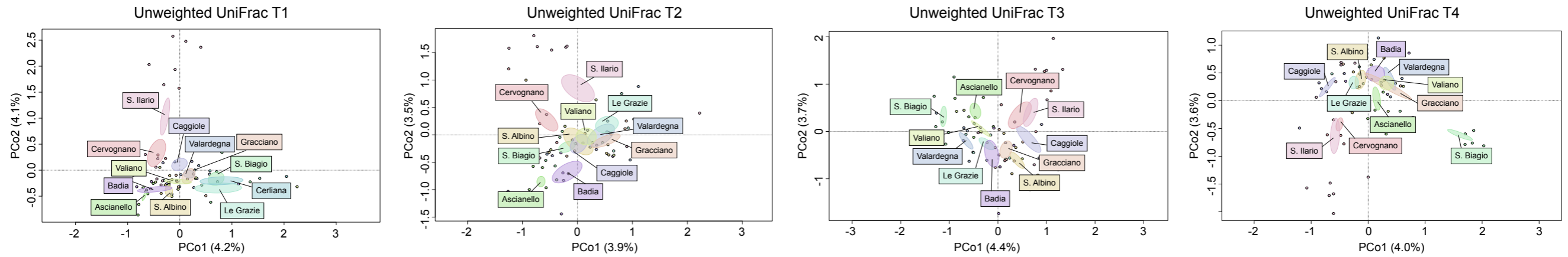


# **Zonation of the *Vitis vinifera* microbiome in Vino Nobile di Montepulciano PDO production area**

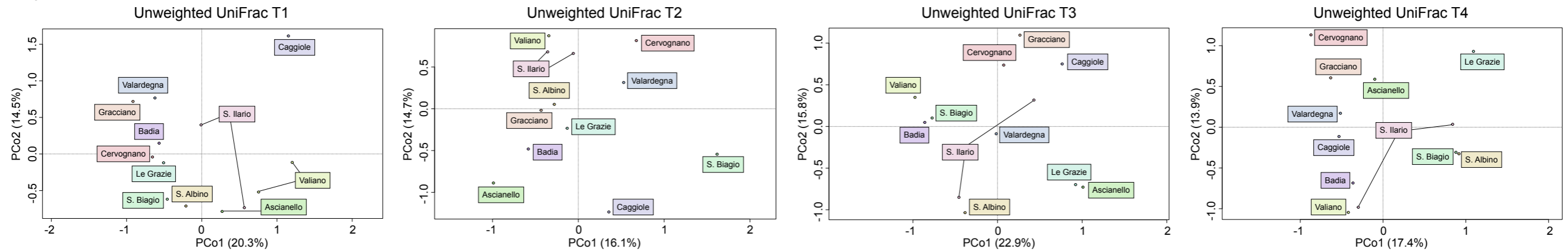
Giorgia Palladino, Enrico Nanetti, Daniel Scicchitano, Nicolò Cinti, Lucia Foresto, Alice Cozzi, Antonio Gonzalez Vara Rodriguez, Nicolò Interino, Jessica Fiori, Silvia Turrone, Marco Candela, Simone Rampelli

