;g__Akkermansia;s__Akkermansia sp017477935				
V02T3_bin.5	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae;g__RGIG8367;s__				
G08FT3_bin.1	d__Bacteria;p__Verrucomicrobiota;c__Verrucomicrobiae;o__Verrucomicrobiales;f__Akkermansiaceae;g__Akkermansia;s__Akkermansia sp015061985				
G12FT3_bin.2	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g__RUG420;s__				
V16T3_bin.2	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Alistipes;s__				
V16T3_bin.9	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__UBA4372;s__UBA4372 sp017622815				
G12FT3_bin.5	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Tidjanibacter;s__				
V02T3_bin.4	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Oscillospiraceae;g__Faecousia;s__				
V16T3_bin.3	d__Bacteria;p__Verrucomicrobiota;c__Verrucomicrobiae;o__Verrucomicrobiales;f__Akkermansiaceae;g__Akkermansia;s__Akkermansia sp017435365				