Microscale analysis of soil characteristics and microbiomes reveals potential impacts on plants and fruit: vineyard as a model case study

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Abstract

Aims

Soil microbiome roles in agriculture is becoming more and more important. This importance is also reflected on the way plants are seen: complex organisms formed by the plant itself plus the microbes inhabiting its tissues, including the ones on the surface of every organ and the ones adhered or in proximity to the roots. In addition, as already demonstrated, the microbial community associated with a specific soil is able to predetermine the health status of crops. For all the above mentioned reasons, defining the microbial composition of agricultural soils and the factors driving the assemblage is pivotal to achieve more sustainable agriculture and viticulture.

Methods

We aimed to investigate how the soil geological characteristics influence the microbiome composition associated with close geographically related vineyards. Moreover, we studied both the top (15 cm in depth) and deep (120 cm in depth) soil layers as anthropically influenced and almost-undisturbed soil, respectively.

Results

We observed slightly different microbial communities despite the close geographical proximity of the two vineyards, which is considered one of the main determinants of the soil microbiome composition. In addition, we found that the geological characteristics of the two soils influence both the root distribution and the accumulation of pathogen- and symbiont-related genera. Sensory profiles of the Grillo wines from the two different soils confirmed the tight link between soil origin and wine traits.

Conclusions

In the present study, we highlight that the geological characteristics of soil can influence soil microbial composition and assemblage in close geographically related vineyards, with a potential effect on wine features.

Introduction

Soil microbial biomass has crucial roles in Earth's biogeochemical cycles in both natural and human-managed ecosystems (Fierer 2017). Despite the challenges that living organisms face to survive in such environments, if we consider viruses, bacteria and fungi, we can estimate that each gram of soil can contain up to millions of individual microorganisms (Fierer et al. 2009). These organisms play key roles in nutrient cycling, soil fertility and soil carbon sequestration, and they show both direct and indirect effects on plant and animal health (Fierer et al. 2009; Serna-Chavez et al. 2013; Fierer 2017). In this respect, some microorganisms have evolved the ability to associate with plants, forming mutualistic symbioses (e.g. arbuscular mycorrhizal fungi [AMF] and rhizobacteria). Furthermore, the importance of interactions between plants and microorganisms has been described as an additive ecological function that can be a major trait in extending a plant's ability to adapt to many stressful environmental conditions (Bulgarelli et al. 2012). Given this wide importance of microbes on plant health, they are seen as a reservoir of additional genes and functions for their host; plants with their interacting microbes are defined as the so-called holobiont (Zilber-Rosenberg and Rosenberg 2008).

Grapevine, as do many other crops that are represented by the same or very similar genotypes, display differential geographical phenotypes in terms of morphological and sensorial signatures; these differences are generally described as the *terroir* (Van Leeuwen and Seguin 2006). Viticulturists have been selectively growing vine cultivars from local wild *Vitis vinifera* subsp. *sylvestris* varieties, which present differences among grape size and shape, berry colour, flavour, yield and many other phenotypic aspects (Arroyo-García et al. 2006). The most interesting individuals have been multiplied by vegetative propagation for years, during which time genetic and somatic modifications have spontaneously occurred. Those events have given rise to an intra-varietal variability associated with phenotypic and biochemical variation, which has led to the description of grapevine clones (Pelsy 2010). Despite this very detailed characterisation, the same grape clones (which are still vegetatively propagated) can show differences among phenotypic characteristics and biochemical traits when grown in different environments, confirming that the plant genome is not the only player able to shape the phenotype. If

we look at the final product (i.e. wine), it is well known that indigenous yeast and bacteria inhabiting the berries' surface can have a wide impact on the flavour and aroma of typical wines (Pretorius 2000; Capece et al. 2010; Tristezza et al. 2014; Knight et al. 2015). From this view, on their journey from the vineyard to the winery, grapes are transformed into wine through microbial biochemical processes, with unquestionable consequences for wine quality parameters.

Due to the above-mentioned importance of microbes associated with grape plants and their impacts on wine characteristics, researchers have tried to address what are the main factors driving the microbial *terroir* composition of the holobiont. Among all the parameters, one of the most important is the geographical location. It has been demonstrated that there are clear delineations among local natural populations of yeast inhabiting berries (Gayevskiy and Goddard 2012; Bokulich et al. 2014). Similarly, a study analysed the impact of geographical distance on both fungal and bacterial communities (Miura et al. 2017). The latter work clearly showed that spatial processes play an important role in structuring the biogeographical pattern of grape-associated fungal communities but do not influence bacterial communities. Interestingly, another main factor influencing the microbial *terroir* is the soil. Researchers have demonstrated that the majority of organ-associated microbes reflect the ones found in the surrounding soil, and their distribution is in turn influenced by the highly localised biogeographical factors and vineyard management practices (Zarraonaindia et al. 2015). The strict relationship among geographical location and microbiome structure was also recently reported among the famous Italian wine region of Trentino (Coller et al. 2019). The authors suggested an inverse correlation between the geographical location and the bacterial and fungal community structure.

As an additional level of complexity, we need to mention that *V. vinifera* cultivars are commonly grown using rootstocks. Rootstocks are used because of their ability to cope with certain biotic and abiotic factors, such as phylloxera (Granett et al. 1987), nematodes (Stirling and Cirami 1984), salinity (Upreti and Murti 2010) and water limitations (Berdeja et al. 2015), and rootstocks also play a role in the growth-defence trade-off balance (Chitarra et al. 2017). It has been demonstrated that the microbiome of the soil surrounding a plant is strongly influenced by the rootstock genotype (Marasco

et al. 2018). Those authors reported that the genotype of the grape root system can select and recruit microbes that will then colonise the aboveground organs, ultimately influencing both fruit and wine qualities.

For all the above-mentioned reasons, we decided to study the microbial composition of bulk soil associated with the root of the grapevine cultivar Grillo in a limited geographical area to determine what are the most important players able to shape the wine typicity. We selected two different soil types that lead to the production of the same Grillo wine but with different organoleptic features (Scienza and Giorgianni 2015), and we analysed the soil chemical and microbial compositions, including the wine organoleptic profile. Furthermore, to understand the anthropic-mediated impact on the microbial communities, we analysed the soil at depths of 15 and 120 cm.

Methods

Vineyard location and sampling

Our study was conducted in two different vineyards located in the municipality of Menfi (Ag), Sicily, Italy. The two vineyards are characterized by the cultivation of the same Grillo RS297 clone, grafted onto 1103 Paulsen (1103P). Grillo is one of the most popular Sicilian varieties and it is the offspring of a natural cross between Catarratto bianco and Muscat of Alessandria. Nowadays Grillo is mainly cultivated in the Trapani province and in Sicily it accounts for more than 6.500 ha.

The first field that we took in consideration is located in Contrada Finocchio (37°37'06.0"N 12°54'54.9"E) on a marly-limestone substratum (ML) at 115 meters above the see level. The second field is located eastward of Menfi, in Contrada Bertolino (37°35'02.7"N 13°00'38.3"E) on a calcarenitic substratum (C) at 140 m a.s.l. The two vineyards are about 8 km far from each other, both of about 1 hectare (10.000 m^2) and the sampling was performed in both vineyards at 15 cm in depth (designated as superficial = S) and 120 cm of depth (designated as deep = D) in correspondence of root profiling trenches.

In Menfi area the summers are warm, muggy and dry and the winters are long, cold, windy, and partly cloudy defining a harsh Mediterranean environment. Over the course of the year the rainfall are

about 490 mm and the temperature typically vary from a minimum of 8°C during the winter to the maximum of 35°C in the summer season. In both vineyards we have chosen, vines were planted in 2002 and were subjected to standard cultural practices (soil, nutrition, irrigation, canopy and pest management) routinely used in the Menfi area. The training system was Guyot consisted of one fruit cane of 8/10 buds and its total length was about 0.6-0.8 m per vine. Vine spacing was 2.5 m × 0.9 m (intra row and inter vines) equal to 4.444 vines per hectare and the fruit cane was trained 0.7 m above ground with one pairs of surmounting catch wires for a canopy wall extending about 1.5 m above the fruit cane.

Grapevine root development

As the root systems of grapevines are capable to reach a large volume of soil exploration, influenced mostly by soil conditions, the classical profile wall method was chosen as the most appropriate one to determine root distribution and density (Böhm and Köpke, 1977; Böhm, 1979). In February 2017, during the dormant period, six vines per vineyards with similar scion circumferences (considering the first 50 cm above the scion-rootstock junction) were selected randomly along the field. For each two vines, a trench of approximately 1.20 m deep was dug parallel to the vine row, first at 1.00 m and then at 0.40 m distances from the vine trunk. At each distance, roots were counted by using a 1.2 m high and 2.0 m wide grid system placed against the profile wall, the grid was divided in sub-grid block with a size of 0.2 m x 0.2 m, two vines for each trench have been considered. Roots were plotted in five depths (0-20 cm, 20-40 cm, 40-60cm, 60-80 cm, 80-100 cm) and were classified into three root diameter classes according to size: Ø < 1.0 mm = fine roots; Ø 1.0-3.0 mm = medium roots and Ø > 3.0 mm = permanent roots. Each thickness class had its own symbol to distinguish between the roots drawn on the plan. Processed data are expressed as root number /m^2. A rooting index has been calculated according to (Van Zyl, 1984). A high index reflects more thin roots relative to medium and thick roots as a result of more favourable soil conditions.

Two soil moisture sensors (WaterScout SM100 Spectrum Technologies - Aurora , IL, USA) has been placed in both soils at two depths (40 and 110 cm), providing the Volumetric Water Content (VWC) of soil (Resolution: 0.1% VWC; Accuracy: 3% VWC @ EC < 8 mS/cm). The two sensors were connect to one station FL SENS USB (GMR Strumenti , Scandicci, IT) data logger.

Wine profiles

Grape composition at harvest was measured on a sample of 0.5 kg of berries collected randomly from each one of the two Grillo vineyards. Soluble solids were measured by refractometer (Atago PR32) at 20 °C, pH and titratable acidity (expressed as g/L of tartaric acid) were measured using an automatic titrator (Crison Micro TT 2022, Riera Principal, 34-36 08328, Alella (Barcelona), Spain) by titration with 0.1N NaOH. Winter pruning wood from 3 replications of 15 vines of each vineyard (total 45 vines) was weighed electronically by means of a hanging scale (CH, Kern, Germany) on 26th February 2018 as an indicator of vine canopy growth in 2017.