*Supplementary Information*

**A)**

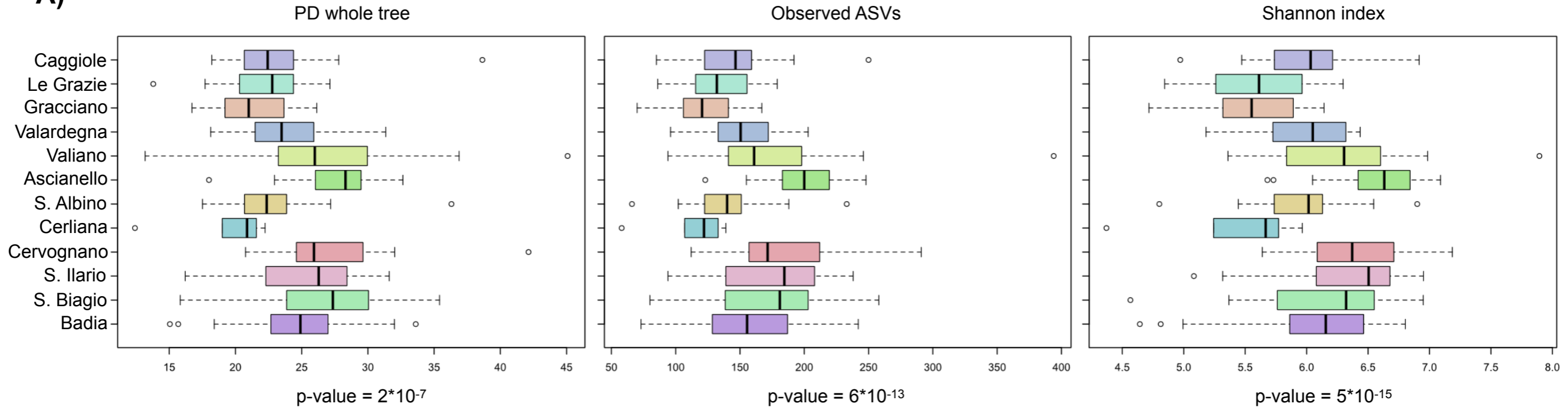


**B)**

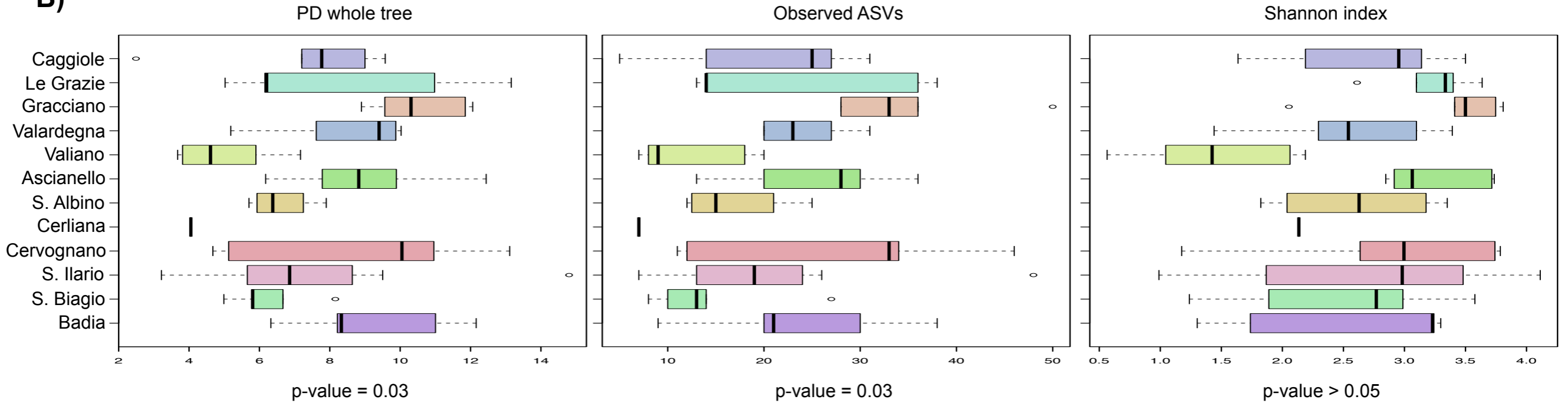


**Supplementary Figure 1 - Comparison of bacterial and fungal composition of rhizospheric soil samples of Montepulciano AGUs (Additional Geographical Units) across time points (T1 to T4).** (A) Principal Coordinate Analysis (PCoA) based on unweighted UniFrac distances showing the variations of *Vitis vinifera* rhizospheric soil bacterial composition in the different AGUs and time points. Procrustes test between time points, p-value = 0.005. (B) Principal Coordinate Analysis (PCoA) based on unweighted UniFrac distances showing the variations of *Vitis vinifera* rhizospheric soil fungal composition in the different AGUs and time points. Procrustes test between time points, p-value = 0.01. The first and second principal components are plotted and the percentage of variance in the dataset explained by each axis is highlighted.

