



Project LIFE16 ENV/IT/000566 LIFE GREEN GRAPES



STUDY REPORT

Bioinformatics analysis of microbial communities associated with vines

[Analisi Bioinformatica delle comunità microbiche associate ai vitigni]

- Deliverable 3 – Action A3-



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Microbiological Analysis REPORT

Analyzed samples and quantities/reads count

The samples collected were taken to the laboratory.

For each sample 0.5 g of material was weighed, and used for DNA extraction using the commercial kit Fast DNA[®] Spin Kit for Soil[™] (MP Biomedicals, CA, USA)

Once the DNA was extracted, an electrophoresis on agarose gel was performed to quantify the DNA available for each sample.

The quantified DNA was sent to an external company (IGA Technology Services, Udine, Italy) which proceeded to carry out a massive sequencing using the Illumina platform.

For each sample, a massive sequencing of the 16S rRNA gene and the ITS gene was performed, which represent respectively the molecular markers for the bacterial and fungal community.

Table 1 resumes the numbers of bacterial and fungal sequences (reads) obtained from each sample

Table 1 :Reads count obtained for each sample.

Sample	16S	ITS
GabbianoTesi5-foglia	27270	-
GabbianoTesi1-suolo	223355	15300
GabbianoTesi2-suolo	187981	52025
GabbianoTesi3-suolo	189138	60779
GabbianoTesi4-suolo	176287	28175
GabbianoTesi5-suolo	87139	156528
CiproPlotA-suolo	227052	54715
CiproPlotB-suolo	123541	22634
CiproPlotC-suolo	113592	32909
CiproPlotC-foglia	180415	-
<u>Total</u>	<u>1535770</u>	<u>423065</u>

The table shows that all the amplifications of the DNA samples gave positive results for the sequencing of the bacterial gene, while 8 samples out of a total of 10 gave a positive result for the sequencing of the ITS gene for fungi. The 2 samples that have not been amplified are those related to the leaves.

Below are the analyses of the two bacterial and fungal communities. The results were obtained following the suggested pipeline of the bioinformatic software QIIME (Caporaso et al., 2010). Statistical data processing was obtained using the PAST software (Hammer et al., 2001).

16S rRNA gene analysis: the bacterial community

The analysis of the bacterial community was performed following the suggested pipeline on the QIIME software website.

In Figure 1, the relative abundances of the different bacterial Phyla identified in each sample are reported. As can be seen from the results, the two samples related to the LEAVES material (Cipro Plot 5 foglia e Gabbiano Tesi 5 foglia) are very similar and characterized by the dominance of the Cyanobacteria. In fact, the sequences related to cyanobacteria are attributable to the chloroplast sequences; this is due to the fact that the primers chosen for the amplification of the 16S rRNA gene have cyanobacteria among their targets, and for this reason they are not suitable for amplifying DNA from leaves. They were nevertheless used because the leaves were not the only type of samples available, and the same primers are commonly used in literature for samples such as soil and roots.

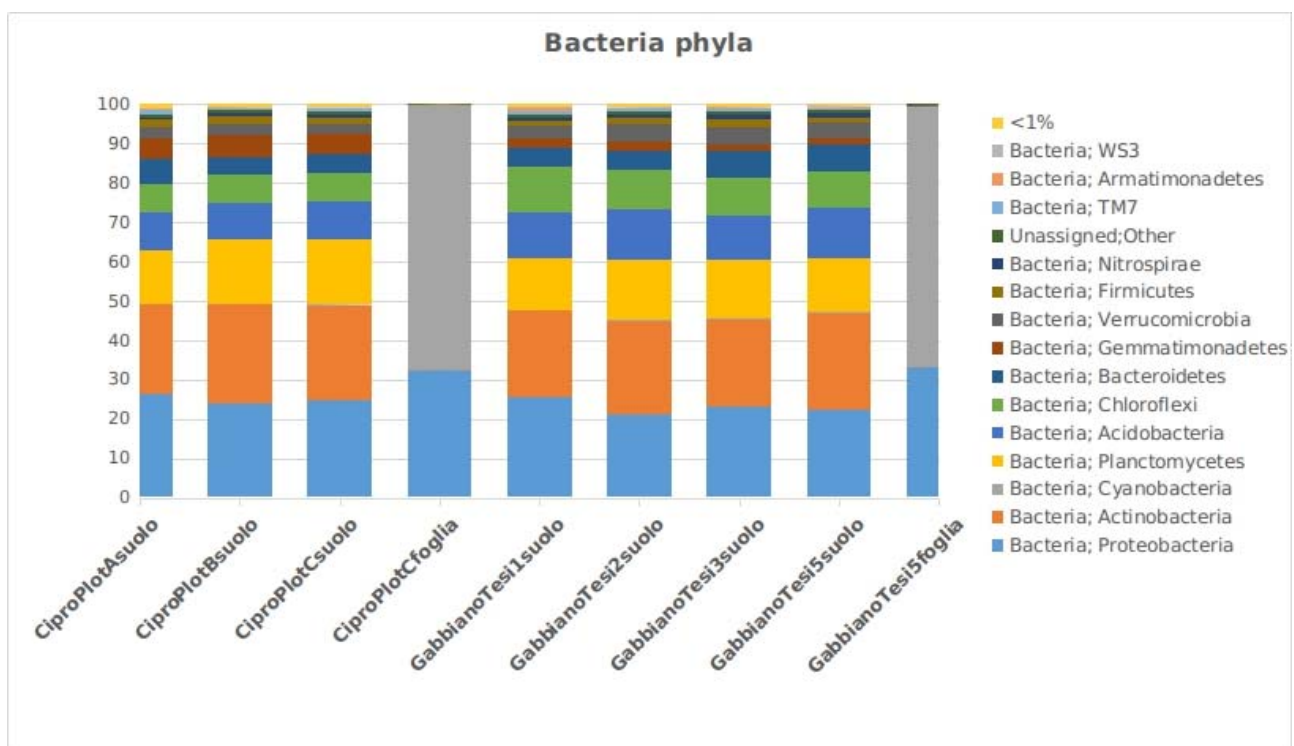


Figure 1: composition of bacterial communities at the phylum level

The data matrix related to the bacterial community was also analyzed at the genus level. The main diversity indices have been calculated (Table 2), whose values have confirmed that the samples related to the leaf material are the poorest in terms of biodiversity (lower values of Chao1

index, especially for the sample “Gabbiano tesi5 foglia”). At the same time, the values of the Shannon index for these two samples are the lowest, indicating that they are characterized by a very low number of bacterial genera, each one with many individuals inside, compared to the other samples, which instead are characterized by a higher number of bacterial genes each with fewer individuals inside.

Table 2: diversity indices calculated for the genus-data matrix.

	Cipro PlotA suolo	Cipro PlotB suolo	Cipro PlotC suolo	Cipro PlotC foglia	Gabbiano Tesi1 suolo	Gabbiano Tesi2 suolo	Gabbiano Tesi3 suolo	Gabbiano Tesi4 suolo	Gabbiano Tesi5 suolo	Gabbiano Tesi5 foglia
Simpson 1-D	0,9648	0,9646	0,9635	0,4393	0,9685	0,969	0,9674	0,9699	0,9688	0,4474
Shannon H	4,155	4,092	4,096	0,6459	4,184	4,192	4,213	4,198	4,192	0,6605
Evenness e^H/S	0,4688	0,4433	0,4421	0,0829 5	0,4824	0,4866	0,497	0,4893	0,4862	0,3872
Chao-1	136	135	136	23	136	136	136	136	136	5

Figure 2 shows the Cluster Analysis related to the bacterial community at the genus level. From the graph it is clear how the community related to the leaf is completely different from that of the soil. Furthermore, the samples related to the "Gabbiano" site form a very distinct cluster, as well as the “Cipro” site samples, all supported by high bootstrap values.