Using a standard protocol (Alabi et al., 2016), Settesoli winery provided a separate vinification of the grapes coming from the two soils (about 7,000 kg of grapes per vinification). The tasting analysis was carried out in Settesoli using the internal trained panel test made up of 13 oenologists (8 males, 5 females). Demographic aspects were recorded at the beginning of the first session and no information about the nature of the study was provided in order to reduce bias. Twenty-five mL aliquots of each wine at 20 ± 1°C were poured into wineglasses coded with a random three-digit number and covered to avoid dispersion of volatiles. Wines were then presented during four evaluation sessions using a randomized design with three replicates for each wine. For the quantitative evaluation of the intensity of attributes (visual, olfactory, gustatory and retro-olfactory) a questionnaire providing discrete scale responses with intervals from 1 to 9 has been used.

Soil DNA isolation and sequencing

177 Total nucleic acids were obtained as previously reported from 1 g of soil (Angel, 2012). DNA was
178 then cleaned using the commercial ZymoBIOMICS DNA Kit (Zymo Research, CA, USA) according to
179 manufacturer's protocols yielding 3 to 5 µg of DNA per extraction quantified using a NanoDrop One
180 spectrophotometer (Thermo Fisher Scientific). DNA integrity was evaluated by electrophoresis on a 1%
181 agarose gel in 1x TAE buffer (40 mM Tris-HCl, 20 mM acetic acid, 1 mM EDTA, pH 7.5) stained with
182 Red Safe Nucleic Acid Staining Solution (Labotaq, Sevilla, Spain) and then visualized under UV light.
183 Three biological replicates for each condition were obtained and used as independent samples.
184 Illumina tag screening of the V3-V4 hypervariable regions of the 16S rRNA gene was performed
185 on the DNA by Macrogen Inc. (South Korea), using primers 341f and 785r to build the bacterial
186 amplicon libraries (Kuczynski et al., 2012). The primer ITS3-ITS4 were used to amplify the highly
187 variable spacer ITS2 of the rDNA fungal operon (Lindahl et al., 2013) by Macrogen, Inc. (South Korea).
188 Sequencing of both bacterial and fungal libraries were done with the MiSeq Illumina apparatus.
189
190 ***Metaphylogenomic analyses, taxonomic distributions***
191 PrinSeq v0.20.4 (Schmieder and Edwards, 2011) was used for a first strict quality control on raw
192 data that were then processed in Qiime 2 (Caporaso et al., 2010). A specific pipeline was used for fungal
193 analysis: retained reads were then used to identify the start and stop sites for the ITS region using the
194 hidden Markov models (HMMs) (Rivers et al., 2018), created for fungi and 17 other groups of
195 eukaryotes. Briefly, the software allows to distinguish true sequences from sequencing errors. In order
196 to distinguish true sequences from those containing errors, sequences have been sorted by abundance
197 and then clustered in a greedy fashion at a threshold percentage of identity (97%). Trimmed sequences
198 are then analysed with DADA2 (Callahan et al., 2016), which models and corrects Illumina-sequenced
199 amplicon errors. Sequence variants are then taxonomically classified through the UNITE database (we
200 selected the reference database built on a dynamic use of clustering thresholds) (Abarenkov et al., 2010).
201 For graphic representation, only genera with an average relative abundance higher than the settled
202 threshold (0.1%) were retained.

A different pipeline was used for bacteria: quality filtering was performed with DADA2 which is able to perform chimera removal, error-correction, sequence variant calling with reads truncated at 260 bp and displaying a quality score above 20. Obtained feature sequences were summarized and annotated using the RDP classifier (Cole et al., 2013) trained to the full length 16S database retrieved from the curated NCBI database.

Statistical analyses

Statistical analysis for microbiome data was performed with R (Version 3.4.4) using phyloseq (version 1.24.0) to import, store and analyse data (McMurdie and Holmes, 2014). To convert data from phyloseq, the official extension phyloseq to_deseq2 was used (Love et al., 2014). Fungal and bacterial communities were used to evaluate beta-diversity deriving from the Bray-Curtis distance matrix (complete dataset was considered at the OTU level clustered with a cut-off threshold of 97% identity). The matrix was further used as input to run a non-parametric multivariate analysis (PERMANOVA) (p values were corrected with sequential Bonferroni significance) and non-metric multidimensional scaling (NMDS) was calculated among both the fungal and bacterial diversity in the samples using PAST (Hammer et al., 2001). Statistical analysis for the determination of significant differences between roots number was carried out using Student-Newman-Keuls test ($p \leq 0.05$) Statistica version 8 (StatSoft, Inc.).

For the wine attributes, the relative differences between wines were analysed and confirmed submitting the judgements to statistical analysis using the ANOVA method according to Alabi et al., 2016.

Results

Soil geological description

In the area of Contrada Finocchio, where the first vineyard is located, a middle-upper Oligocene lithological succession outcrops; it consists of open shelf carbonates, mostly represented by thick-

bedded whitish marly limestones, alternating with white or greyish marls. The observed thickness of each level varies from a few centimetres up to 80–100 cm. Intercalations of nummulitic and resedimented biocalcarenites are sometimes present. The above-described lithological complex is ascribed to the lower portion of the so-called Ragusa Formation, particularly to the Leonardo Member. The general characteristics of the succession are strongly influenced by the predominant presence of compact limestones or of the more or less marly levels containing 20%–60% clay, in addition to other constituents. The permeability degree of the lithological complex is generally scarce due to the presence of marly levels with very low permeability; however, the local presence of limestone with high secondary permeability, due to fissuration induced by tectonic processes, allows a moderate drainage of the groundwater and the formation of small aquifers with local importance.

East the town of Menfi, where Contrada Bertolino and the second vineyard are located, outcrops a middle-upper Pliocene terrigenous succession locally known as the Marnoso-arenacea del Belice formation. From the bottom, it comprises several tens of metres of fine to medium sandstones; the sandy particles are mainly rounded quartz grains, but there are also levels of resedimented biocalcarenites. They extent upwards to sandstones and coastal calcarenites and conglomerates. The substratum in the area is formed of up to 50 m of a thick package of hemipelagic shales and marls as well as brownish siltstones with interbedded siltstones and calcarenite mudstones. The Marnoso-arenacea del Belice formation underlies the quaternary marine deposits, which are mostly represented by lower Pleistocene yellowish, partially cemented calcarenites and biocalcarenites alternating with thick beds of biocalcirudites, with thin intercalations of marl levels, conglomerate lenses and calcareous sand and gravel. The total thickness of the quaternary complex varies from a few metres, as in the area of interest, up to tens of metres. The natural porosity characteristics of the above-mentioned lithological complexes, especially of the calcarenite levels and of the small cemented sands, allows good drainage and

252 circulation of the groundwater, which is partially confined by marly and loam/clay grain thin-bedded
253 intercalations with low permeability.
254
255 ***Soil chemical analyses***
256 The average values of physical and chemical characteristics of the two soils are reported in
257 Supplementary Table 1. The pH is alkaline, but it is in an optimal pH range for plant nutrient uptake
258 (Proffitt and Campbell-Clause 2012).
259
260 ***Root system distribution and development***
261 Table 1 reports the root density at two distances from the vine trunk (0.40 and 1 m), dividing the
262 roots into three diameter classes, and the average number of roots/m^2 found in the two soils along the
263 dug trench. In those soil conditions, we found that the total root number of C soil was higher compared
264 with ML soil 0.40 m from the vine trunk, while the mean root density did not vary significantly at 1 m
265 from the vine trunk. Considering the root size, we found thinner roots (Ø < 1 mm) in ML soil 1 m from
266 the vine trunk and more abundant, thicker roots (Ø 1–3 mm and Ø > 3 mm) for both distances (0.4 and
267 1 m) in C soil, where the sandy texture allowed more root diffusion (Table 1). We found some significant
268 differences: an average ratio of 7.1 versus 3.8 at 0.4 m from the vine trunk ($p < 0.05$) and 10.9 versus
269 4.3 at 1 m from the vine trunk ($p < 0.05$) for ML and C soil, respectively (Supplementary Table 2).
270 Regarding the vertical distribution, in C soil, most roots (62 roots/0.04 m^2) were located between
271 0.40 and 0.60 m of soil depth and 0.40 m from the vine trunk. Indeed, for ML soil, we observed most
272 roots at the same depth (0.40–0.60 m) but at 1 m from the vine trunk (48 roots/0.2 m^2).
273 Soil humidity was monitored during the season at two depths, 40 and 110 cm. The higher
274 humidity in silt, owing to marly limestone, permits a better water holding capacity. In C soil, due to its
275 high sandy texture and high drainage, the lower humidity indicates a reduced ability to water retain
276 (Supplementary Fig. 1).
277

278 ***Bacterial community diversity and composition***

279 The bacterial community was analysed at both the family and the genus level: the number of
280 retained sequences after chimera removal and taxonomical assignment ranged from 29,924 to 51,428.
281 The diversity indices (Shannon and Simpson) indicate a significant difference between superficial and
282 deep microbial composition in both soil types (Table 2). Interestingly both indices suggest that
283 calcarenite superficial (CS) displays the same diversity as deep marly limestone (MLD).