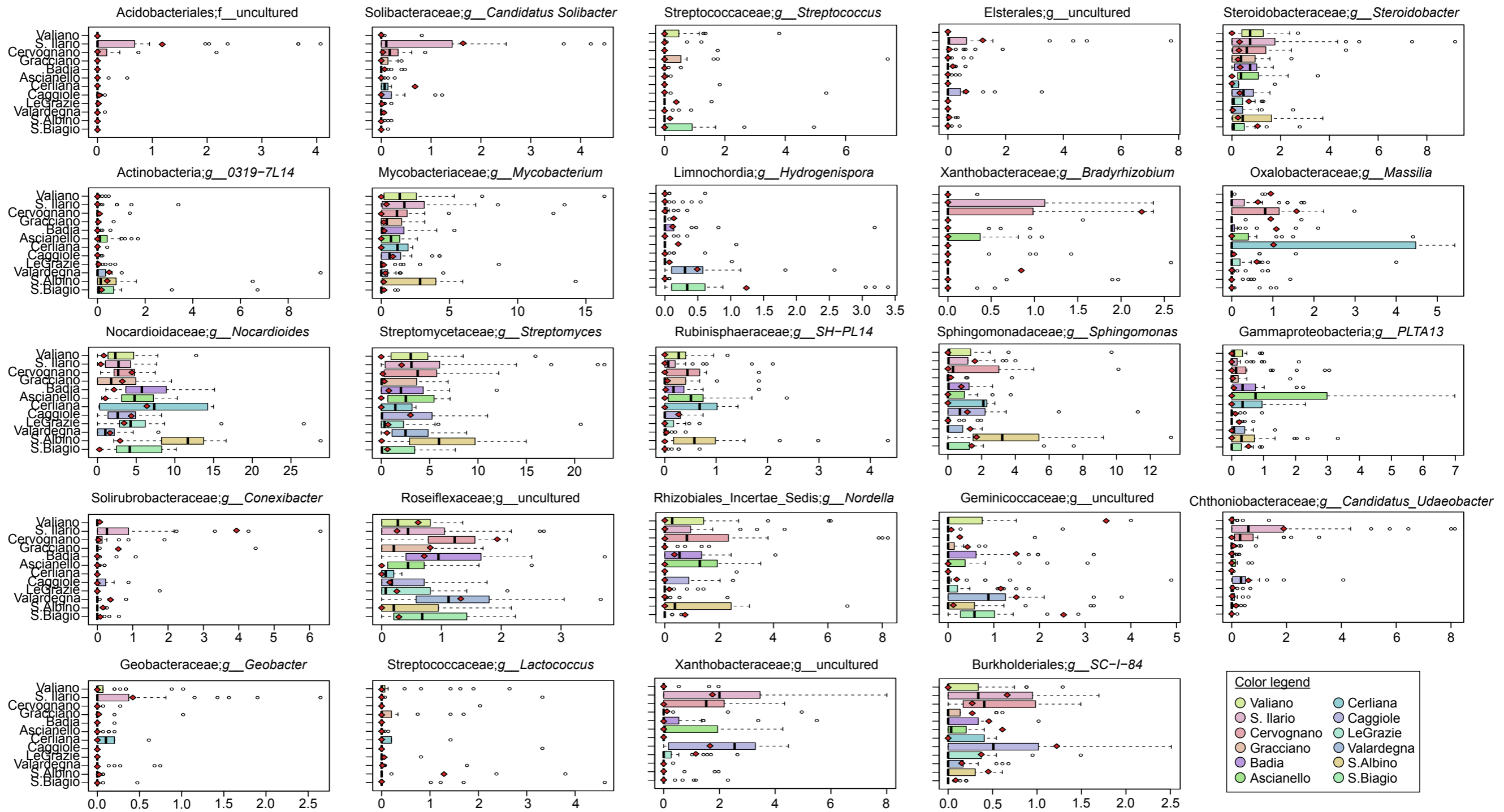
**A)**



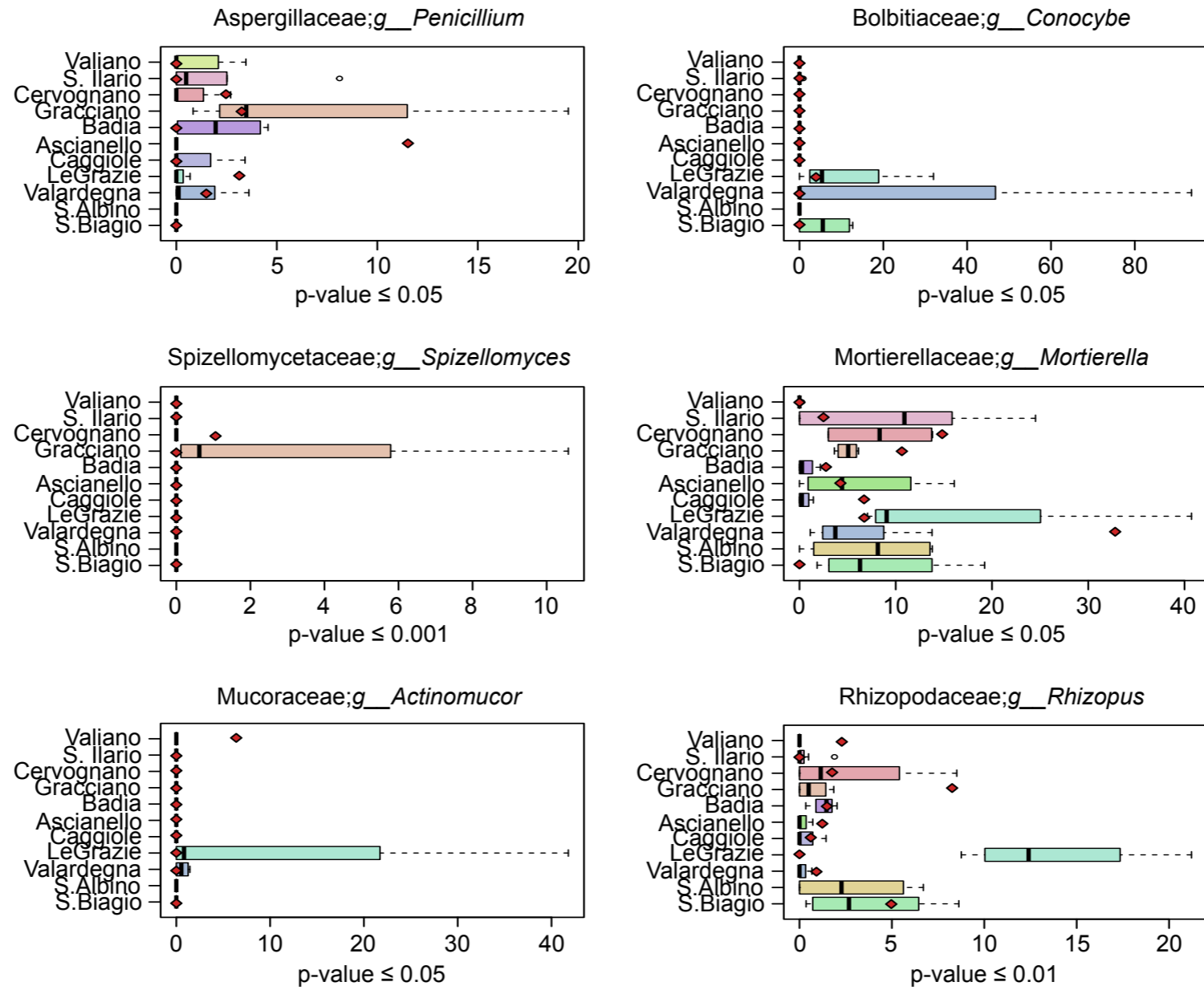
**B)**



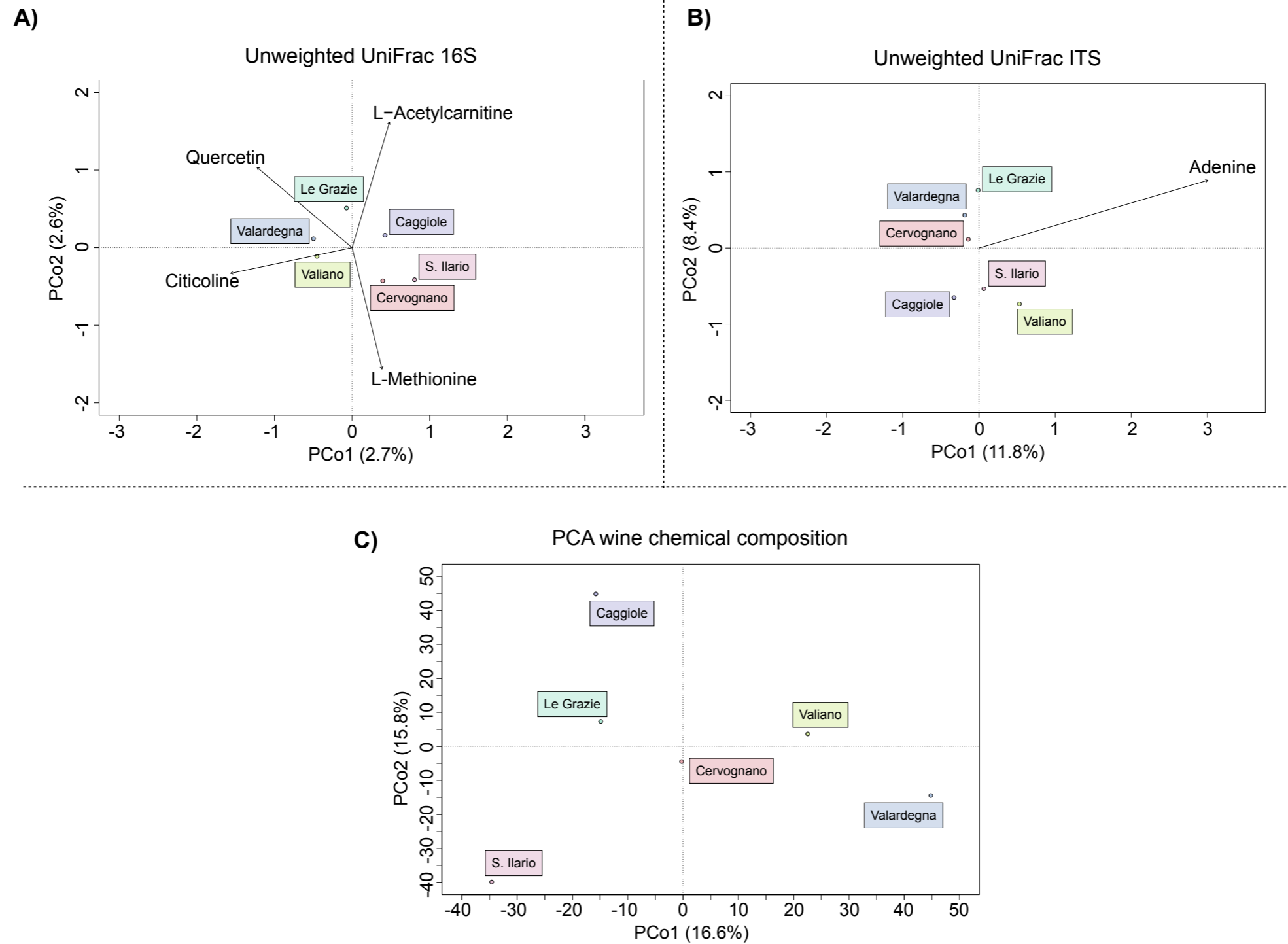
**Supplementary Figure 2 - Diversity of microbial communities across Montepulciano AGUs (Additional Geographical Units).** Boxplots showing the alpha-diversity distributions of the microbiome associated with samples from different AGUs for bacterial (A) and fungal (B) communities, based on the Faith's phylogenetic diversity (PD whole tree), the number of observed amplicon sequence variants (ASVs), and the Shannon index. Significant differences between AGUs are indicated below each graph (Kruskal-Wallis test).



**Supplementary Figure 3 - Relative abundance of the 24 bacterial taxa that discriminate among Additional Geographical Units (AGUs).** The relative abundance distribution of the discriminant bacterial taxa in the rhizosphere is represented in each boxplot, whereas the mean relative abundance of the same taxa in the respective soil samples is represented with a red rhombus. Significant variations in relative abundance were assessed by Kruskal-Wallis test controlled for multiple testing using False Discovery Rate (FDR) ( $p$ -value  $\leq 0.001$  for all taxa). AGUs are reported in the first column of boxplots and in the color legend at the bottom right.



**Supplementary Figure 4 - Relative abundance of the 6 fungal taxa that discriminate among Additional Geographic Units - AGUs.** The relative abundance distribution of the discriminant fungal taxa in the rhizosphere is represented in each boxplot, whereas the mean relative abundance of the same taxa in the respective soil samples is represented with a red rhombus. Significant variations in relative abundance were assessed by Kruskal-Wallis test controlled for multiple testing using False Discovery Rate (FDR) (p-values are reported below each boxplot). AGUs are indicated next to each boxplot.