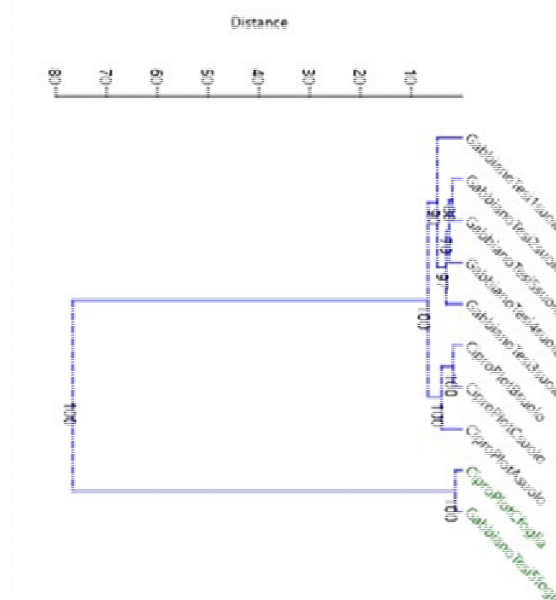


Figure 2: Cluster Analysis performed with Ward’s method, Euclidean distance, and 100 bootstrap, on 16S data matrix.

The separation of these three groups of samples is also confirmed by Non-metric Multidimensional Scaling (NMDS) analysis reported in Figure 3.

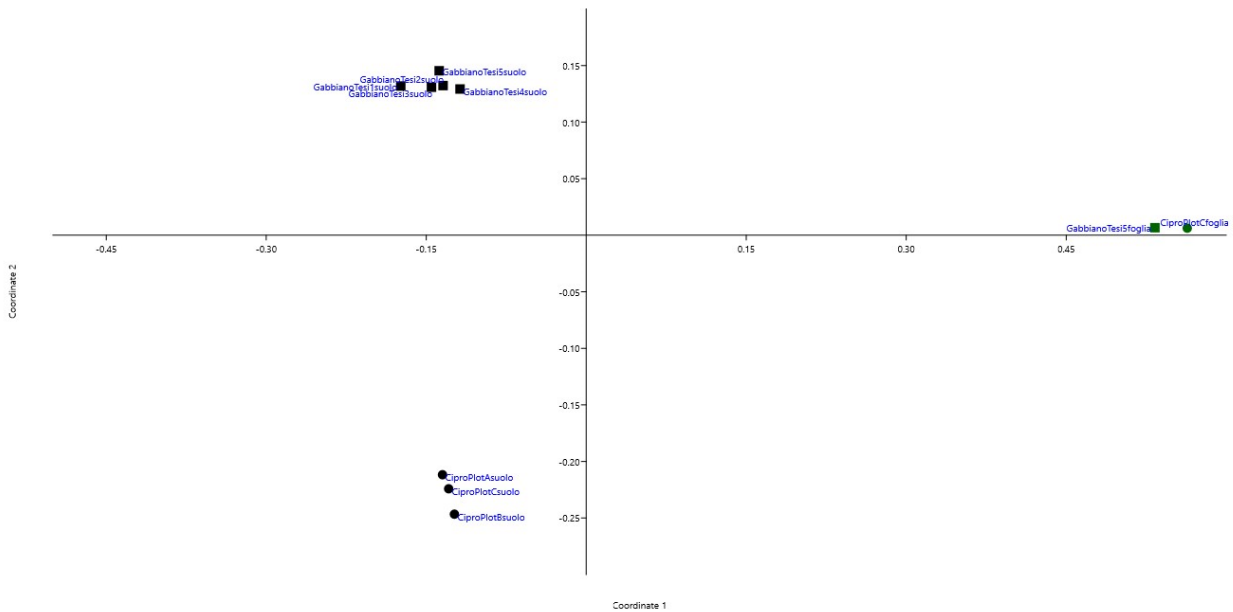


Figure 3: Non-metric Multidimensional Scaling (NMDS) analysis performed on bacterial-genera data-matrix (stress=0.08484, Bray-Curtis distance measure).

If we analyse more in details the abundance data of each identified bacterial genus (Table 3), we can make some interesting observations, such as a greater presence of bacteria belonging to the *Gaiellaceae* and *Gemmataceae* families on the "Gabbiano" site. compared to "Cipro" (in bold black), or, on the contrary, higher percentages of bacteria belonging to the genus *Skermanella* sp. on the "Cyprus" site compared to "Gabbiano" (in bold red). From Table 3, it is possible to observe that from the samples Cipro Plot C foglie and Gabbiano Tesi 5 foglie, chloroplast and mitochondria sequences (highlighted in green) were predominantly amplified; the same sequences were found in very low percentages in all the other samples.

Table 3: Relative abundances (%) of bacterial genera detected in each sample.

	Cipro PlotA suolo	Cipro PlotB suolo	Cipro PlotC suolo	Cipro PlotC foglie	Gabbiano Tesi1 suolo	Gabbiano Tesi2 suolo	Gabbiano Tesi3 suolo	Gabbiano Tesi4 suolo	Gabbiano Tesi5 suolo	Gabbiano Tesi5 foglie
Cyanobacteria; Chloroplast; Streptophyta;	0,051	0,056	0,035	67,634	0,011	0,216	0,106	0,368	0,092	66,630
Planctomycetes; Phycisphaerae; WD2101;	8,992	10,996	11,312	0,000	7,740	7,726	7,928	6,607	6,863	0,000
Proteobacteria; Alphaproteobacteria; Rickettsiales; mitochondria; Other	0,008	0,004	0,002	32,134	0,002	0,030	0,032	0,037	0,008	32,954
Acidobacteria; Acidobacteria-6; iii1-15	4,392	4,847	4,808	0,001	4,522	5,656	4,405	5,763	5,771	0,000
Actinobacteria; Thermoleophilia; Gaiellales; Gaiellaceae;	1,401	1,488	1,826	0,001	2,658	3,897	3,142	3,617	4,530	0,000
Actinobacteria; Rubrobacteria; Rubrobacteriales; Rubrobacteraceae; Rubrobacter	3,183	4,878	4,639	0,000	1,927	1,603	1,480	2,124	1,447	0,000
Actinobacteria; Thermoleophilia; Solirubrobacteriales	1,591	1,997	1,752	0,000	1,842	2,347	2,028	2,761	2,487	0,000
Chloroflexi; Thermomicrobia; JG30-KF-CM45	1,541	2,161	2,370	0,000	2,065	1,789	1,699	2,281	1,689	0,000