284 The complete bacterial community composition for each sample type at the family level is
285 reported in Supplementary Table 3, with statistical results of pairwise comparisons reported in
286 Supplementary Table 4. To simplify, we describe results for the families that represent at least 2% of
287 the bacterial community (Fig. 1a). Regarding C soil, we found that the composition of superficial (CS)
288 and deep (CD) samples is quite different, as also indicated by the statistical analysis in Supplementary
289 Table 4 and in the PCoA (Supplementary Fig. 2). It is worth noting that CD displays more taxa and the
290 most variable composition compared with all the other samples. In detail, the superficial sample is richer
291 in *Nitrososphaeraceae* and *Bacillaceae*, whereas *Sphingobacteriaceae* and *Comamonadaceae* are more
292 abundant in the 120-cm depth sample (Supplementary Table 4). Based on pairwise comparison of
293 superficial calcarenite and marly limestone samples, only three families showed significant differences
294 in abundance. Among the three families, *Bacillaceae* and *Nitrososphaeraceae* are more abundant in CS
295 than MLS. Regarding ML soil, we observed 21 families that are more abundant in the superficial (MLS)
296 sample and 28 families that are more represented at the 120-cm depth (MLD) sample. Among the
297 differentially abundant families, *Acidobacteriaceae*, *Sphingomonadaceae*, *Gemmatimonadaceae*,
298 *Nitrospiraceae*, *Acidimicrobiaceae* and *Verrucomicrobia* subdivision 3 are overrepresented in MLS; in
299 comparison, *Bacillaceae*, *Nitrososphaeraceae*, *Streptomycetaceae* and *Sphingobacteriaceae* are
300 overrepresented in MLD. We then also compared the two deep soil types and observed a larger number
301 of differentially abundant families. There are 36 overrepresented families in MLD, including
302 *Nitrososphaeraceae*, *Bacillaceae*, *Hyphomicrobiaceae*, *Rhodospirillaceae*, *Chitinophagaceae* and
303 *Sinobacteraceae*, which belong to the top selected families representing about the 60% of microbial

304 composition. In parallel, 32 families are more abundant in CD, including *Sphingobacteriaceae*,
305 *Comamonadaceae*, *Pseudomonadaceae*, *Nitrospiraceae*, *Acidobacteriaceae*, *Sphingomonadaceae* and
306 *Cytophagaceae*.

307 Regarding the bacterial composition at the genus level (Supplementary Table 5), we decided to
308 retain only taxa representing at least 2% of the overall community (Fig. 1b). Pairwise comparisons were
309 done to determine the significant differences among sample types (Supplementary Table 6). First, we
310 started by comparing CS and CD. There are eight genera that are more abundant in CS, whereas 21
311 genera are more abundant in CD. Some of the genera more abundant in CD soil are *Pedobacter*,
312 *Flavobacterium*, *Variovorax* and *Pseudomonas*. When comparing CS and MLS, we observed only a
313 few differences: four genera are more abundant in CS, among them *Flavobacterium*, and only one is
314 more abundant in MLS. Then, we compared MLS to MLD, observing a large number of differentially
315 abundant genera: 37 are more abundant in MLS whereas 50 are more abundant in MLD soil. Some of
316 the more abundant genera more in MLS are *Acidobacterium*, *Sphingomonas*, *Nordella*,
317 *Stenotrophobacter*, *Nitrospira* and *Vicinamibacter*. In parallel, some of the more abundant genera in
318 MLD are *Flavobacterium*, *Pedobacter* and *Steroidobacter*. Finally, we also compared the genera of
319 MLD and CD. Similarly to what we found for the families, this comparison produced the largest number
320 of differentially abundant genera: 61 are more abundant in MLD and 62 are more abundant in CD.
321 Among them, *Steroidobacter* and *Thiobacter* are more abundant in MLD and *Pedobacter*,
322 *Flavobacterium*, *Variovorax*, *Acidobacterium*, *Nitrospira*, *Nordella*, *Vicinamibacter*, *Sphingomonas*
323 and *Stenotrophobacter* are more abundant in CD.

324

325 ***Fungal community diversity and composition***

326 We also analysed the fungal communities at both the family and the genus level: the number of
327 retained sequences after chimera removal and taxonomical assignment ranged from 40,294 to 73,785.
328 The diversity indices (Shannon and Simpson) indicate that in both soil types there is a significant

difference between superficial and deep microbial composition (Table 3). The difference is more marked in calcarenite than in marly limestone soil.

The complete fungal community composition for each sample type at the family level is reported in Supplementary Table 5, with statistical results of pairwise comparisons reported in Supplementary Table 6. For simplicity, we describe results for the families that represent at least the 2% of the fungal community (Fig. 2a). As a first, general observation, we can note that at least the family level, the composition of both CD and MLD is almost the same. There is only one family (*Phaffomycetaceae*, which does not represent at least 2% of the fungal community) that shows a differential abundance (Supplementary Table 6). When comparing CS and CD, four families are more abundant in CS and six are more abundant in CD. Among the more abundant families in CD samples are *Botryosphaeriaceae*, *Togniniaceae* and *Chaetomiaceae*, each of which represents at least 2% of the fungal community. We then compared CS and MLS: eight families are more abundant in CS (among them the *Clodosporiaceae*) and 13 are more abundant in MLS. Among the latter are *Dermataceae* and *Togniniaceae*. Finally, we compared MLS and MLD: 13 families are more abundant in MLS (among them *Dermataceae*) and 10 families are more abundant in MLD. Among the latter group, *Botryosphaeriaceae* and *Chaetomiaceae* are above the 2% threshold.

The complete fungal community composition for each sample type at the genus level is reported in Supplementary Table 9, with statistical results of pairwise comparisons reported in Supplementary Table 10. For simplicity, we describe results for the families that represent at least the 2% of the fungal community (Fig. 2b). Similarly to what we observed for the family level, there are minimal genus-level differences between CD and MLD. We observed only one genus that is more abundant in CD (*Stemphylium*) and one more abundant in MLD (*Cyberlindnera*); both genera account for less than the selected 2% threshold of the fungal community. When comparing CS and CD, 18 genera are more abundant in CS (among them, only *Dactylonectria* is above the 2% threshold) and six genera are more abundant in CD. Among the latter are *Neofusicoccum*, *Camarosporium*, *Phaeoacremonium* and *Humicola*. When comparing the composition of the two superficial soils (CS vs MLS), we found more

355 differentially abundant genera (54 in total): 25 more abundant in CS and 29 more abundant in MLS.
356 Regarding genera that represent at least 2% of the fungal community, *Chaetomium* is more abundant in
357 CS whereas *Laetinaevia* and *Phaeoacremonium* are more abundant in MLS. For the MLS and MLD
358 comparison, there are 28 more abundant genera in MLS (only *Laetinaevia* and *Dactylonectria* are above
359 the 2% threshold), whereas there 19 are more abundant genera in MLD (only *Camarosporium* and
360 *Neofusicoccum* are above the 2% threshold).

361 Specific fungal genera can have an important impact on plant development; hence, we also
362 focussed on pathogenic (Fig. 3) and mycorrhizal (Fig. 4) fungi. Regarding pathogenic fungi – we
363 selected the genera that are usually reported as grape pathogens and which are already reported in soil
364 – in both soils, *Neofusicoccum* species are more abundant in the 120-cm depth samples than in
365 superficial ones. On the contrary, *Ilyonectria* is more abundant in both soil types in the superficial layer.
366 In addition, in calcarenite soil, we observed a significant accumulation of *Phaeoacremonium* in CD.
367 Moreover, the genus *Cadophora* is more abundant in MLS than MLD. Regarding mycorrhiza, in both
368 soils *Glomus* is more abundant in MLD. *Rhizophagus* shows an opposite pattern: it is more abundant in
369 deep samples (CD and MLD) than superficial (CS and MLS) samples. Finally, we detected
370 *Funneliformis* only in MLS.

371

372 ***Community structure***

373 Community structure is always represented by two independent factors: the diversity and the
374 complexity of taxa present in each sample. Diversity indices (Taxa, Shannon, Simpson and Evenness),
375 which represent species richness and evenness, were calculated for both bacteria (Table 2) and fungi
376 (Table 3) in CS, CD, MLS and MLD. In addition, after the bioinformatics classification of amplicons
377 for both the 16S, ITS and the two communities analysed together, we reduced the dataset of each
378 biological replicate to a bidimensional scaling using a Bray-Curtis distance matrix and plotting the
379 results in corresponding non-metric multidimensional scaling (NMDS), as reported in Supplementary
380 Fig. 3. A cluster heatmap of 16S and ITS communities considered together is reported in Supplementary

Fig. 4. Moreover, co-occurrence analyses of 16S, ITS and the two communities analysed together are reported in Supplementary Figs. 5, 6 and 7. Statistical analyses of co-occurrence relationship are reported in Supplementary Tables 11, 12 and 13.

Wine sensory analyses

Berry and must features are reported in Table 4. Considering that the two vineyards have the same row and interrow spacing, there was higher productivity in the vineyard grown in C soil. On the other hand, the must chemical composition signalled a richer sugar content where the yield was lower (ML soil), combined with a lower level in total acidity. There were no differences when considering must YAN (yeast assimilable nitrogen).

The tasting results are reported in Fig. 5. The results show a good differentiation between the two wines. Regarding the olfactory scents, the wine obtained from grapes grown in C soil resulted was richer and generally more interesting, with higher scores for orange blossom and elegance, but also in terms of pleasantness and floral retro olfactory sensations. The wine obtained from grapes grown in ML soil was richer in ripe fruit notes such as melon, pear and citrus. In the mouth, there were no differences for sapidity, body and acidity.

Discussion

We aimed to investigate the possibility that not only the geographical distance, but also the soil geological characteristics are involved in the definition of the microbial composition. That factor can, together with the physiochemical characteristic of soil, impact wine typicity. Furthermore, we also considered that the vineyard is an anthropic environment; hence, we analysed both the superficial (anthropic disturbed) and deep (almost undisturbed) soils to understand how the microbial composition shifts along the profile. It is well known that one of the most important factors influencing the microbial composition associated with grapevine plants is the geographical region in which they are growing

406 (Berendsen et al. 2012; Mezzasalma et al. 2018; Coller et al. 2019). On the other hand, it is also well
407 known that soil microbes strongly influence the microbial composition of their hosts, playing an
408 important role in shaping the vine-associated microorganisms and the microbial community structures
409 (Manici et al. 2017; Nerva et al. 2019). For these reasons, we decided to analyse two close
410 geographically related soils with different geological characteristics: calcarenite and marly limestone
411 soil.