**Supplementary Figure 5 - Impact of wine chemical composition on rhizospheric microbial communities of Montepulciano AGUs (Additional Geographical Units).** Principal Coordinate Analysis (PCoA) based on unweighted UniFrac distances between bacterial (A) and fungal (B) profiles of *Vitis vinifera* cultivar Sangiovese rhizospheres in the different Additional Geographical Units (AGUs) of the Montepulciano territory. Only the six AGUs selected for wine chemical composition are shown. Superimposition of wine analytical components through the envfit function are shown ( $p$ -values  $\leq 0.05$ ). (C) Principal Component Analysis of wine analytical components in the six selected AGUs. In all graphs, the first and second principal components are plotted and the percentage of variance in the dataset explained by each axis is highlighted.

**Supplementary Table 1 - Site and plant characteristics of all vineyards in the Montepulciano consortium.** Additional Geographic Units (AGUs) are given in the first column. Plant age is given in years (y), altitude in meters above sea level (masl), and soil composition in percentage of soil type (gravel, sand, silt and clay). Soil composition was retrieved by Costantini et al. (2021). "--", no information available.

AGU	Vineyard	Plant age (y)	Rootstock	Rootstock type	Clone family	Agronomical practice	Management	Latitude	Longitude	Altitude (masl)	Soil composition (%)			
											Gravel	Sand	Silt	Clay
Valiano	Tenuta Tre Rose	>15	420 A - Gravesac	Berlanderi x Riparia	CG5 - CV20	Plow sole	Biological	43.16022892	11.93597164	337	5	47	29	24
	Palazzo Vecchio	>15	1103P, 110R, 775P	Berlanderi x Rupestris	VCR	Partial grassing	Conventional	43.1657283	11.90092418	320	5	47	29	24
Cervognano	Boscarelli	>15	1103, 110R, 420A	Berlanderi x Rupestris	CH20	Partial grassing	Integrated	43.10599529	11.85076717	301	9	42	36	22
Cerliana	La Ciarliana	>15	420A	Berlanderi x Riparia	R	Plow sole	Conventional	43.11531862	11.81196374	340	9	42	36	22
S. Albino	Fattoria del Cerro	>15	K5BB	Berlanderi x Riparia	CH20	Plow sole	Integrated	43.07135327	11.80867656	470	9	42	36	22
Valardegna	Salcheto	>15	1103P	Berlanderi x Rupestris	Prugnolo Grifo/Bravio	Partial grassing	Biological	43.08663725	11.80031461	422	9	42	36	22
S. Biagio	Le Bertille	>15	--	--	--	--	--	43.10096085	11.76677398	380	9	42	36	22
S. Ilario	Podere Casanova	>15	3309C	Riparia x Rupestris	R	Grassing	Biological	43.07415305	11.89457831	260	10	77	14	9
	Bindella	>15	110R	Berlanderi x Rupestris	VCR	Partial grassing	Integrated	43.09568433	11.86674385	310	9	42	36	22
Le Grazie	Fattoria della Talosa	>15	110R	Berlanderi x Rupestris	G76	Partial grassing	Biological	43.11157039	11.78225739	410	9	42	36	22
Ascianello	De' Ricci	>15	1103P, K5BB	Berlanderi x Rupestris, Berlanderi x Riparia	CH20	Partial grassing	Biological	43.140338	11.791788	350	9	42	36	22
Gracciano	Fattoria Svetoni	>15	--	--	--	Partial grassing	Biological	43.13461785	11.8367263	280	10	77	14	9
Caggiolo	Azienda agricola Tiberini	>15	1103P, 110R, 140R, 157.11	Berlanderi x Rupestris, Berlanderi x Riparia	SG	Partial grassing	Biological	43.12371226	11.80025501	310	9	42	36	22
Badia	Avignonesi	>15	--	--	--	--	Biodynamic	43.157433	11.81136	280	9	42	36	22

**Supplementary Table 2 - Chemical soil properties in the vineyards from different AGUs.** Abbreviations: N = nitrogen, P = phosphorous, K = potassium, Hum = humidity, EC = electrical conductivity,

Temp = temperature

AGU	N (mg/Kg)	P (mg/Kg)	K (mg/Kg)	pH	Hum (%)	EC	Temp °C
<i>S. Ilario</i>	6	9	18	8.10	14.23	91	24.6
<i>Ascianello</i>	27	37	75	6.73	20	375	21.2
<i>Badia</i>	12	17	37	6.72	13	171	24.1
<i>Caggiolo</i>	6	9	19	6.63	15	97	17.5
<i>Cerliana</i>	1	1	3	6.72	18.4	15	24.1
<i>Cervognano</i>	NA	NA	NA	NA	NA	NA	NA
<i>Gracciano</i>	12	17	36	8.25	13.2	177	24.3
<i>Le Grazie</i>	10	14	30	6.42	15.7	143	17.6
<i>S.Albino</i>	10	14	30	7.44	15.44	149	22.1
<i>S.Biagio</i>	42	59	119	6.67	17.4	596	29.6
<i>Valardegna</i>	10	15	31	8.45	14.60	150	23.6
<i>Valiano</i>	NA	NA	NA	NA	NA	NA	NA



**Supplementary Table 3 - Functional plant growth-promoting (PGP) profile of the rhizosphere and soil microbiome of Additional Geographic Units (AGUs).** Abundance of PGP functions identified in the rhizosphere and bulk soil samples of different AGUs using a read-mapping approach. Data were normalized in copies per million ((reads count for an enzyme in a given sample/(gene length/1000))/(n° reads per sample/10<sup>6</sup>).