		Cipro PlotA suolo	Cipro PlotB suolo	Cipro PlotC suolo	Cipro PlotC foglia	Gabbiano Tesi1 suolo	Gabbiano Tesi2 suolo	Gabbiano Tesi3 suolo	Gabbiano Tesi4 suolo	Gabbiano Tesi5 suolo	Gabbiano Tesi5 foglia
Proteobacteria; Rhodospirillales; Skermanella	Alphaproteobacteria; Rhodospirillaceae;	2,788	2,914	2,299	0,000	1,746	1,014	1,097	1,475	1,004	0,000
Proteobacteria; Myxococcales	Deltaproteobacteria;	1,867	1,526	1,697	0,000	1,713	1,588	1,749	1,686	1,805	0,000
Bacteria; Alphaproteobacteria; Rhodospirillaceae;	Proteobacteria; Rhodospirillales;	1,751	1,945	1,792	0,002	1,314	1,848	1,452	1,756	1,644	0,004
Bacteroidetes; Cytophagia; Cytophagales; Cytophagaceae;		2,032	1,538	1,932	0,000	0,958	1,404	1,692	1,498	1,901	0,000
Actinobacteria; MB-A2-108; 0319-7L14		1,469	1,643	1,415	0,001	1,175	1,746	1,425	1,735	2,055	0,000
Bacteroidetes; [Saprosirae]; [Saprosirales]; Chitinophagaceae;		1,314	0,934	1,046	0,001	1,327	1,584	1,925	1,709	2,286	0,000
Planctomycetes; Gemmatales; Gemmataceae;	Planctomycetia;	0,621	0,776	0,823	0,000	1,713	2,323	2,104	1,479	2,110	0,000
Actinobacteria; Actinomycetales; Micrococcaceae;	Actinobacteria;	1,986	0,884	1,046	0,000	3,260	0,825	0,980	1,665	0,796	0,000
Planctomycetes; Gemmatales; Gemmataceae;	Planctomycetia;	0,638	0,782	0,836	0,001	1,520	1,952	1,987	1,710	1,825	0,000
Planctomycetes; Pirellulales; Pirellulaceae;	Planctomycetia;	1,654	1,766	1,525	0,001	1,025	1,410	1,264	1,146	1,245	0,000
Acidobacteria; Acidobacteria-6; mb2424;	iii1-15;	0,989	1,059	1,033	0,000	1,409	1,534	1,454	1,568	1,730	0,000
Bacteria; [Chloracidobacteria]; RB41	Acidobacteria;	1,033	0,919	0,923	0,000	1,662	1,600	1,362	1,459	1,086	0,000
Chloroflexi; Ellin6529;		0,799	0,748	0,692	0,000	1,268	1,619	1,358	1,901	1,501	0,000
Firmicutes; Bacilli; Bacillales; Bacillaceae; Bacillus		1,362	1,780	1,372	0,014	0,769	1,050	1,447	0,537	0,959	0,000
Gemmatimonadetes; Gemmatimonadetes;		1,535	2,057	1,413	0,001	0,854	0,793	0,639	0,651	0,734	0,000
Proteobacteria; Sphingomonadales; Kaistobacter	Alphaproteobacteria; Sphingomonadaceae;	1,358	1,007	1,246	0,000	1,095	0,958	0,925	1,253	0,792	0,000
Proteobacteria; Xanthomonadales; Sinobacteraceae;	Gammaproteobacteria;	0,960	0,887	1,056	0,001	0,741	1,264	1,127	1,121	1,327	0,000
Actinobacteria; Acidimicrobiales; C111;	Acidimicrobiia;	0,501	0,560	0,646	0,000	0,983	1,343	1,497	1,407	1,365	0,000
Gemmatimonadetes; Gemm-1;		1,284	1,419	1,063	0,000	0,825	0,857	0,642	0,875	0,690	0,000
Proteobacteria; Rhizobiales; Rhodoplanes	Alphaproteobacteria; Hyphomicrobiaceae;	0,700	0,862	0,831	0,000	0,867	1,055	1,047	1,037	1,202	0,000
Bacteria; Actinomycetales; Streptomycetaceae;	Actinobacteria; Actinomycetales;	0,779	0,816	0,598	0,000	0,945	1,181	0,714	1,072	0,880	0,000
Proteobacteria; Betaproteobacteria; MND1		0,951	1,165	1,478	0,000	0,651	0,754	0,563	0,617	0,717	0,000
Acidobacteria; Solibacteres; Solibacterales		0,774	0,628	0,695	0,000	0,873	0,825	0,823	0,965	0,969	0,000
Actinobacteria; Actinomycetales; Nocardioideaceae;	Actinobacteria;	0,753	0,695	0,667	0,000	0,591	0,781	0,837	1,007	0,799	0,000
Unassigned;Other		0,730	0,694	0,699	0,054	0,809	0,628	0,710	0,705	0,709	0,378
Proteobacteria; Rhizobiales; Bradyrhizobiaceae;	Alphaproteobacteria;	0,701	0,808	0,821	0,000	0,718	0,761	0,755	0,772	0,752	0,000
Chloroflexi; Anaerolineae; Caldilineales; Caldilineaceae;		0,733	0,575	0,524	0,000	1,017	0,730	0,891	0,851	0,699	0,000
Actinobacteria; Acidimicrobiales	Acidimicrobiia;	0,663	0,897	0,773	0,000	0,652	0,725	0,658	0,726	0,659	0,000
Proteobacteria; Rhizobiales; Balneimonas	Alphaproteobacteria; Bradyrhizobiaceae;	0,796	0,924	0,760	0,001	0,906	0,527	0,459	0,779	0,579	0,000
Chloroflexi; [Kouleoethrixaceae];	Chloroflexi; [Roseiflexales];	0,310	0,217	0,205	0,001	1,206	0,878	0,920	1,246	0,741	0,000
Acidobacteria; [Chloracidobacteria]; RB41; Ellin6075;		0,740	0,549	0,813	0,000	0,944	0,577	0,764	0,807	0,506	0,000
Bacteria; Actinomycetales; Geodermatophilaceae;	Actinobacteria; Actinomycetales;	1,203	1,332	1,389	0,000	0,386	0,234	0,213	0,372	0,267	0,000
Bacteria; Alphaproteobacteria; Sphingomonadales; Sphingomonadaceae;	Proteobacteria; Sphingomonadales;	1,177	0,822	0,957	0,000	0,487	0,421	0,436	0,622	0,444	0,000