412 An interesting observation is the root ratio between thin versus medium and thick roots (rooting
413 index): ML soil displayed much higher rooting indexes than C soil for both distances (0.4 and 1 m) from
414 the vine. Based on this finding, we propose that ML soil conditions are more favourable for wide and
415 diffuse soil root exploration. Furthermore, it is interesting to observe that in the vertical root profile, C
416 soil displayed the highest root concentration in the 0–0.60-m soil depth for both trunk distances (140
417 roots/m^2 in total), suggesting an impairment in colonising the deep soil. In ML soil, the roots were able
418 to explore a wider soil area (from 0–0.8 m) because there was no physical resistance and a lower root
419 density, especially considering the closer trunk distance (0.40 m). In addition, for both soil types in the
420 deeper layers (0.80–1.0 m), the root density decreased significantly. Considering the deeper soil level,
421 both soils have the same humidity in spring, but as the seasons progress, the differences between soils
422 are more and more evident. This different behaviour of soil to retain humidity markedly influences the
423 root distribution and density. Indeed, water scarcity boosts root growth and diffusion, phenomena that
424 our data confirmed, namely more medium roots close to the vine trunk and thicker roots far from the
425 vine trunk in C soil.

426 As a first, general consideration of the bacterial community, we found that, as previously
427 reported by other researchers (Zarraonaindia et al. 2015; Coller et al. 2019), the superficial layer displays
428 quite similar bacterial features, with some minor differences between the two geographically related
429 sites. Interestingly, this pattern seems to disappear completely in the deep samples: the bacterial analysis
430 showed 123 differentially abundant genera between the two soil types. This result is quite interesting
431 because the majority of papers have always looked at the superficial layer, which is highly perturbed by

human activities, agricultural residues and climatic features. Of note, this pattern seems to be the complete opposite for the fungal community: the two superficial soil types displayed 55 differentially abundant genera, but there were only two differentially abundant genera for the deep soil types.

It is worth noting that the wines produced by the two vineyards displayed different organoleptic characteristics: the wine produced from grapes grown in C soil showed a higher score in terms of appreciation for its complexity in terms of olfactory sensory and pleasantness, while the wine from grapes grown in ML soil displayed different olfactory scents. Furthermore, the yield and quality of berries showed minor differences, with a significantly higher sugar content in wine from grapes grown in ML soil and a significantly higher acidity level for must for wine from grapes grown in C soil. Accordingly, plant microbiomes can widely impact wine features, and this aspect is well described by the microbial *terroir* concept (Van Leeuwen and Seguin 2006; Gilbert et al. 2014; Knight et al. 2015). Based on our results, we can speculate that, although the chemical composition of the two soil types is quite different and impacts the organoleptic qualities of wines, the different soil microbial composition can play a part by affecting quality features in light of the soil–plant *continuum*. For example, the presence of different mycorrhizal species between the two soil types can result in different physiological and biochemical modulation of the plant metabolism. Mycorrhizal fungi are known to play crucial ecological services, for example, the enhancement of the plant nutritional status (both for water and minerals) and the induction of the priming state triggering growth-defence tradeoff responses (Balestrini and Lumini 2018; Alagna et al. 2020). From this perspective, it is also well known that some mycorrhizal species can differentially impact plant behaviours (Volpe et al. 2018). Thus, in this experiment, the presence of different mycorrhizal species can impact the grape behaviour, inducing physiological and biochemical adjustment between the two selected sites.

The main factors that are considered to drive plant biochemical responses are: i) the ecology of plant-associated microbes, ii) viticulture management and iii) environmental conditions. The impact(s) on grape metabolomics and wine flavour is likely due to complex interactions between them that need further investigation to be dissected. In this respect, a recent work (Vadakattu et al. 2019) clearly

458 demonstrated that fungal and bacterial communities analysed in different soils are associated with
459 diverse levels of rotundone concentration in grape berries. In detail, the authors demonstrated that the
460 microbial structure and the well-connected soil bacterial community co-occurrence networks were
461 linked to the high rotundone areas with respect to lower ones. In addition, a recent work highlighted
462 how the soil fungal communities of Pinot noir cv. played the principal role in shaping wine aroma
463 profiles and regional distinctiveness more than soil or climatic features (Liu et al. 2020). These findings
464 further support the strong connection between soil microbial communities and plant performance,
465 including fruit production yield and quality characteristics.

466 The microbial *continuum* between soil and plants can have also detrimental effects, favouring
467 colonisation by fungal pathogens (Manici et al. 2017; Nerva et al. 2019). Therefore, we decided to
468 examine wood pathogens, which can penetrate through trunk wounds (especially the ones caused by
469 agricultural practices and that are close to the soil surface) and through the root system, causing plant
470 illness (Whiteman et al. 2003; Giménez-Jaime et al. 2006; Aroca et al. 2010; Gramaje and Armengol
471 2011). In C soil, two wood pathogen genera (*Neofusicoccum* and *Phaeoacremonium*) are significantly
472 overrepresented in the 120-cm depth sample (both > 4 times more abundant in the deep soils than in the
473 superficial one), whereas in ML soil, only *Neofusicoccum* is overrepresented in the 120-cm depth soil
474 (about 3.5 times more abundant than in superficial sample). This result is quite interesting because, as
475 previously reported, the accumulation of fungal pathogens in vineyards occurs i) after long term
476 cultivation (Manici et al. 2017) and ii) where infected plants are present (Nerva et al. 2019). On the
477 contrary, the *Ilyonectria* genus, which is associated with black foot disease (Cabrala et al. 2012), is more
478 abundant in the superficial samples of both soil types; this phenomenon represents an issue for the plant
479 health status and for young plants, which are routinely used as replacement for died and/or compromised
480 vines (Agustí-Brisach et al. 2014). Interestingly, in the analysed vineyards, we did not observe any sign
481 of esca or black foot diseases, suggesting that the complexity of the plant-pathogen-environment
482 interaction does not lead to symptom development.

We also detected some genera of fungal and bacterial plant symbionts. We first focused on mycorrhizal fungi, which have a relevant impact due to the involvement in complex interactions with plants (Balestrini and Lumini 2018). They are able to induce physiological changes such as the resistance induction against biotic (Pozo et al. 2013; Volpe et al. 2018; Alagna et al. 2020) and abiotic (Balestrini et al. 2017; Balestrini et al. 2018; Mannino et al. 2020) stresses, which fall into the so-called mycorrhiza induced resistance (MIR) (Pozo and Azcón-Aguilar 2007; Jung et al. 2012; Cameron et al. 2013). The *Glomus* genus is more abundant in both CD and MLD, suggesting that the higher proportion of fine roots found in the deep layers are the ones more active in recruiting microbial symbionts. It is worth noting that *Glomus* species are often associated with an enhanced tolerance to salinity and water deficit stresses (Fileccia et al. 2017; Harshavardhan and Kumar 2018; Zhang et al. 2018), suggesting that this genus can play important roles for vine adaptation to the semi-arid Mediterranean areas. In parallel, *Funneliformis* and *Rhizophagus* genera, which are well-known plant symbionts (Chitarra et al. 2017), are significantly overrepresented in the MLS compared with MLD and C samples. This result is consistent with the root distribution, where in the ML vineyard we observed wide root diffusion up to 80 cm form the surface and then a strong reduction in root density. Moreover, we also observed a significant difference for the fine roots (< 3 mm diameter), which are more abundant in ML than in C soil, allowing the establishment of more symbioses.

Flavobacteriaceae, which comprises the *Flavobacterium* genus (among others), is a ubiquitous family of bacteria, commonly found in agricultural soils, and is often associated with positive features such as the ability to degrade pesticides (Nayarisseri et al. 2015; Parte et al. 2017) and the ability to induce resistance against fungal and bacterial pathogens in several crops (Kolton et al. 2016; Kwak et al. 2018). We observed an overrepresentation of the above-mentioned family and genus in both CD and MLD, suggesting that this group of microbes can also play important role in protecting plants against pathogens. Of note, the *Bacillus* genus, which also encompasses several species that exert beneficial effects on plants (Fendrihan et al. 2016; Shafi et al. 2017), is differentially represented in the two soil types. For C soil, we observed a significant accumulation of such genus in the superficial samples,

509 whereas for ML soil, it overaccumulates in the deep layer. This result suggests different soil propensities
510 to host this genus, and this eventuality deserves further investigation.

511 We also aimed to enrich the knowledge about the soil microbiome because it is a frontier
512 research field. Crop losses due to plant pathogens are an ever-increasing threat for agricultural
513 production. While food demands increase, there is a compelling need to reduce the use of
514 environmentally harmful pesticides and agrochemicals (Pennock et al. 2015). To initiate more
515 sustainable agriculture, manipulation of microbiomes associated with plants and soils has been
516 suggested as a reliable alternative for impairing pathogens development and to obtain suppressive
517 conditions (Raaijmakers and Mazzola 2016; Fierer 2017; Trivedi et al. 2020). However, we still need
518 to unravel how the complex interactions among plant, pathogen and soil features determine the
519 development of plant diseases under field conditions. In this respect, researcher provisions about all the
520 drivers involved in determine the assembly of the host-associated microbiome, such as the genetic
521 background of the pathogen and the host and the presence of biotic and abiotic stresses, are decisive to
522 decipher the outcome of plant-pathogen interactions (Kwak et al. 2018). Among all the field drivers, it
523 was recently demonstrated that the initial soil microbiome composition can predetermine whether plants
524 are able to survive or succumb to diseases (Wei et al. 2019). Considering these data, the descriptions of
525 soil microbiomes are of pivotal importance to develop new agricultural strategies and to achieve the
526 needs of more sustainable viticulture.

527

528 **Conclusions**

529 In this study, we have highlighted that the geological characteristics of soil can impact both plant
530 root development and soil microbial composition and assemblage in the different soil layers with a final
531 effect on wine features (summarised in Fig. 6). The grapevine is regarded as one of the most impactful
532 crop species in terms of environmental sustainability, and thus the soil microbial composition can play
533 a pivotal role in enhancing the ability of vines to cope with biotic and abiotic stresses. The ability to
534 exploit the native microbial diversity of soils will became one of the most important agricultural

practices in the future; hence, describing the microbial species associated to the semi-arid Mediterranean environment has become essential. Furthermore, the soil-feature-mediated root distribution play also important role for the plant water availability, because the possibility to explore the deep layers increase the possibility to exploit tasks of soil where the water is more available. This is an additional detail to take into account when considering a soil for the successful establishment of a vineyard, especially if it is located in semi-arid regions where water availability is limited and its use in agriculture plays a fundamental role in defining the sustainability. To date, it is still impossible, at least in a natural environment, to define the contribute of the single feature (soil characteristics, microbial composition, physiochemical parameters, etc.) on the plant performances and for this reason we can only suppose that the differences observed between the two vineyards are due to the combination of such interacting parameters. Due to the potential biotechnological application of microbes in agriculture, additional studies to link the soil microbial composition and the wines features are still ongoing.