AGU	Valardegna		S.Biagio		LeGrazie		Badia		Ascianello	
Vineyard	Salcheto		Bertille		Talosa		Avignonesi		DeRicci	
Ecosystem	Rhizosphere	Bulk soil	Rhizosphere	Bulk soil	Rhizosphere	Bulk soil	Rhizosphere	Bulk soil	Rhizosphere	Bulk soil
N fixation	253.33244	253.56081	247.72462	248.112129	251.914698	242.144023	246.841611	245.896123	272.236875	255.770062
P solubilization	519.693471	513.847621	517.117393	469.877291	539.80944	520.801887	541.353565	525.087623	502.753704	477.580141
Siderophore production	117.709424	102.601028	95.1561947	86.858134	83.7740471	89.4248764	106.68441	116.10761	136.425328	133.276813
IAA production	485.630976	451.86668	494.393106	376.625372	504.947659	474.766202	492.37769	494.630377	484.131827	458.895643
ACC deaminase production	23.007601	22.7278197	21.6513326	19.5976165	22.9139776	24.6880899	21.4839648	18.7107619	18.0837785	20.6455929

AGU	S.Albino		Valiano				Cerliana		Gracciano	
Vineyard	Cerro		PalazzoVecchio		TreRose		Ciarliana		Svetoni	
Ecosystem	Rhizosphere	Bulk soil	Rhizosphere	Bulk soil	Rhizosphere	Bulk soil	Rhizosphere	Bulk soil	Rhizosphere	Bulk soil
N fixation	245.161867	237.297965	261.4787	258.75021	247.017013	289.026902	267.87289	231.754579	270.074223	266.946281
P solubilization	504.167645	427.029998	462.810473	507.034069	490.230313	499.636566	525.466851	421.721006	534.439308	530.437528
Siderophore production	92.5650446	86.6199499	145.151287	104.084526	107.48817	119.575935	108.305706	93.9227997	100.63267	130.501276
IAA production	485.80206	414.209339	461.286801	448.963185	439.289388	478.49473	559.174905	414.594039	527.122714	481.003453
ACC deaminase production	20.4542001	19.1470931	20.4485896	20.7340903	13.7356376	15.1534794	19.678259	20.6158577	22.3434965	25.2639428

AGU	Caggiolo		Cervognano		S. Ilario			
Vineyard	Tiberini		Boscarelli		Bindella		Casanova	
Ecosystem	Rhizosphere	Bulk soil	Rhizosphere	Bulk soil	Rhizosphere	Bulk soil	Rhizosphere	Bulk soil
N fixation	254.70967	244.997058	248.1592	248.150919	278.514379	249.270456	259.064404	224.670145
P solubilization	532.123067	502.172868	553.042669	488.304118	545.11962	468.293636	581.538473	622.797182
Siderophore production	94.4612051	119.183709	104.591244	137.015835	120.366355	96.079104	83.2131811	108.087523
IAA production	478.426958	436.466634	524.096431	462.368216	457.238688	405.289537	497.46969	461.164674
ACC deaminase production	21.1471873	21.6917165	20.8084552	25.5832629	18.9627404	17.1426964	17.9646773	27.108519

**Supplementary Table 4 - Metagenome-assembled genomes (MAGs) taxonomically assigned to previous Additional Geographic Unit (AGU)-associated genera. Only MAGs with more than 50% completeness and less than 5% contamination were considered. For each MAG (column 1), the PhyloPhlAn assignment is reported in column 2, the respective 16S taxonomy is reported in column 3, completeness and contamination percentages are reported in column 4 and 5, and bp size is reported in column 6.**