	Cipro PlotA suolo	Cipro PlotB suolo	Cipro PlotC suolo	Cipro PlotC foglia	Gabbiano Tesi1 suolo	Gabbiano Tesi2 suolo	Gabbiano Tesi3 suolo	Gabbiano Tesi4 suolo	Gabbiano Tesi5 suolo	Gabbiano Tesi5 foglia
Bacteroidetes; [Saprosirae]; [Saprosirales]; Chitinophagaceae; Flavisolibacter	0,920	0,578	0,574	0,000	1,105	0,562	0,545	0,603	0,372	0,000
Proteobacteria; Alphaproteobacteria; Rhodospirillales	0,792	1,009	0,924	0,001	0,368	0,508	0,527	0,421	0,584	0,000
Proteobacteria; Betaproteobacteria;	0,630	0,662	0,532	0,000	0,631	0,629	0,532	0,694	0,665	0,000
Actinobacteria; Thermoleophilia; Solirubrobacterales; Solirubrobacteraceae;	0,698	0,948	0,753	0,000	0,454	0,478	0,468	0,600	0,446	0,000
Proteobacteria; Deltaproteobacteria; Syntrophobacterales; Syntrophobacteraceae;	0,512	0,539	0,477	0,001	0,519	0,739	0,574	0,628	0,788	0,000
Verrucomicrobia; [Spartobacteria]; [Chthoniobacterales]; [Chthoniobacteraceae]; Candidatus Xiphinematobacter	0,014	0,038	0,011	0,000	0,668	1,433	1,090	0,576	0,929	0,000
Proteobacteria; Gammaproteobacteria; Xanthomonadales; Sinobacteraceae; Steroidobacter	0,619	0,674	0,569	0,000	0,520	0,679	0,538	0,522	0,567	0,000
Bacteria; Verrucomicrobia; [Spartobacteria]; [Chthoniobacterales]; [Chthoniobacteraceae];	0,486	0,355	0,436	0,000	0,591	0,624	0,736	0,672	0,412	0,000
Bacteria; Nitrospirae; Nitrospira; Nitrospirales; 0319-6A21;	0,340	0,287	0,429	0,000	0,576	0,691	0,619	0,568	0,653	0,000
Bacteria; Verrucomicrobia; [Pedosphaerae]; [Pedosphaerales]	0,470	0,628	0,477	0,000	0,451	0,579	0,491	0,456	0,588	0,000
Actinobacteria; Actinobacteria; Actinomycetales; Propionibacteriaceae;	0,694	0,660	0,609	0,000	0,303	0,388	0,364	0,456	0,645	0,000
Proteobacteria; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas	0,171	0,060	0,095	0,000	2,825	0,095	0,516	0,128	0,102	0,000
Proteobacteria; Betaproteobacteria; Burkholderiales; Comamonadaceae;	0,539	0,265	0,393	0,000	0,539	0,450	0,598	0,601	0,519	0,000
Proteobacteria; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae;	0,603	0,382	0,514	0,000	0,362	0,361	0,966	0,371	0,322	0,000
Chloroflexi; TK10; AKYG885; Dolo_23;	0,393	0,532	0,484	0,000	0,459	0,533	0,441	0,511	0,512	0,000
TM7; TM7-1;	0,648	0,363	0,443	0,000	0,480	0,411	0,512	0,434	0,358	0,000
Chloroflexi; Chloroflexi; AKIW781	0,341	0,248	0,242	0,000	0,985	0,357	0,407	0,745	0,272	0,000
Proteobacteria; Deltaproteobacteria; [Entotheonellales]; [Entotheonellaceae];	0,542	0,812	0,643	0,000	0,205	0,380	0,331	0,193	0,434	0,000
Chloroflexi; S085;	0,309	0,232	0,228	0,000	0,481	0,606	0,418	0,493	0,714	0,000
Proteobacteria; Alphaproteobacteria; Rhizobiales; Beijerinckiaceae;	0,452	0,610	0,628	0,000	0,442	0,281	0,268	0,375	0,293	0,000
Proteobacteria; Betaproteobacteria; Burkholderiales; Oxalobacteraceae;	0,555	0,284	0,335	0,000	0,812	0,272	0,327	0,427	0,259	0,000
Actinobacteria; Rubrobacteria; Rubrobacterales; Rubrobacteraceae;	0,431	0,628	0,459	0,000	0,244	0,472	0,417	0,183	0,386	0,000
Actinobacteria; Actinobacteria; Actinomycetales; Micromonosporaceae;	0,295	0,351	0,287	0,000	0,280	0,511	0,502	0,460	0,512	0,000
Gemmatimonadetes; Gemm-5;	0,841	0,842	0,977	0,001	0,094	0,106	0,093	0,067	0,105	0,000
Actinobacteria; Acidimicrobiia; Acidimicrobiales; AKIW874;	0,301	0,296	0,330	0,000	0,272	0,596	0,475	0,388	0,467	0,000
Planctomycetes; Planctomycetia; Pirellulales; Pirellulaceae; Pirellula	0,351	0,380	0,335	0,000	0,304	0,510	0,372	0,410	0,423	0,000
Proteobacteria; Alphaproteobacteria; Rhizobiales	0,427	0,360	0,325	0,000	0,371	0,361	0,367	0,445	0,386	0,000
Verrucomicrobia; [Pedosphaerae]; [Pedosphaerales]; Ellin517;	0,601	0,596	0,487	0,000	0,270	0,248	0,306	0,268	0,226	0,000
Verrucomicrobia; Opitutae; Opitutales; Opitutaceae; Opitutus	0,387	0,342	0,276	0,000	0,470	0,343	0,446	0,398	0,324	0,000
Planctomycetes; Planctomycetia; Gemmatales; Isosphaeraceae;	0,330	0,423	0,687	0,000	0,215	0,279	0,351	0,307	0,317	0,000
Actinobacteria; Actinobacteria; Actinomycetales; Micromonosporaceae;Other	0,296	0,255	0,235	0,000	0,258	0,411	0,457	0,427	0,489	0,000
Planctomycetes; Planctomycetia; Planctomycetales; Planctomycetaceae; Planctomyces	0,432	0,464	0,404	0,001	0,237	0,335	0,330	0,326	0,293	0,000

		Cipro PlotA suolo	Cipro PlotB suolo	Cipro PlotC suolo	Cipro PlotC foglia	Gabbiano Tesi1 suolo	Gabbiano Tesi2 suolo	Gabbiano Tesi3 suolo	Gabbiano Tesi4 suolo	Gabbiano Tesi5 suolo	Gabbiano Tesi5 foglia
Actinobacteria; Actinobacteria; Actinomycetales; Nocardioideae; Kribbella		0,251	0,294	0,259	0,000	0,329	0,505	0,381	0,379	0,368	0,000
Actinobacteria; Actinobacteria; Actinomycetales; Mycobacteriaceae; Mycobacterium		0,355	0,362	0,319	0,000	0,268	0,346	0,398	0,315	0,334	0,000
Actinobacteria; Actinobacteria; Actinomycetales; Nocardioideae; Nocardioidees		0,242	0,363	0,356	0,000	0,203	0,396	0,349	0,477	0,308	0,000
Chloroflexi; Chloroflexi; [Roseflexales]		0,194	0,104	0,138	0,000	0,585	0,513	0,481	0,370	0,291	0,000
Bacteria; Acidobacteria; Sva0725; Sva0725		0,266	0,317	0,220	0,000	0,383	0,371	0,394	0,393	0,310	0,000
Proteobacteria; Deltaproteobacteria; Myxococcales; Haliangiaceae;		0,451	0,403	0,479	0,000	0,221	0,274	0,304	0,229	0,283	0,000
Actinobacteria; Actinobacteria; Actinomycetales; Microbacteriaceae; Cryocolla		0,436	0,487	0,419	0,000	0,165	0,236	0,245	0,268	0,272	0,000
Actinobacteria; Actinobacteria; Actinomycetales; Streptomycetaceae; Streptomyces		0,387	0,474	0,412	0,000	0,443	0,228	0,168	0,160	0,222	0,000
Actinobacteria; Acidimicrobia; Acidimicrobiales; EB1017;		0,216	0,234	0,201	0,000	0,227	0,379	0,366	0,338	0,488	0,000
Bacteria; Chloroflexi; Gitt-GS-136;		0,230	0,199	0,143	0,000	0,293	0,410	0,303	0,471	0,393	0,000
Bacteria; Proteobacteria; Alphaproteobacteria; Sphingomonadales; Sphingomonadaceae; Sphingomonas		0,184	0,126	0,137	0,000	0,308	0,337	0,416	0,351	0,458	0,000
Bacteria; Proteobacteria; Alphaproteobacteria; Rhodospirillales; Acetobacteraceae;		0,349	0,369	0,404	0,000	0,292	0,184	0,210	0,270	0,205	0,000
Bacteria; Acidobacteria; iii1-8; DS-18		0,387	0,362	0,419	0,000	0,160	0,227	0,180	0,342	0,194	0,000
Actinobacteria; Actinobacteria; Actinomycetales; Pseudonocardiaceae; Pseudonocardia		0,382	0,411	0,349	0,000	0,201	0,228	0,226	0,220	0,232	0,000
Bacteroidetes; Cytophagia; Cytophagales; Cytophagaceae; Adhaeribacter		0,625	0,361	0,399	0,000	0,274	0,125	0,111	0,195	0,137	0,000
Actinobacteria; Actinobacteria; Micrococcales		0,500	0,281	0,447	0,000	0,124	0,211	0,168	0,191	0,238	0,000
Actinobacteria; Actinobacteria; Actinomycetales; Microbacteriaceae; Agromyces		0,107	0,078	0,067	0,000	0,270	0,317	0,444	0,369	0,339	0,000
Proteobacteria; Betaproteobacteria; Ellin6067		0,227	0,261	0,358	0,000	0,182	0,237	0,238	0,280	0,202	0,000
Acidobacteria; Acidobacteria-6; iii1-15; RB40;		0,173	0,120	0,138	0,000	0,156	0,416	0,283	0,186	0,496	0,000
Nitrospirae; Nitrospira; Nitrospirales; Nitrospiraceae; Nitrospira		0,215	0,180	0,222	0,000	0,287	0,280	0,211	0,259	0,313	0,000
Chloroflexi; Anaerolineae; SBR1031; A4b;		0,260	0,159	0,184	0,000	0,240	0,230	0,284	0,279	0,256	0,000
Actinobacteria; Actinobacteria; Actinomycetales; Micromonosporaceae; Actinoplanes		0,101	0,059	0,089	0,000	0,559	0,220	0,352	0,133	0,313	0,000
Bacteria; Acidobacteria; [Chloracidobacteria]; PK29		0,056	0,084	0,029	0,000	0,343	0,422	0,329	0,204	0,344	0,000
Chloroflexi; Anaerolineae; envOPS12		0,188	0,154	0,131	0,000	0,318	0,223	0,265	0,270	0,255	0,000
Proteobacteria; Alphaproteobacteria; Rhizobiales; Hyphomicrobiaceae; Devosia		0,360	0,156	0,216	0,000	0,146	0,206	0,244	0,175	0,231	0,000
Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae; Erwinia		0,041	0,015	0,014	0,001	0,588	0,043	0,935	0,022	0,055	0,000
Bacteroidetes; Flavobacteriia; Flavobacteriales; Flavobacteriaceae; Flavobacterium		0,244	0,056	0,132	0,000	0,112	0,299	0,339	0,149	0,366	0,000
Proteobacteria; Betaproteobacteria; Burkholderiales; Comamonadaceae; Ramlibacter		0,249	0,099	0,185	0,000	0,210	0,183	0,279	0,269	0,216	0,000
Actinobacteria; Actinobacteria; Actinomycetales; Intrasporangiaceae; Phycococcus		0,101	0,141	0,102	0,000	0,209	0,224	0,275	0,307	0,300	0,000
Proteobacteria; Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae;		0,010	0,000	0,005	0,001	1,298	0,009	0,271	0,002	0,055	0,000