Declarations

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Conflicts of Interests

The authors declare that they have no competing interests.

Ethics approval and consent to participate

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

The SRA accession numbers of the NGS reported in this paper are deposited in NCBI under the BioProject PRJNA655455; BioSample SAMN15735114 and SAMN15735115; SRA accession SRR12436974 and SRR12436973.

Code availability

Not applicable.

Author contributions

L.N. and W.C. designed the experimental system, carried out the wet lab experiments, analysed data and wrote the manuscript draft. D.T., L.N. and W.C. worked out vine roots system. D.T. analysed wines sensory description. D.T., A.G, L.M. and G.G. helped to design the experiments, contributed to the writing and carefully revised the manuscript.

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548

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FIGURE LEGENDS

Figure 1. Relative abundances of bacterial families (A) and genera (B) among soil samples. CS: calcarenite superficial; CD: calcarenite 120 cm deep; MLS: marly-limestone superficial; MLD: marly-limestone 120 cm deep. Only orders or genera representing at least the 1% over the total number of classified amplicons were retained (n=3).

Figure 2. Relative abundances of fungal families (A) and genera (B) among soil samples. CS: calcarenite superficial; CD: calcarenite 120 cm deep; MLS: marly-limestone superficial; MLD: marly-limestone 120 cm deep. Only orders or genera representing at least the 1% over the total number of classified amplicons were retained (n=3).

Figure 3. Relative abundances of the main trunk pathogens among soil samples. CS: calcarenite superficial; CD: calcarenite 120 cm deep; MLS: marly-limestone superficial; MLD: marly-limestone 120 cm deep (n=3).

Figure 4. Relative abundances of the main mycorrhizal fungi soil samples. CS: calcarenite superficial; CD: calcarenite 120 cm deep; MLS: marly-limestone superficial; MLD: marly-limestone 120 cm deep (n=3).

Figure 5. Average wine quality features determined by the tasting profile. Asterisks denote significant differences according to ANOVA, * = $p < 0.05$ and ** = $p < 0.01$.

Figure 6. Summary of the main differences in terms of wine features, root index and microbiome composition observed between the two soil types. In the centre top part differences among aromas and root index are highlighted. Downstream, in the centre of the figure are reported the microbial differences for both bacterial and fungi occurring between C and ML at 15 cm and 120 cm depth from the soil surface. Mycorrhizal genera are reported in C or ML where they are more abundant in respect to the other samples (either soil type or layer). Green up-facing arrows means a higher score/value whereas red down-facing arrows means lower score/value (left arrows refer to C soil whereas right arrows refer to ML soil).

SUPPLEMENTARY FIGURE LEGENDS

Figure S1. PCoA representing the four soil samples analysed for 16S sequences (n=3).
Figure S2. NMDS algorithm based on Bray-Curtis distances matrixes were used to reduce into a bi-dimensional scaling data obtained for bacteria (A), fungi (B) and the overall microbial community (C) (n=3).
Figure S3. Cluster heatmap built using data of both 16S and ITS amplicon sequencing to highlight relationship among samples.
Figure S4. Significant co-occurrence network of bacterial communities and interactions among OTUs in soil samples. The nodes are sized according to degree of connection (Supplementary Table 11). Green lines indicate that there are co-occurrence interactions and red lines indicate mutualistic exclusions (n=3).
Figure S5. Significant co-occurrence network of fungal communities and interactions among OTUs in soil samples. The nodes are sized according to degree of connection (Supplementary Table 12). Green lines indicate that there are co-occurrence interactions and red lines indicate mutualistic exclusions (n=3).
Figure S6. Significant co-occurrence network of both bacterial and fungal communities considered together and interactions among OTUs in soil samples. The nodes are sized according to degree of

790 connection (Supplementary Table 13). Green lines indicate that there are co-occurrence interactions and
791 red lines indicate mutualistic exclusions (n=3).

TABLES

Table 1. Root density of small (< 1 mm), medium (1 - 3 mm) and permanent (> 3 mm) roots for marly limestone (ML) and calcarenite (C) soils at 0.4 and 1 meter from the trunk. Asterisks indicate statistical significance as attested by Student-Newman-Keuls test ($p < 0.05$) (n=3).

Distance from vine trunk	Treatment	Root density (number of roots/m^2 profile wall/root size)			Root density (number of roots/m2 profile wall)
		Ø < 1 mm	Ø 1 - 3 mm	Ø > 3 mm	
0.4 m	**ML**	81	26 b	15 b	121 b
	C	72	57 a	34 a	164 a
	Significance	ns	**	**	**
1 m	**ML**	123 a	30	14 b	168 a
	C	85 b	36	28 a	149 b
	Significance	*	ns	**	*

800 **Table 2**. Richness estimators and diversity indices for bacterial (16S) communities sampled in the four different soil types. Statistical ANOVA was
801 conducted to detect significant differences, different letter in each row means significant differences according to Tukey's HSD test (n=3).
802

	CS				CD				MLS				MLD			
	Av.		SD		Av.		SD		Av.		SD		Av.		SD	
Taxa	133.33	±	0.575	b	137.667	±	0.577	c	131.667	±	0.577	a	132.000	±	0.000	a
Simpson	0.970	±	0.002	a	0.955	±	0.002	a	0.964	±	0.002	b	0.974	±	0.001	c
Shannon	3.985	±	0.038	c	3.727	±	0.018	a	3.900	±	0.033	b	4.062	±	0.032	c
Evenness	0.404	±	0.017	b	0.302	±	0.006	a	0.375	±	0.012	b	0.440	±	0.014	c