Genomic bin	PhyloPhlAn assignment	16S taxonomy	Completeness %	Contamination n %	Size bp
bin.290	uSGB_61472:Other:k_Bacteria p_Actinobacteria c_CFGB38726 o_OFGB38726 f_FGB38726 g_GGB44089 s_GGB44089_SGB61472 t_SGB61472:0.247037	Actinobacteria Unclassified	54.47	2.91	3027411
bin.412	uSGB_61528:Other:k_Bacteria p_Actinobacteria c_CFGB35837 o_OFGB35837 f_FGB35837 g_GGB44133 s_GGB44133_SGB61528 t_SGB61528:0.250557	Actinobacteria Unclassified	53.09	3.02	1503685
bin.26	uSGB_55849:Other:k_Bacteria p_Actinobacteria c_CFGB10712 o_OFGB10712 f_FGB10712 g_GGB32443 s_GGB32443_SGB55849 t_SGB55849:0.265725	Actinobacteria Unclassified	76.83	3.51	2438761
bin.320	kSGB_35016:Species:k_Bacteria p_Actinobacteria c_Actinobacteria o_Micrococcales f_Microbacteriaceae g_Agromyces s_Agromyces_ramosus t_SGB35016:0.152843	Agromyces	57.4	4.10	2789788
bin.340	kSGB_31955:Species:k_Bacteria p_Proteobacteria c_Alphaproteobacteria o_Rhizobiales f_Bradyrhizobiaceae g_Bradyrhizobium s_Bradyrhizobium_algeriense t_SGB31955:0.26546	Bradyrhizobium	96.65	1.80	4623281
bin.202	uSGB_49283:Genus:k_Bacteria p_Actinobacteria c_Actinobacteria o_Micrococcales f_Cellulomonadaceae g_Cellulomonas s_Cellulomonas_SGB49283 t_SGB49283:0.312952	Cellulomonas	57.18	0.78	1649748
bin.1	uSGB_13269:Family:k_Bacteria p_Actinobacteria c_Thermoleophila o_Solirubrobacterales f_Conexibacteraceae g_GGB8534 s_GGB8534_SGB13269 t_SGB13269:0.283502	Conexibacter	61.91	1.76	5199929
bin.176	uSGB_13270:Family:k_Bacteria p_Actinobacteria c_Thermoleophila o_Solirubrobacterales f_Conexibacteraceae g_GGB8535 s_GGB8535_SGB13270 t_SGB13270:0.243761	Conexibacter	65.18	4.97	1715402
bin.348	uSGB_13270:Family:k_Bacteria p_Actinobacteria c_Thermoleophila o_Solirubrobacterales f_Conexibacteraceae g_GGB8535 s_GGB8535_SGB13270 t_SGB13270:0.192258	Conexibacter	67.54	1.71	2165037
bin.396	kSGB_16435:Species:k_Bacteria p_Actinobacteria c_Actinobacteria o_Cryptosporangiales f_Cryptosporangiaceae g_Cryptosporangium s_Cryptosporangium_aurantiacum t_SGB16435:0.276697	Cryptosporangium	52.12	0.85	1495776
bin.144	kSGB_33795:Species:k_Bacteria p_Actinobacteria c_Rubrobacteria o_Gaiellales f_Gaiellaceae g_Gaiella s_Gaiella_occulta t_SGB33795:0.256381	Gaiella	73.59	4.78	1669372
bin.83	kSGB_33795:Species:k_Bacteria p_Actinobacteria c_Rubrobacteria o_Gaiellales f_Gaiellaceae g_Gaiella s_Gaiella_occulta t_SGB33795:0.339586	Gaiella	69.63	3.03	2860656
bin.228	kSGB_12835:Species:k_Bacteria p_Proteobacteria c_Betaproteobacteria o_Burkholderiales f_Oxalobacteraceae g_Massilia s_Massilia_yuzhufengensis t_SGB12835:0.35328	Massilia	54.81	2.81	2796737
bin.164	kSGB_32229:Species:k_Bacteria p_Proteobacteria c_Alphaproteobacteria o_Rhizobiales f_Phyllobacteriaceae g_Mesorhizobium s_Mesorhizobium_sp_YMIC_6_2 t_SGB32229:0.212502	Mesorhizobium	59.82	3.92	5194556
bin.349	uSGB_61376:Genus:k_Bacteria p_Actinobacteria c_Actinobacteria o_Corynebacteriales f_Mycobacteriaceae g_Mycobacterium s_Mycobacterium_SGB61376 t_SGB61376:0.35328	Mycobacterium	98.54	0.97	2070293
bin.19	kSGB_853:Species:k_Archaea p_Thaumarchaeota c_Nitrososphaeria o_Nitrososphaerales f_Nitrososphaeraceae g_Nitrososphaera s_Candidatus_Nitrososphaera_gargensis t_SGB853:0.320287	Archaea	64.29	2.76	2948997
bin.378	uSGB_55847:Family:k_Bacteria p_Actinobacteria c_Actinobacteria o_Corynebacteriales f_Nocardiaceae g_GGB38223 s_GGB38223_SGB55847 t_SGB55847:0.265526	Nocardiaceae Unclassified	56.04	3.61	1668073
bin.111	kSGB_34149:Species:k_Bacteria p_Actinobacteria c_Actinobacteria o_Propionibacteriales f_Nocardioidaceae g_Nocardioides s_Nocardioides_iriomotensis t_SGB34149:0.26546	Nocardioides	77.70	4.37	1248512
bin.197	kSGB_55752:Species:k_Bacteria p_Actinobacteria c_Actinobacteria o_Propionibacteriales f_Nocardioidaceae g_Nocardioides s_Nocardioides_bacterium t_SGB55752:0.300994	Nocardioides	51.66	2.59	1740489
bin.92	kSGB_15965:Species:k_Bacteria p_Actinobacteria c_Actinobacteria o_Propionibacteriales f_Nocardioidaceae g_Nocardioides s_Nocardioides_sp_Root122 t_SGB15965:0.254359	Nocardioides	57.42	4.42	2834555