	Cipro PlotA suolo	Cipro PlotB suolo	Cipro PlotC suolo	Cipro PlotC foglia	Gabbiano Tesi1 suolo	Gabbiano Tesi2 suolo	Gabbiano Tesi3 suolo	Gabbiano Tesi4 suolo	Gabbiano Tesi5 suolo	Gabbiano Tesi5 foglia
Proteobacteria; Alphaproteobacteria; Rhizobiales; Hyphomicrobiaceae;	0,176	0,106	0,132	0,000	0,212	0,232	0,225	0,233	0,317	0,000
Bacteroidetes; Sphingobacteriia; Sphingobacteriales; Sphingobacteriaceae;	0,183	0,073	0,090	0,000	0,123	0,218	0,328	0,192	0,388	0,000
Armatimonadetes; Chthonomonadetes; SJA-22	0,108	0,075	0,065	0,000	0,602	0,157	0,180	0,302	0,100	0,000
Proteobacteria; Betaproteobacteria; Burkholderiales; Alcaligenaceae;	0,057	0,026	0,060	0,000	0,140	0,319	0,318	0,178	0,484	0,000
Gemmatimonadetes; Gemm-3;	0,417	0,274	0,526	0,000	0,104	0,055	0,073	0,084	0,050	0,000
Proteobacteria; Betaproteobacteria; Burkholderiales; Comamonadaceae; Other	0,177	0,106	0,156	0,000	0,243	0,193	0,209	0,239	0,256	0,000
Acidobacteria; Acidobacteria-6; CCU21	0,207	0,218	0,238	0,000	0,145	0,187	0,168	0,188	0,221	0,000
Proteobacteria; Alphaproteobacteria; Rhizobiales; Rhizobiaceae; Agrobacterium	0,220	0,068	0,087	0,000	0,228	0,195	0,302	0,152	0,277	0,000
Bacteroidetes; Sphingobacteriia; Sphingobacteriales	0,303	0,167	0,331	0,000	0,101	0,119	0,179	0,112	0,205	0,000
Chloroflexi; TK17; mle1-48	0,165	0,190	0,161	0,000	0,205	0,163	0,182	0,194	0,144	0,000
Chloroflexi; Anaerolineae; Caldilineales; Caldilineaceae; Caldilinea	0,098	0,078	0,086	0,000	0,192	0,238	0,294	0,159	0,255	0,000
Proteobacteria; Alphaproteobacteria; Rhizobiales; Rhodobiaceae; Afifella	0,190	0,245	0,190	0,000	0,142	0,175	0,121	0,179	0,130	0,000
Bacteroidetes; Sphingobacteriia; Sphingobacteriales; Sphingobacteriaceae; Pedobacter	0,282	0,034	0,065	0,000	0,072	0,084	0,557	0,065	0,188	0,000
Actinobacteria; Thermoleophilia; Solirubrobacterales; Conexibacteraceae;	0,180	0,208	0,208	0,000	0,171	0,133	0,152	0,163	0,109	0,000
Bacteria; Gemmatimonadetes; Gemmatimonadetes; C114	0,304	0,436	0,384	0,000	0,040	0,044	0,028	0,043	0,040	0,000
Bacteria; Verrucomicrobia; Verrucomicrobiae; Verrucomicrobiales; Verrucomicrobiaceae;	0,158	0,114	0,138	0,000	0,107	0,167	0,231	0,156	0,240	0,000
Chloroflexi; TK10; B07_ WMSP1	0,138	0,145	0,156	0,000	0,171	0,199	0,147	0,137	0,177	0,000
Planctomycetes; Phycisphaerae; Phycisphaerales	0,182	0,176	0,193	0,001	0,104	0,159	0,141	0,093	0,194	0,000
Gemmatimonadetes; Gemmatimonadetes; Gemmatimonadales; Ellin5301;	0,193	0,211	0,212	0,000	0,174	0,072	0,104	0,172	0,083	0,000
Acidobacteria; Solibacteres; Solibacterales; Solibacteraceae; Candidatus Solibacter	0,080	0,090	0,067	0,000	0,109	0,214	0,161	0,167	0,290	0,000
Proteobacteria; Alphaproteobacteria; Sphingomonadales; Erythrobacteraceae;	0,209	0,131	0,244	0,000	0,083	0,106	0,123	0,147	0,115	0,000
Verrucomicrobia; [Pedosphaerae]; [Pedosphaerales]; OPB35;	0,187	0,322	0,194	0,000	0,084	0,114	0,095	0,069	0,091	0,000
Proteobacteria; Betaproteobacteria; SC-I-84	0,099	0,068	0,152	0,000	0,143	0,153	0,141	0,206	0,181	0,000
Proteobacteria; Alphaproteobacteria; Rhizobiales; Phyllobacteriaceae;	0,127	0,186	0,175	0,000	0,079	0,140	0,140	0,150	0,126	0,000
Proteobacteria; Gammaproteobacteria; Thiotrichales; Piscirickettsiaceae;	0,174	0,224	0,212	0,000	0,059	0,128	0,069	0,093	0,146	0,000
Proteobacteria; Alphaproteobacteria; Ellin329	0,157	0,151	0,156	0,000	0,128	0,147	0,109	0,128	0,123	0,000
Actinobacteria; Actinobacteria; Actinomycetales; Micromonosporaceae; Catellatospora	0,078	0,116	0,105	0,000	0,112	0,204	0,209	0,152	0,092	0,000
Verrucomicrobia; [Spartobacteria]; [Chthoniobacteriales]; [Chthoniobacteraceae]; DA101	0,059	0,002	0,015	0,000	0,090	0,239	0,258	0,082	0,317	0,000
Actinobacteria; Thermoleophilia; Solirubrobacterales; Other; Other	0,111	0,135	0,136	0,000	0,090	0,143	0,099	0,167	0,160	0,000
Actinobacteria; Actinobacteria; Actinomycetales; Pseudonocardaceae;	0,140	0,133	0,133	0,000	0,128	0,151	0,133	0,081	0,134	0,000
Chloroflexi	0,108	0,150	0,122	0,000	0,164	0,126	0,100	0,169	0,092	0,000
Actinobacteria; Actinobacteria; Actinomycetales; Geodermatophilaceae; Modestobacter	0,304	0,331	0,259	0,000	0,028	0,028	0,023	0,025	0,018	0,000
<1%	13,108	10,650	11,173	0,151	12,062	11,439	12,829	11,527	11,757	0,034

ITS gene analysis: the fungal community

The fungal community analysis was performed following the pipeline suggested on the QIIME software website, similar to the bacterial one, with the only difference in the reference database for the alignment of the sequences and the taxonomic analysis.