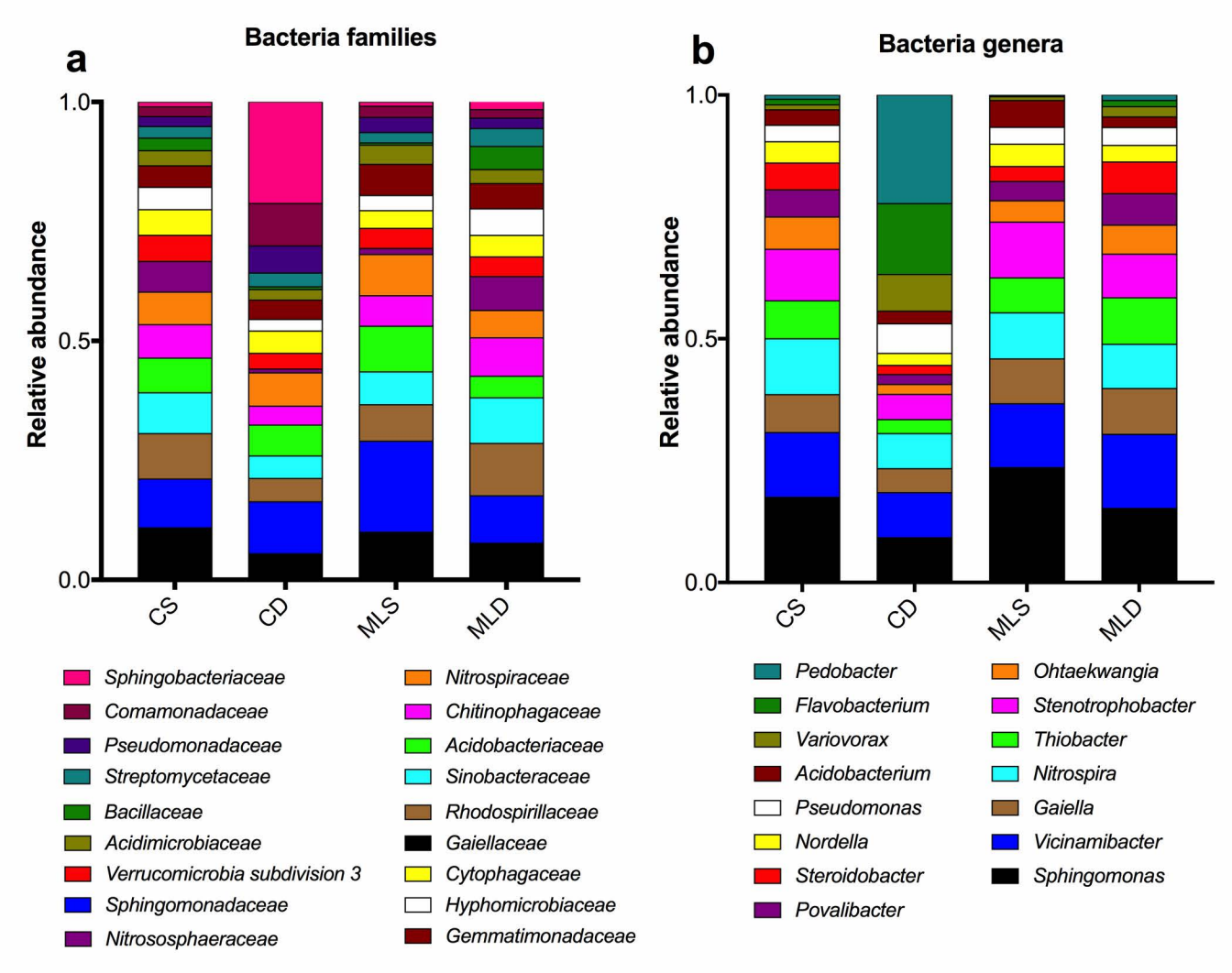
803
804

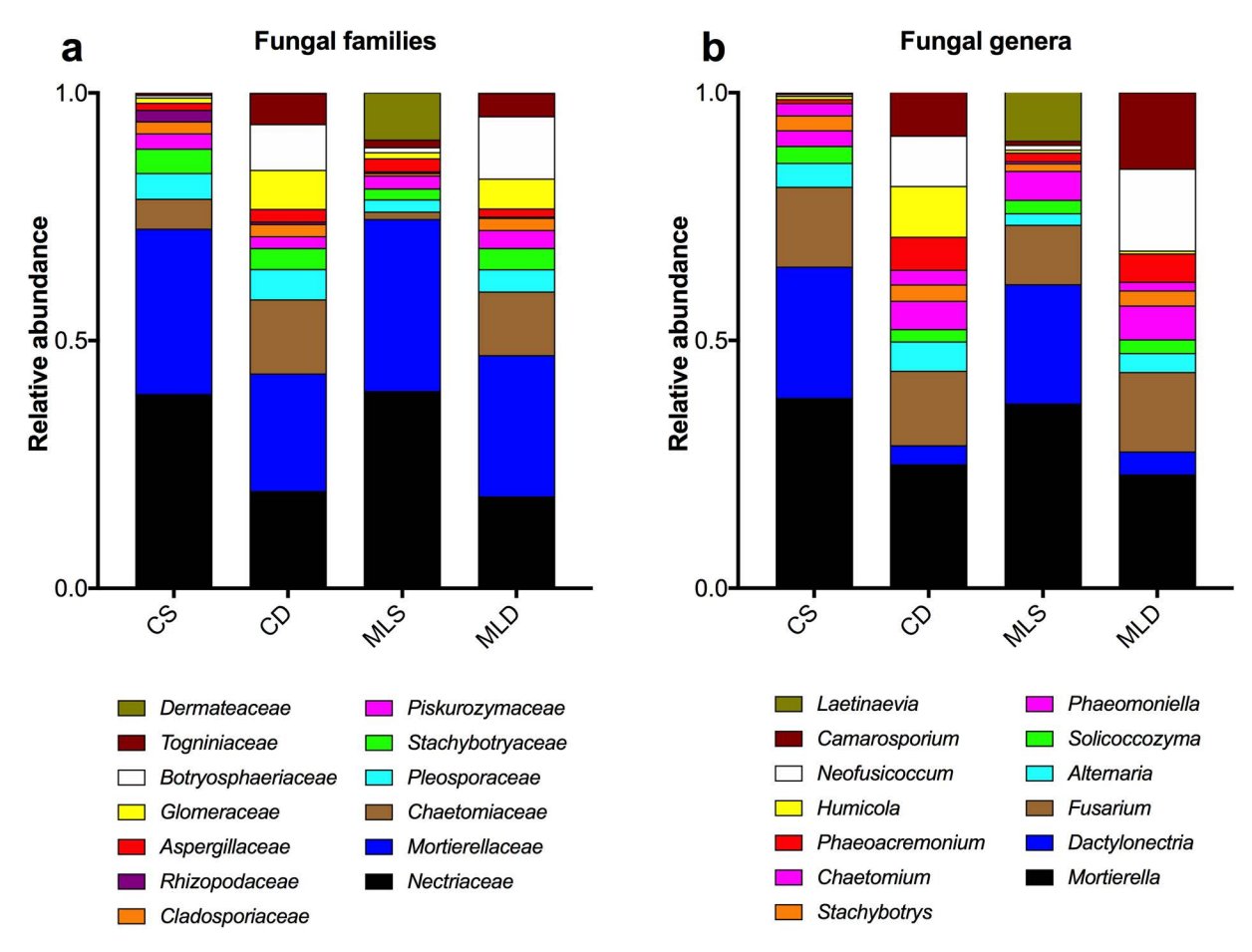
Table 3. Richness estimators and diversity indices for fungal (ITS) ommunities sampled in the four different soil types. Statistical ANOVA was conducted to detect significant differences, different letter in each row means significant differences according to Tukey's HSD test (n=3).

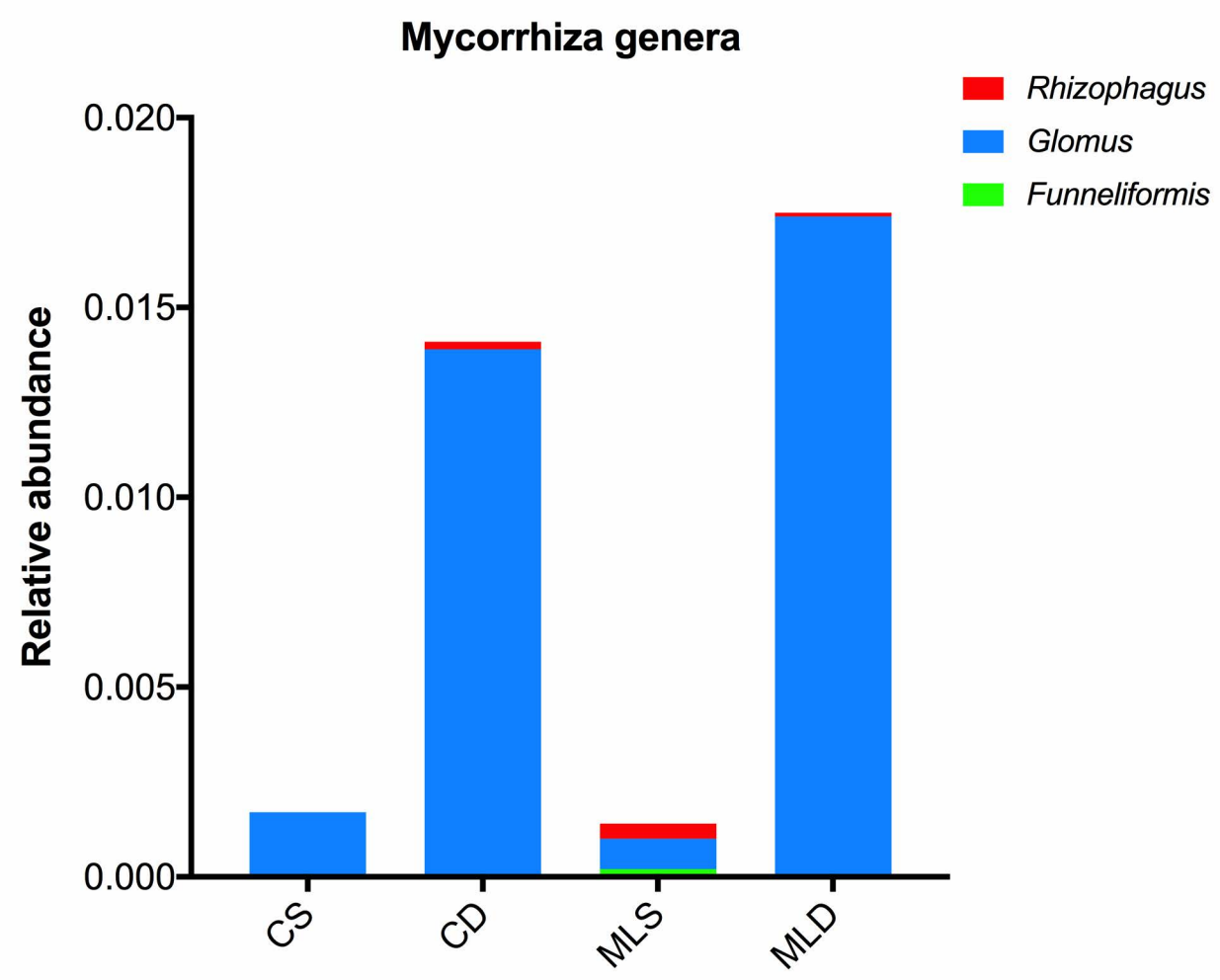
	CS			CD			MLS			MLD		
	Av.	SD		Av.	SD		Av.	SD		Av.	SD	
Taxa	122.67 ±	3.21	c	99.00 ±	0.00	a	119.33 ±	2.31	c	105.00 ±	1.73	b
Simpson	0.86 ±	0.01	a	0.92 ±	0.00	c	0.86 ±	0.00	a	0.90 ±	0.00	b
Shannon	2.77 ±	0.06	ab	3.02 ±	0.01	c	2.66 ±	0.09	a	2.93 ±	0.04	bc
Evenness	0.13 ±	0.01	a	0.21 ±	0.00	c	0.12 ±	0.01	a	0.18 ±	0.00	b

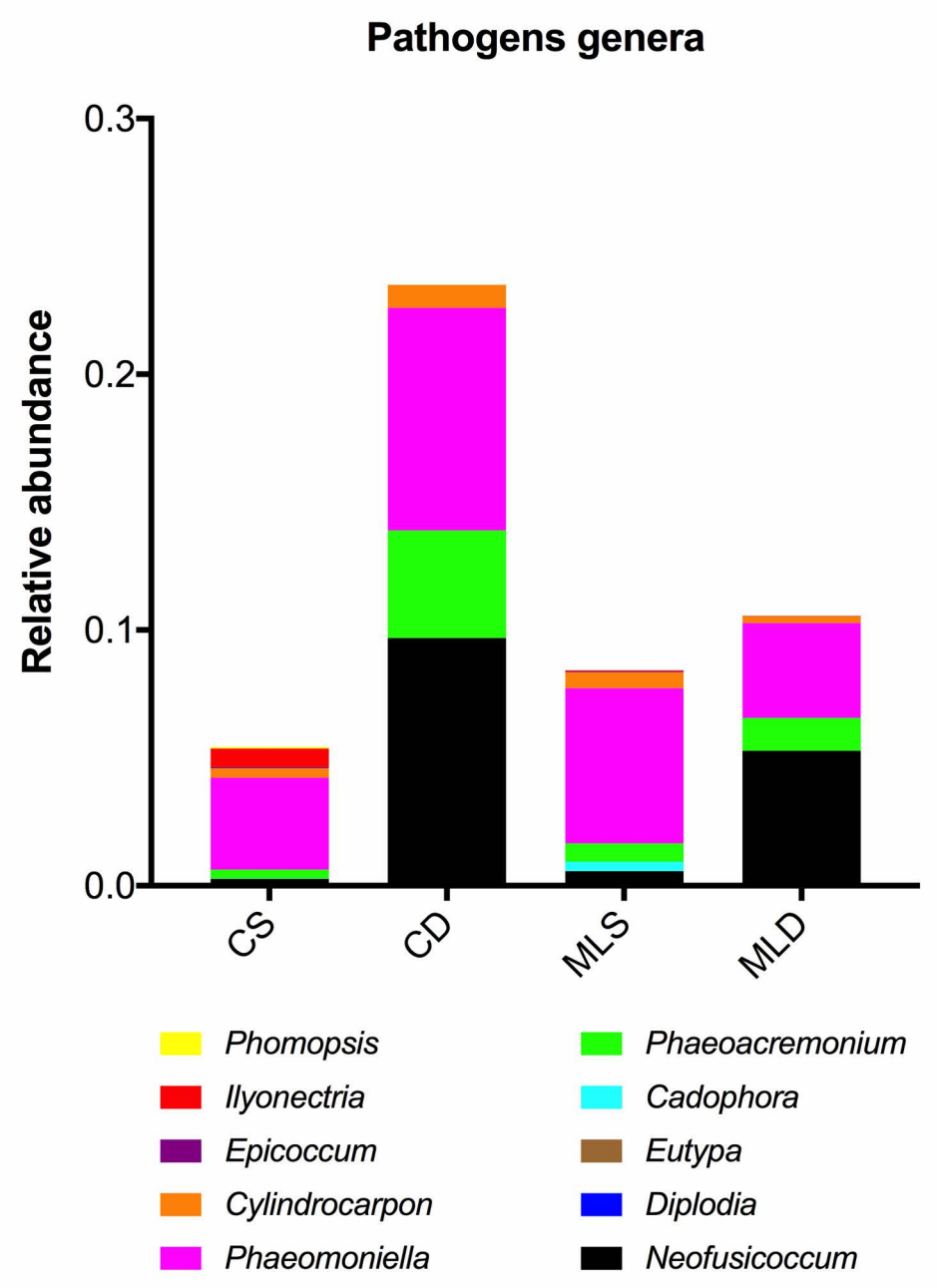
Table 4. Single plant productivity and grape chemical composition for the two vineyards analysed in 2017.