bin.278	kSGB_11537:Species:k_Bacteria p_Proteobacteria c_Alphaproteobacteria o_Sphingomonadales f_Sphingomonadaceae g_Novosphingobium s_Novosphingobium_sp_Rr_2_17 t_SGB11537:0.193331	Novosphingobium	57.43	4.67	2453724
bin.307	kSGB_16407:Species:k_Bacteria p_Actinobacteria c_Actinobacteria o_Streptosporangiales f_Thermomonosporaceae g_Thermomonospora s_Thermomonospora_curvata t_SGB16407:0.328965	Novosphingobium/Thermomonospora	52.49	2.58	2643601
bin.321	uSGB_55819:Family:k_Bacteria p_Cyanobacteria c_Cyanobacteria_unclassified o_Synechococcales f_Prochloraceae g_GGB41164 s_GGB41164_SGB55819 t_SGB55819:0.248764	Synechococcales Unclassified	83.49	2.91	2104713
bin.325	uSGB_61813:Other:k_Bacteria p_Proteobacteria c_CFGB35932 o_OFGB35932 f_FGB35932 g_GGB44394 s_GGB44394_SGB61813 t_SGB61813:0.273629	Proteobacteria Unclassified	62.1	4.73	2190116
bin.96	uSGB_61813:Other:k_Bacteria p_Proteobacteria c_CFGB35932 o_OFGB35932 f_FGB35932 g_GGB44394 s_GGB44394_SGB61813 t_SGB61813:0.26803	Proteobacteria Unclassified	62.22	1.03	1350259
bin.128	kSGB_67219:Species:k_Bacteria p_Proteobacteria c_Alphaproteobacteria o_Rhodobacterales f_Rhodobacteraceae g_Rhodobacteraceae_unclassified s_Rhodobacteraceae_bacterium t_SGB67219:0.372583	Rhodobacter	52.21	2.04	1857802
bin.355	uSGB_52610:Genus:k_Bacteria p_Actinobacteria c_Actinobacteria o_Micrococcales f_Microbacteriaceae g_Rhodoluna s_Rhodoluna_SGB52610 t_SGB52610:0.300994	Microbacteriaceae	74.22	3.53	2002286
bin.121	kSGB_17577:Species:k_Bacteria p_Actinobacteria c_Rubrobacteria o_Rubrobacterales f_Rubrobacteraceae g_Rubrobacter s_Rubrobacter_xylanophilus t_SGB17577:0.300994	Rubrobacter	54.39	0.50	1851211
bin.36	kSGB_17577:Species:k_Bacteria p_Actinobacteria c_Rubrobacteria o_Rubrobacterales f_Rubrobacteraceae g_Rubrobacter s_Rubrobacter_xylanophilus t_SGB17577:0.258493	Rubrobacter	60.04	3.45	3175285
bin.178	kSGB_55872:Species:k_Bacteria p_Actinobacteria c_Thermoleophila o_Solirubrobacterales f_Solirubrobacteraceae g_Solirubrobacter s_Solirubrobacter_sp_CPCC_204708 t_SGB55872:0.295981	Solirubrobacter	74.11	1.94	1593893
bin.119	kSGB_24610:Species:k_Bacteria p_Proteobacteria c_Alphaproteobacteria o_Sphingomonadales f_Sphingomonadaceae g_Sphingomonas s_Sphingomonas_sp_URHD0007 t_SGB24610:0.35328	Sphingomonas	66.50	3.23	2636012
bin.123	kSGB_10471:Species:k_Bacteria p_Proteobacteria c_Gammaproteobacteria o_Nevskiales f_Sinobacteraceae g_Steroidobacter s_Steroidobacter_denitrificans t_SGB10471:0.295981	Steroidobacter	78.95	3.41	2177673
bin.42	kSGB_10471:Species:k_Bacteria p_Proteobacteria c_Gammaproteobacteria o_Nevskiales f_Sinobacteraceae g_Steroidobacter s_Steroidobacter_denitrificans t_SGB10471:0.312952	Steroidobacter	74.23	4.68	3459230
bin.126	kSGB_16111:Species:k_Bacteria p_Actinobacteria c_Actinobacteria o_Streptomycetales f_Streptomycetaceae g_Streptomyces s_Streptomyces_sp_1114_5 t_SGB16111:0.270747	Streptomyces	68.45	3.45	3676162
bin.173	kSGB_34439:Species:k_Bacteria p_Actinobacteria c_Actinobacteria o_Streptomycetales f_Streptomycetaceae g_Streptomyces s_Streptomyces_sp_TAA040 t_SGB34439:0.320287	Streptomyces	63.88	4.34	2875784
bin.43	uSGB_46364:Genus:k_Bacteria p_Actinobacteria c_Actinobacteria o_Streptomycetales f_Streptomycetaceae g_Streptomyces s_Streptomyces_SGB46364 t_SGB46364:0.35328	Streptomyces	78.38	3.39	2183585
bin.276	kSGB_12768:Species:k_Bacteria p_Proteobacteria c_Betaproteobacteria o_Burkholderiales f_Comamonadaceae g_Variovorax s_Variovorax_sp_YR216 t_SGB12768:0.320287	Variovorax	66.22	4.88	2533478



**Supplementary Table 6 - Accession numbers of plant growth-promoting (PGP) proteins from the NCBI database.**

Protein names are given in the first column.

Protein ID	NCBI accession
NifB	WP_011024080
NifE	WP_014404757
NifH	WP_010870393
NifN	WP_011241560
NifV	WP_011241567
NifU	NP_461477
phoA	NP_414917
GDH	NP_388275
EntF	WP_000077784
EntS	NP_415123
FslA	WP_003037766
ipdC	WP_035671558
aro10	NP_010668
aldH	NP_001260290
AcdS	XP_037178185