Figure 4 shows the relative abundances of the different eukaryotic Phyla identified in each sample. In this case, the samples are 8 and not 10 because those related to the leaves are missing. In fact, we find the 3 soil samples from the "Cipro" site, and the 5 from the "Gabbiano" site. In all the samples the dominant group is that of the Ascomycota fungi. In all the samples there are also sequences belonging to the protists groups of Ciliophora and Cercozoa, commonly described in soil.

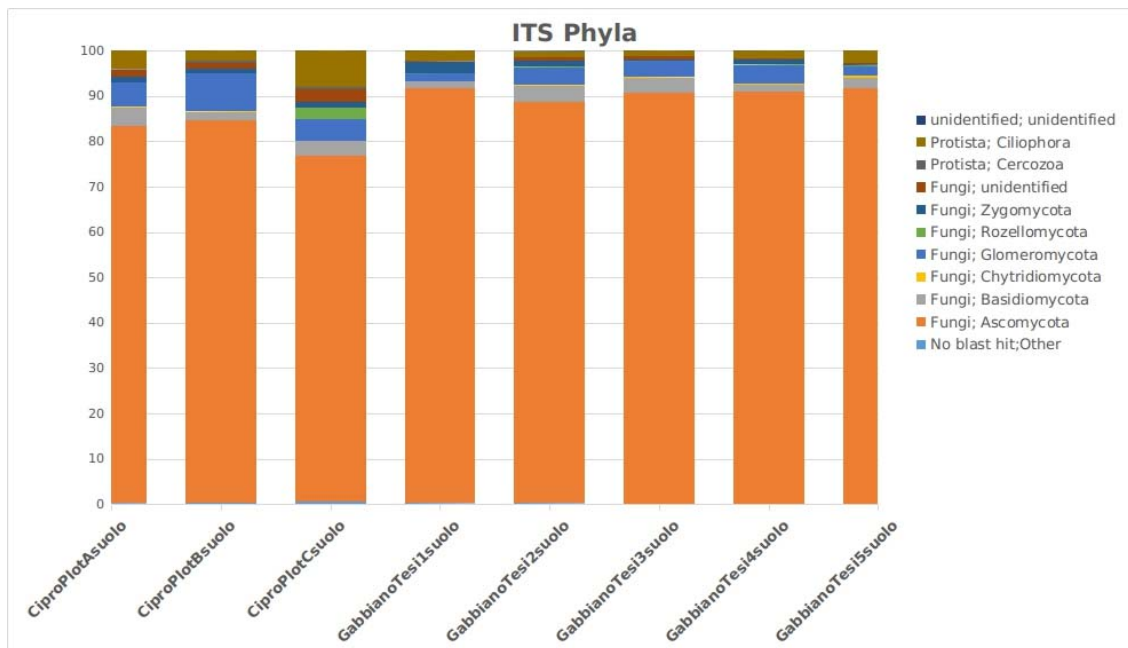


Figure 4: eukaryotic community composition at phylum level.

The data matrix related to the eukaryotic community was also analyzed at the genus level and the main diversity indices were calculated (Table 4) similar to what was done for the bacteria.

Table 4: diversity indices on eukaryotic data matrix at genus level

	Gabbiano Tesi1 suolo	Gabbiano Tesi2 suolo	Gabbiano Tesi3 suolo	Gabbiano Tesi4 suolo	Gabbiano Tesi5 suolo	Cipro PlotA suolo	Cipro PlotB suolo	Cipro PlotC suolo
Simpson 1-D	0,8639	0,937	0,8862	0,9194	0,9387	0,9475	0,9556	0,9538
Shannon H	2,635	3,195	2,85	3,08	3,194	3,356	3,403	3,401
Evenness $e^{H/S}$	0,2405	0,3642	0,2543	0,3296	0,3588	0,4218	0,4846	0,492
Chao-1	58	67	68	66	68	68	62	61

Il campione “Gabbiano Tesi 1”, turns out to be the one with the lower diversity (lower Chao1 index), and with the lowest value of Shannon Index that indicates a community that, compared to the other samples, is characterized by a lower number of taxa (genera) with high number of individuals inside.

In Figure 5, the Cluster Analysis reveals that there are two statistically well supported distinct clusters (bootstrap value = 100), corresponding to the two sampling sites Gabbiano and Cipro. Within the cluster related to the "Gabbiano" site, the sample "Gabbiano Tesi1 suolo" is the one that most differs from the other 4. This observation is confirmed if we analyze Figure 6, which represents the NMDS analysis performed on the same dataset. In this case, in fact, the sample "Gabbiano Tesi1 suolo" is spatially distant from the others 4. As for the "Cipro" samples, it is possible to state that also in this case, the sample taken in plot B is more different from the others two plots (A and C). These differences can be observed in Table 5 too, which shows the relative abundances expressed asv% of the total of the different bacterial genera identified for each sample.

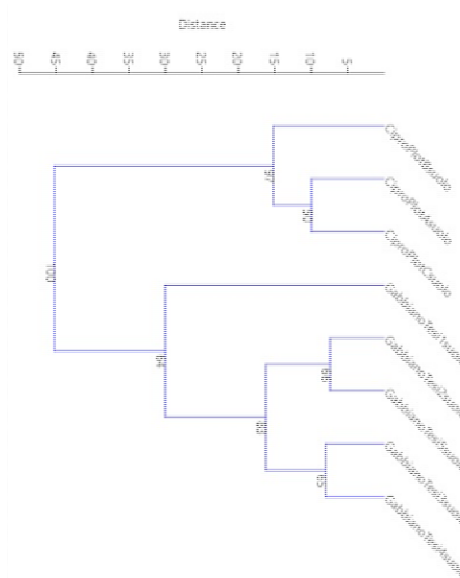


Figura 5: Cluster Analysis performed with Ward’s method, Euclidean distance and 100 bootstrap, on ITS dataset.

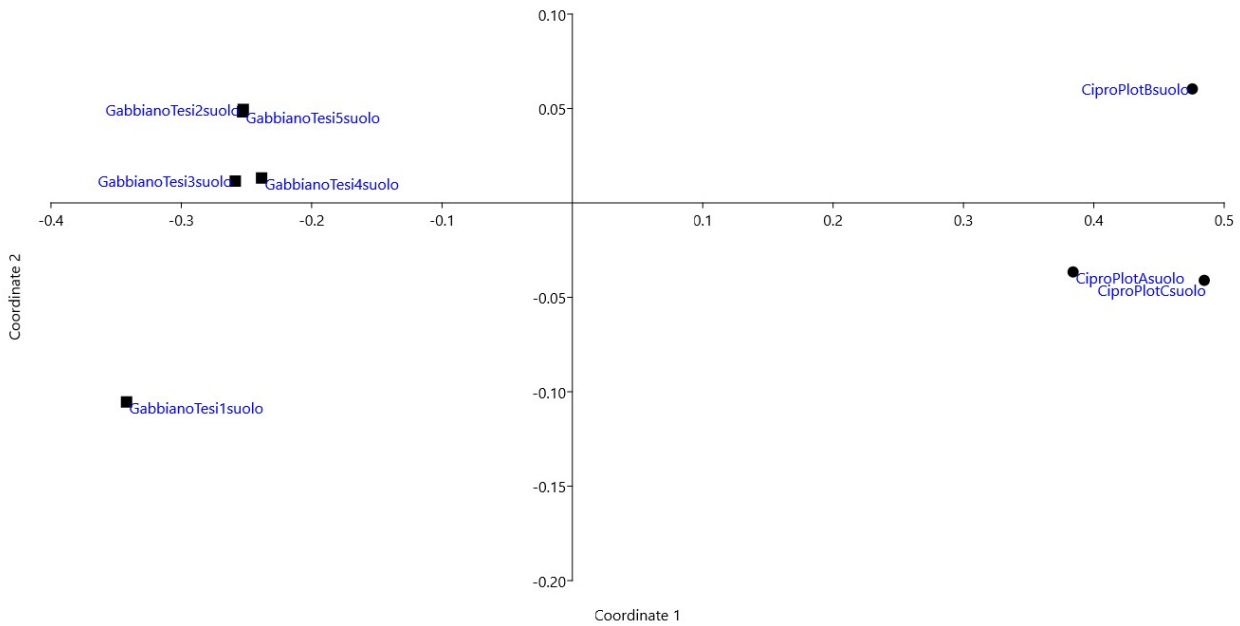


Figure 6: Non-metric Multidimensional Scaling (NMSD) analysis performed on the genus-level data matrix related to eukaryotic sequences (stress=0.07379, Bray-Curtis distance measure).