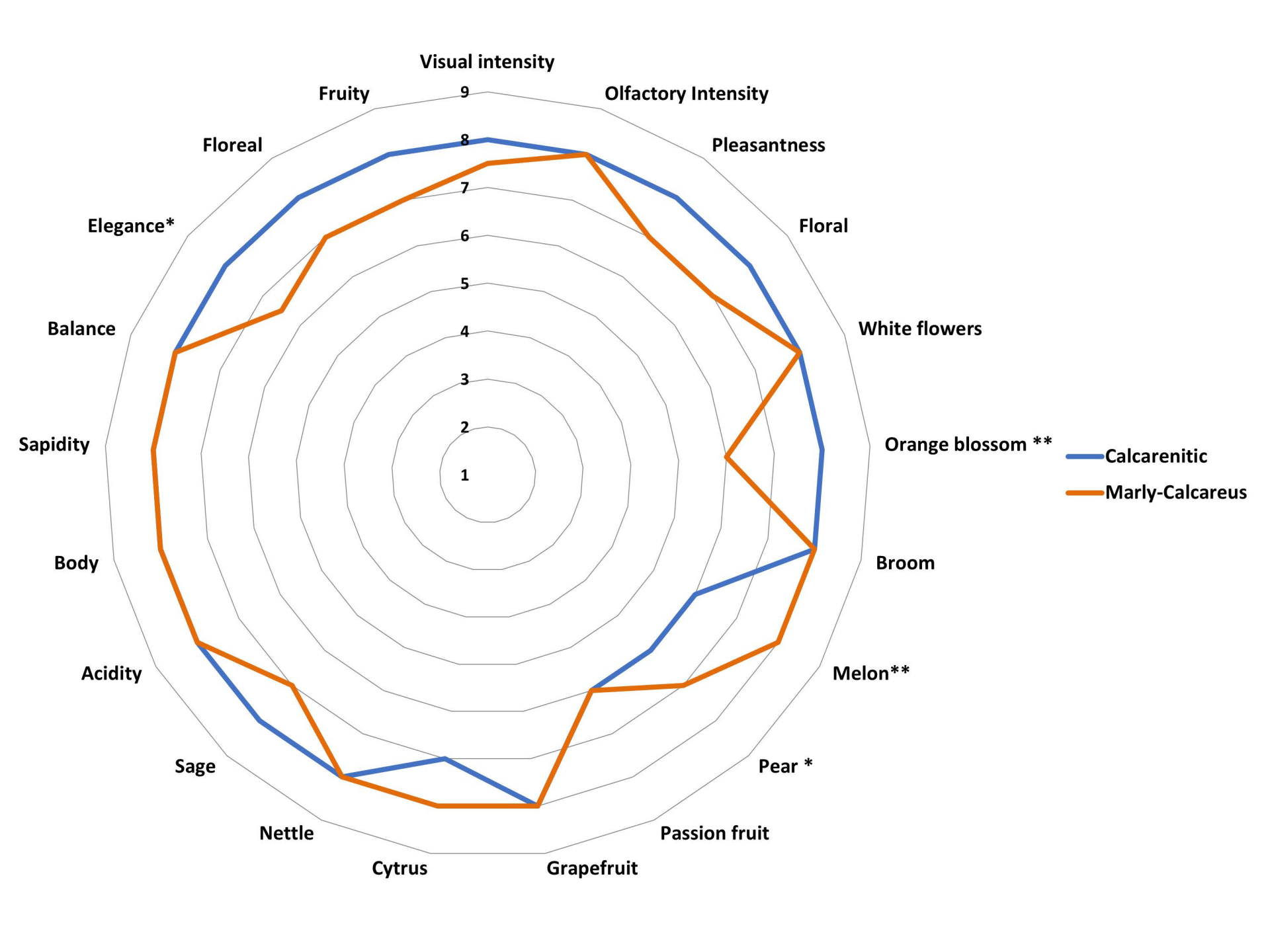
	C		ML		*P*
	Av.	St.Dev	Av.	St.Dev	
Vine yield (kg)	3,4	± 0,57	2,5	± 0,40	*
Bunch weight (g)	197	± 54,00	134	± 46,10	
Berry weight (g)	3,04	± 0,09	2,13	± 0,11	**
Winter wood (vine/kg)	0,87	± 0,33	0,67	± 0,17	
Sugars (°Babo)	16,6	± 0,70	18,1	± 0,65	*
Total Acidity (g/L)	7,37	± 0,33	6,8	± 0,21	*
pH	3,08	± 0,15	3,13	± 0,12	
Malic Acid (g/L)	2,43	± 0,21	1,8	± 0,24	**
Tartaric Acid (g/L)	8,27	± 0,35	7,1	± 0,41	*
Yeast assimilable nitrogen (YAN)	244	± 23,00	219	± 19,00	


a
Bacteria families
Relative abundance
1.0
0.5
0.0
CS
CD
MLS
MLD
Sphingobacteriaceae
Comamonadaceae
Pseudomonadaceae
Streptomycetaceae
Bacillaceae
Acidimicrobiaceae
Verrucomicrobia subdivision 3
Sphingomonadaceae
Nitrososphaeraceae
Nitrospiraceae
Chitinophagaceae
Acidobacteriaceae
Sinobacteraceae
Rhodospirillaceae
Gaiellaceae
Cytophagaceae
Hyphomicrobiaceae
Gemmatimonadaceae
b
Bacteria genera
Relative abundance
1.0
0.5
0.0
CS
CD
MLS
MLD
Pedobacter
Flavobacterium
Variovorax
Acidobacterium
Pseudomonas
Nordella
Steroidobacter
Povalibacter
Ohtaekwangia
Stenotrophobacter
Thiobacter
Nitrospira
Gaiella
Vicinamibacter
Sphingomonas