By analyzing in details the distribution of eukaryotic genera within the samples, it can be observed that the genus *Alternaria* sp. (Ascomycota) despite being present in all the samples, it is much more widespread in the "Gabbiano" site compared to "Cipro" (black bold). The same can be said for the genera *Tetracladium* sp., *Phaeosphaeria* sp. (Ascomycota), and for the groups of *Onygenales*, *Helotiales*.

In contrast, *Ulocladium* sp., *Lophiostoma* sp., *Preussia* sp., *Corynespora* sp., and the group of *Sordariomycetes* (all Ascomycota) are more represented in "Cipro" than in "Gabbiano" (bold red).

Table 5: Relative abundances (%) of eukaryotic genera in each sample.

	Cipro PlotA suolo	Cipro PlotB suolo	Cipro PlotC suolo	Gabbiano Tesi1 suolo	Gabbiano Tesi2 suolo	Gabbiano Tesi3 suolo	Gabbiano Tesi4 suolo	Gabbiano Tesi5 suolo
Ascomycota; Dothideomycetes; Pleosporales; Pleosporaceae; Alternaria	12,47895	6,226111	4,326007	22,81738467	14,90236	26,76535	21,64087	15,1962
Ascomycota; Dothideomycetes; Pleosporales; unidentified	2,245735	5,099578	3,040293	2,144491041	6,124323	14,13682	8,609441	8,356289
Ascomycota; unidentified	2,494062	2,796218	2,59707	12,30461304	7,767788	6,180762	4,34523	8,323881
Ascomycota; Dothideomycetes; Capnodiales; Davidiellaceae; Cladosporium	11,42302	3,324281	9,336996	1,048417842	1,00058	6,211209	3,138459	5,740578
Ascomycota; Dothideomycetes; Pleosporales; Incertae sedis; Phoma	1,207083	3,183464	1,520147	24,59969501	0,444702	0,735415	2,119313	0,226007
Ascomycota; Dothideomycetes; Pleosporales; Phaeosphaeriaceae; Phaeosphaeria	0,047506	0	0	0,381242852	10,27166	7,675012	7,543388	5,536745
Ascomycota; Leotiomycetes; Helotiales; unidentified	0,099331	0,196138	0,637363	0,905451773	6,914637	3,566995	6,750245	6,230971
Protista; Ciliophora; unidentified	4,042323	2,212834	8,128205	2,268394968	1,411446	1,2132	1,778176	2,705261
Ascomycota; Eurotiomycetes; Onygenales; unidentified	5,00108	10,51599	3,820513	0,009531071	0,761311	0,295103	0,579933	0,132193

	Cipro PlotA suolo	Cipro PlotB suolo	Cipro PlotC suolo	Gabbiano Tesi1 suolo	Gabbiano Tesi2 suolo	Gabbiano Tesi3 suolo	Gabbiano Tesi4 suolo	Gabbiano Tesi5 suolo
Ascomycota; Leotiomycetes; Helotiales; Incertae sedis; Tetracladium	1,090477	1,020921	0,879121	4,060236371	3,400522	2,766002	2,417807	3,453217
Ascomycota; Dothideomycetes; Pleosporales; Pleosporaceae; Ulocladium	5,096092	5,300744	8,351648	0	0	0,025763	0	0,017057
Ascomycota; Eurotiomycetes; Eurotiales; Trichocomaceae; Aspergillus	1,636796	3,294106	5,498168	0,838734274	2,088167	0,6511	2,609697	1,092509
Ascomycota; Dothideomycetes; Pleosporales; Pleosporaceae; Curvularia	5,104729	0,080467	0,208791	0,171559283	1,931071	2,609083	2,852757	3,518034
Ascomycota; Sordariomycetes; unidentified	2,012524	4,993965	3,637363	0,552802135	0,942575	0,908729	1,100166	1,69889
Ascomycota; Sordariomycetes; Xylariales; Hyponectriaceae; unidentified	5,374649	6,024945	3,776557	0,114372856	0,07734	0,124131	0,068227	0,096373
Glomeromycota; Glomeromycetes; Glomerales; Glomeraceae; unidentified	1,146621	4,707302	2,245421	0,686237133	2,402359	1,365436	2,494563	0,599558
Ascomycota; Dothideomycetes; unidentified	0,23537	0,186079	0,274725	1,372474266	4,843387	0,73073	4,545648	1,683539
Ascomycota; Dothideomycetes; Pleosporales; Incertae sedis; Letendrea	0,082056	0	0	0,505146778	5,744876	2,203902	0,452006	4,121856
Ascomycota; Dothideomycetes; Dothideales; Dothioraceae; Aureobasidium	2,468149	1,161738	0,56044	1,248570339	0,749227	1,2132	3,12993	2,260923
Basidiomycota; Tremellomycetes; Tremellales; Incertae sedis; Cryptococcus	2,761823	0,558238	1,003663	1,229508197	2,615043	1,812774	0,933862	1,366276
Ascomycota; Dothideomycetes; Pleosporales; Sporormiaceae; Preussia	4,001296	1,93623	5,047619	0,181090355	0,210267	0,182683	0,093813	0,055436
Ascomycota; Eurotiomycetes; Eurotiales; Trichocomaceae; Penicillium	1,9607	2,776101	2,516484	1,019824628	0,679138	0,22484	0,388043	0,294236
Ascomycota; Leotiomycetes; Helotiales; Helotiaceae; Articulospora	0,002159	0,06035	0	4,374761723	2,03983	0,487154	0,208946	1,893342
Ascomycota; Pezizomycetes; Pezizales; Pezizaceae; unidentified	0	4,440756	3,688645	0	0	0,01171	0	0
unidentified	1,561218	1,081271	2,882784	0,181090355	0,799981	0,599574	0,255853	0,363317
Ascomycota; Eurotiomycetes; Eurotiales; unidentified; unidentified	1,129346	4,908469	0,893773	0	0,132927	0,025763	0,456271	0,037526
Ascomycota; Dothideomycetes; Pleosporales; Pleosporaceae; Epicoccum	0,183546	0	0	0,152497141	0,519625	1,520013	0,481856	4,015249
Ascomycota; Dothideomycetes; Pleosporales; Pleosporaceae; Pleospora	1,695098	0,754375	0,644689	0,381242852	2,397525	0,06792	0,852842	0,010234
Ascomycota; Dothideomycetes; Pleosporales; Corynesporaceae; Corynespora	1,410063	3,902635	0,937729	0	0	0	0	0
Ascomycota; Dothideomycetes; Pleosporales; Phaeosphaeriaceae; Stagonospora	0	0	0	0	2,465197	0,482469	0,933862	2,005066
Glomeromycota; Glomeromycetes; Glomerales; Glomeraceae; Glomus	0,57439	2,268155	1,018315	0,095310713	0,297274	0,644073	0,396572	0,518537
Ascomycota; Dothideomycetes; Botryosphaeriales; Incertae sedis; Camarosporium	0,161952	0,799638	1,432234	0,057186428	0,935325	0,370049	0,140719	0,464807
Ascomycota; Sordariomycetes; Hypocreales; Nectriaceae; Fusarium	0,44051	0,442567	0,091575	1,000762486	0,596964	0,548048	0,750501	0,45969
Ascomycota; Leotiomycetes; Helotiales; Incertae sedis; Helicodendron	2,02548	0,150875	0,025641	0,581395349	0,234435	0,374734	0,481856	0,324086
Ascomycota; Leotiomycetes; Helotiales; Incertae sedis; Spirosphaera	0,172749	0,150875	0,065934	2,192146397	0,867653	0,309155	0,144983	0,272914
Ascomycota; Dothideomycetes; Pleosporales; Lophiostomataceae; Lophiostoma	1,051609	0,990746	0,703297	0,047655356	0,181265	0,447338	0,217475	0,421311
Ascomycota; Sordariomycetes; Hypocreales; Nectriaceae; Gibberella	0,498812	0,176021	1,161172	0,114372856	0,473705	0,395812	0,818728	0,390608
Ascomycota; Pezizomycetes; Pezizales; Pezizaceae; Peziza	0,246167	0,311808	0,43956	0,686237133	0,459203	0,747125	0,524498	0,579943
Ascomycota; Pezizomycetes; Pezizales; Pyronemataceae; Geopyxis	0	0,035204	0	0	0,014501	0,025763	3,752505	0,002559
Zygomycota; Incertae sedis; Mucorales; Lichtheimiaceae; Dichotomocladium	0,524725	0,452625	0,761905	0,752954632	0,894238	0,236551	0,025585	0,092109
Basidiomycota; Agaricomycetes; Agaricales; Amanitaceae; Amanita	0,477219	0,578354	1,120879	0,133434998	0,391531	0,348971	0,093813	0,503185
Ascomycota; Dothideomycetes; Pleosporales; Sporormiaceae; Sporormiella	0,414597	2,42406	0,520147	0	0,002417	0	0	0,004264

	Cipro PlotA suolo	Cipro PlotB suolo	Cipro PlotC suolo	Gabbiano Tesi1 suolo	Gabbiano Tesi2 suolo	Gabbiano Tesi3 suolo	Gabbiano Tesi4 suolo	Gabbiano Tesi5 suolo
Rozellomycota; unidentified	0,002159	0	2,520147	0,114372856	0,232019	0,049184	0,196154	0,227713
Ascomycota; Leotiomyces; Helotiales; Incertae sedis; Pilidium	0,002159	0,015088	0	0	0	0,049184	0,012793	3,2093
Ascomycota; Dothideomycetes; Botryosphaerales; unidentified	0,187864	0,276604	0,007326	0,943576058	0,889404	0,278708	0,336873	0,353936
Glomeromycota; Glomeromycetes; Archaeosporales; unidentified	2,761823	0	0,040293	0,019062143	0,08459	0,103052	0,012793	0,082727
Zygomycota; Incertae sedis; Mucorales; Mucoraceae; Mucor	0,302311	0,176021	0,186813	1,47731605	0,183681	0,046842	0,682274	0,046907
Ascomycota; Sordariomycetes; Magnaporthales; Mycoleptodiscus	0,110127	0,352042	0,065934	0,066717499	0,193349	0,892334	0,443478	0,717252
Ascomycota; Dothideomycetes; Pleosporales; Pleosporaceae; unidentified	2,057871	0,020117	0,032967	0,390773923	0,065255	0,131157	0,089548	0,01194
Ascomycota; Sordariomycetes; Sordariales; Chaetomiaceae; Chaetomium	0,552796	0,392275	1,227106	0,085779642	0,111176	0,039815	0,123662	0,11343
Ascomycota; Sordariomycetes; Hypocreales; unidentified	0,041028	0,095554	0,190476	1,810903546	0,16918	0,05621	0,029849	0,1791
No blast hit	0,241848	0,291692	0,578755	0,209683568	0,224768	0,166288	0,140719	0,098079
Glomeromycota; Glomeromycetes; Glomerales; Claroideoglomeraceae; Claroideoglossum	0,231052	0,326896	0,238095	0,07624857	0,215101	0,327892	0,089548	0,329203
Ascomycota; Sordariomycetes; Hypocreales; Incertae sedis; Stachybotrys	0,399482	0,538121	0,461538	0,07624857	0,101508	0,0445	0,051171	0,046907
Ascomycota; Dothideomycetes; Dothideales; unidentified	0,004319	0	0	0	0,014501	0,313839	1,232357	0,153514
Ascomycota; Pezizomycetes; unidentified	0,831354	0,462684	0,333333	0	0	0	0	0
Ascomycota; Dothideomycetes; Pleosporales; Incertae sedis; Pyrenochaeta	0,140358	0,331925	0,769231	0,019062143	0,019335	0,065578	0,017057	0,230271
Glomeromycota; Glomeromycetes; Diversisporales; Diversisporaceae; Diversispora	0,412438	0,155904	0,175824	0,238276782	0,16193	0,306813	0,025585	0,057994
Ascomycota; Dothideomycetes; Pleosporales; Phaeosphaeriaceae; unidentified	0,023753	0	0	0	0,012084	0,243577	0,068227	1,072041
Ascomycota; Dothideomycetes; Botryosphaerales; Botryosphaeriaceae; Diplodia	0,220255	0,070408	0,457875	0	0,04592	0,053868	0,319816	0,218331
Glomeromycota; Glomeromycetes; Glomerales; unidentified	0,138199	0,236371	0,095238	0,238276782	0,113592	0,096025	0,379515	0,075051
Glomeromycota; Glomeromycetes; Glomerales; Glomeraceae; Funneliformis	0,006478	0,246429	0,087912	0,038124285	0,193349	0,259971	0,328344	0,122811
Ascomycota; Dothideomycetes; Pleosporales; Cucurbitariaceae; Pyrenochaetopsis	0	0	0	0	0,13051	0,21313	0,230267	0,670345
Ascomycota; Sordariomycetes; Hypocreales; Incertae sedis; Ilyonectria	0,114446	0,281633	0,347985	0,190621426	0,026585	0,035131	0,157776	0,050319
Ascomycota; Leotiomyces; Incertae sedis; Incertae sedis; Leohumicola	0	0	0	0,038124285	1,065835	0	0,017057	0,034114
Ascomycota; Saccharomycetes; Saccharomycetales; Lipomycetaceae; Lipomyces	0,267761	0,236371	0,373626	0,085779642	0,043503	0,028105	0,012793	0,078463
Ascomycota; Saccharomycetes; unidentified; unidentified; unidentified	0,10149	0,291692	0,205128	0	0	0,477785	0	0
Zygomycota; Incertae sedis; Kickxellales; Kickxellaceae; Linderina	0,291514	0,352042	0,216117	0,009531071	0,043503	0,060894	0,034114	0,046054
Ascomycota; Sordariomycetes; Sordariales; Lasiosphaeriaceae; Schizothecium	0,015116	0,010058	0,150183	0	0,319026	0,234208	0,089548	0,235388
Ascomycota; Dothideomycetes; Capnodiales; Davidiellaceae; Davidiella	0,254805	0,06035	0,117216	0,009531071	0,04592	0,163946	0,196154	0,205538
Ascomycota; Dothideomycetes; Pleosporales; Leptosphaeriaceae; unidentified	0,012956	0	0	1,000762486	0,026585	0,002342	0,004264	0,001706
Protista; Cercozoa; unidentified	0,082056	0,573325	0,307692	0,019062143	0,026585	0	0	0
<1%	5,612179	4,682157	7,018315	3,631338162	6,254834	6,019158	6,549827	6,266791

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