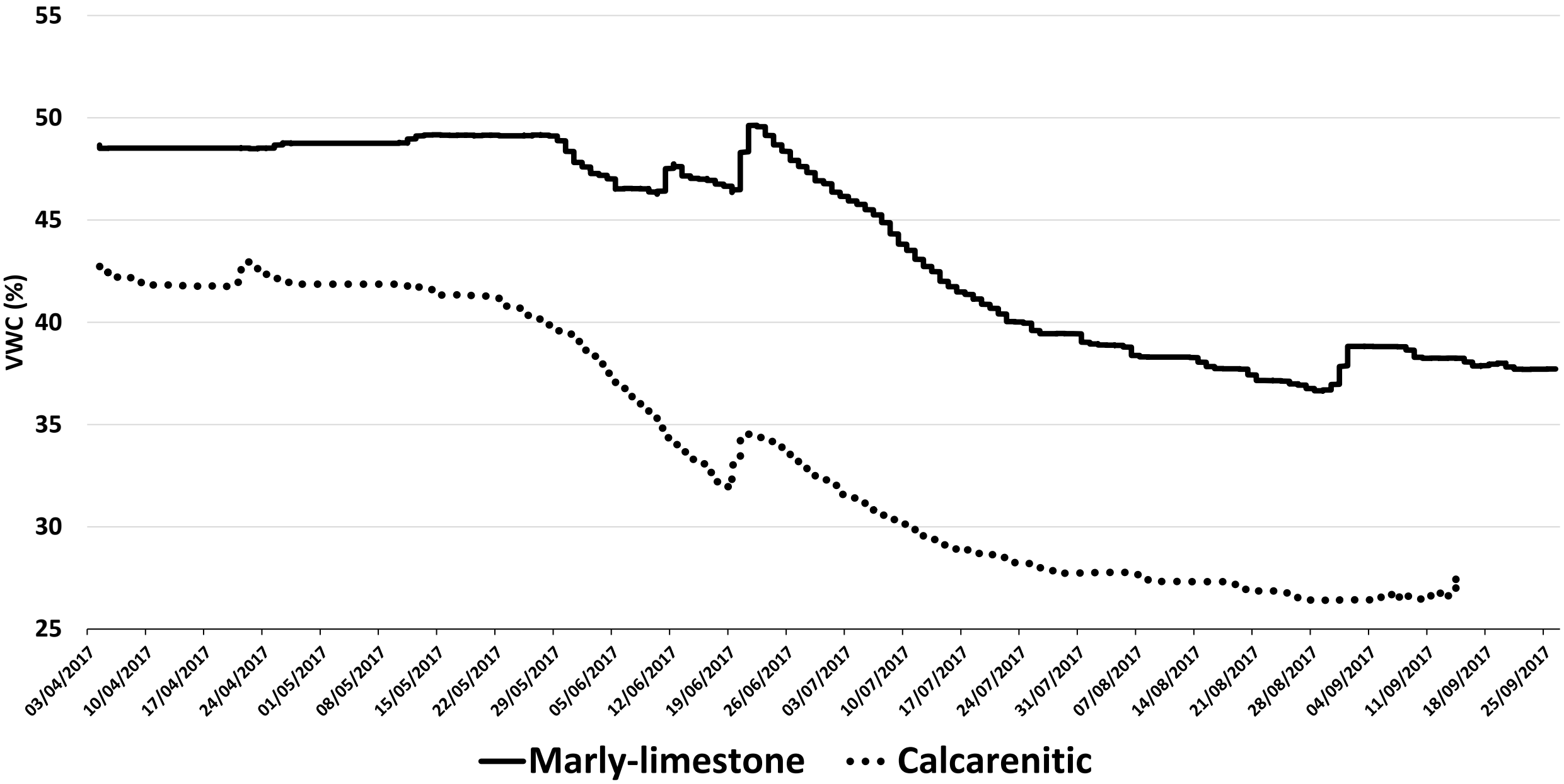

a
Fungal families
Relative abundance
1.0
0.5
0.0
CS
CD
MLS
MLD
Dermateaceae
Togniniaceae
Botryosphaeriaceae
Glomeraceae
Aspergillaceae
Rhizopodaceae
Cladosporiaceae
Piskurozymaceae
Stachybotryaceae
Pleosporaceae
Chaetomiaceae
Mortierellaceae
Nectriaceae
b
Fungal genera
Relative abundance
1.0
0.5
0.0
CS
CD
MLS
MLD
Laetinaevia
Camarosporium
Neofusicoccum
Humicola
Phaeoacremonium
Chaetomium
Stachybotrys
Phaeomoniella
Solicoccozyma
Alternaria
Fusarium
Dactylonectria
Mortierella


Mycorrhiza genera
Rhizophagus
Glomus
Funneliformis
Relative abundance
0.020
0.015
0.010
0.005
0.000
CS
CD
MLS
MLD

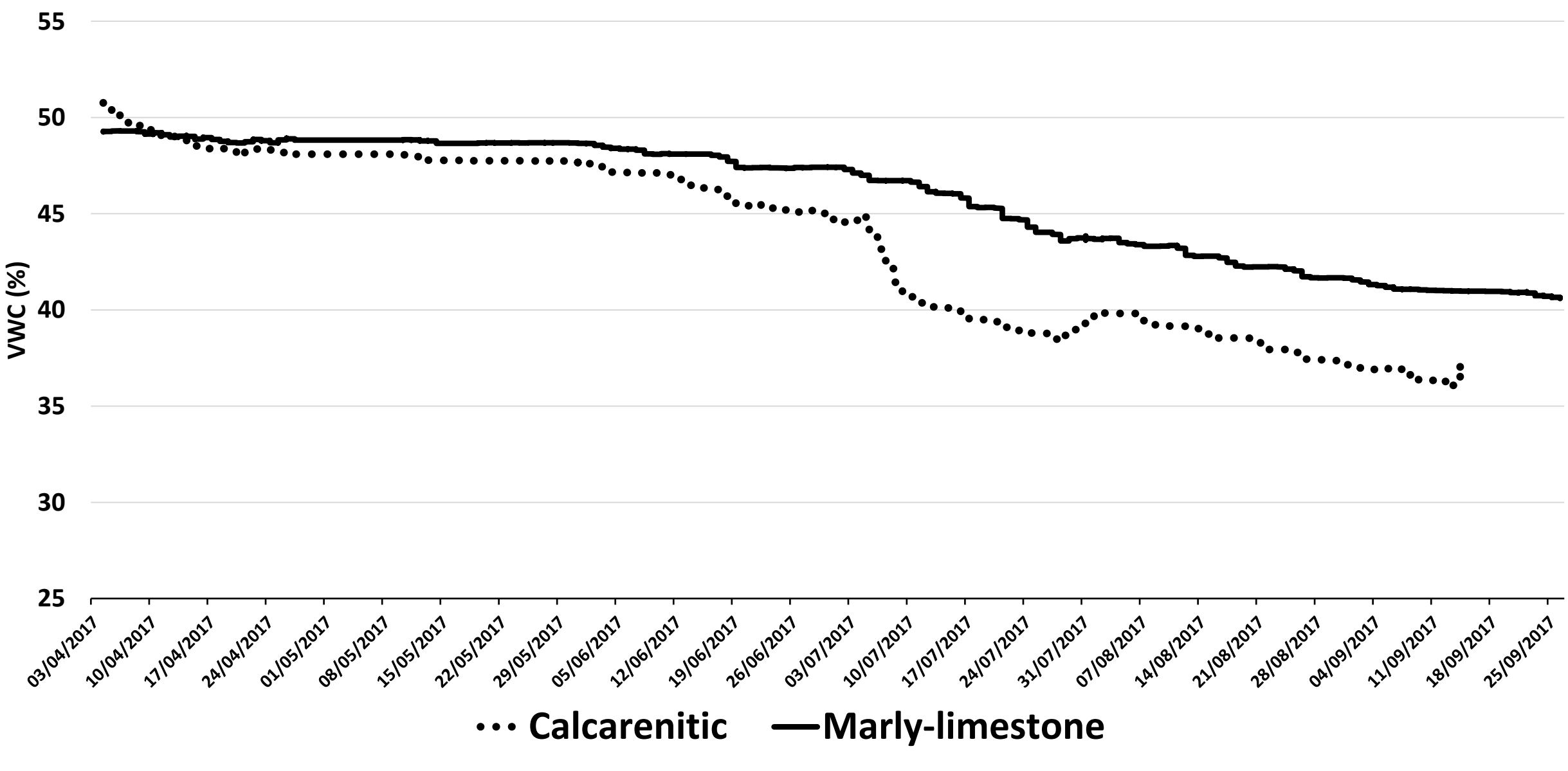

Pathogens genera
Relative abundance
0.3
0.2
0.1
0.0
CS
CD
MLS
MLD
Phomopsis
Ilyonectria
Epicoccum
Cylindrocarpon
Phaeomoniella
Phaeoacremonium
Cadophora
Eutypa
Diplodia
Neofusicoccum

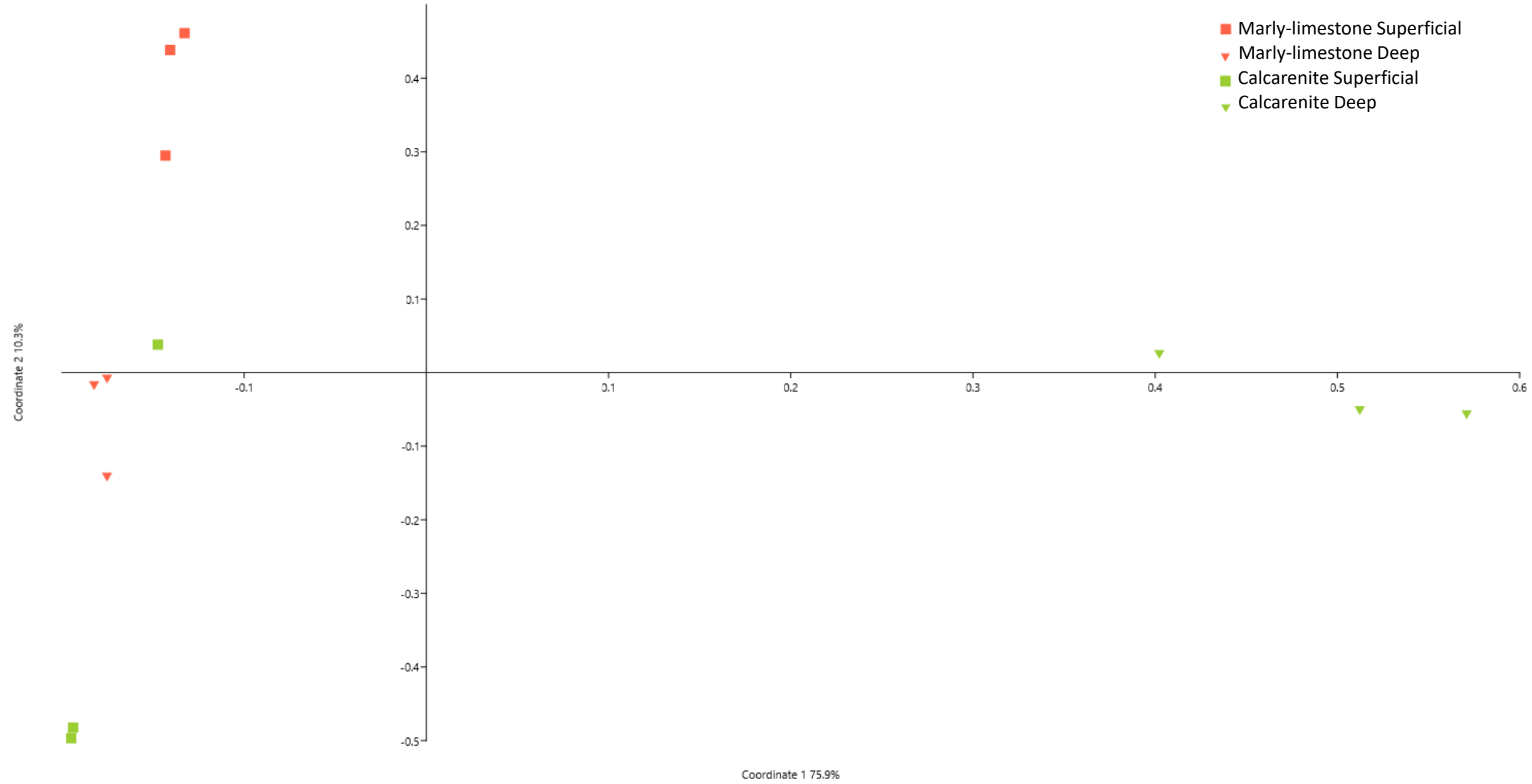

Visual intensity
Olfactory Intensity
Pleasantness
Floral
White flowers
Orange blossom **
Broom
Melon**
Pear *
Passion fruit
Grapefruit
Cytrus
Nettle
Sage
Acidity
Body
Sapidity
Balance
Elegance*
Floreal
Fruity
9
8
7
6
5
4
3
2
1
Calcarenitic
Marly-Calcareus

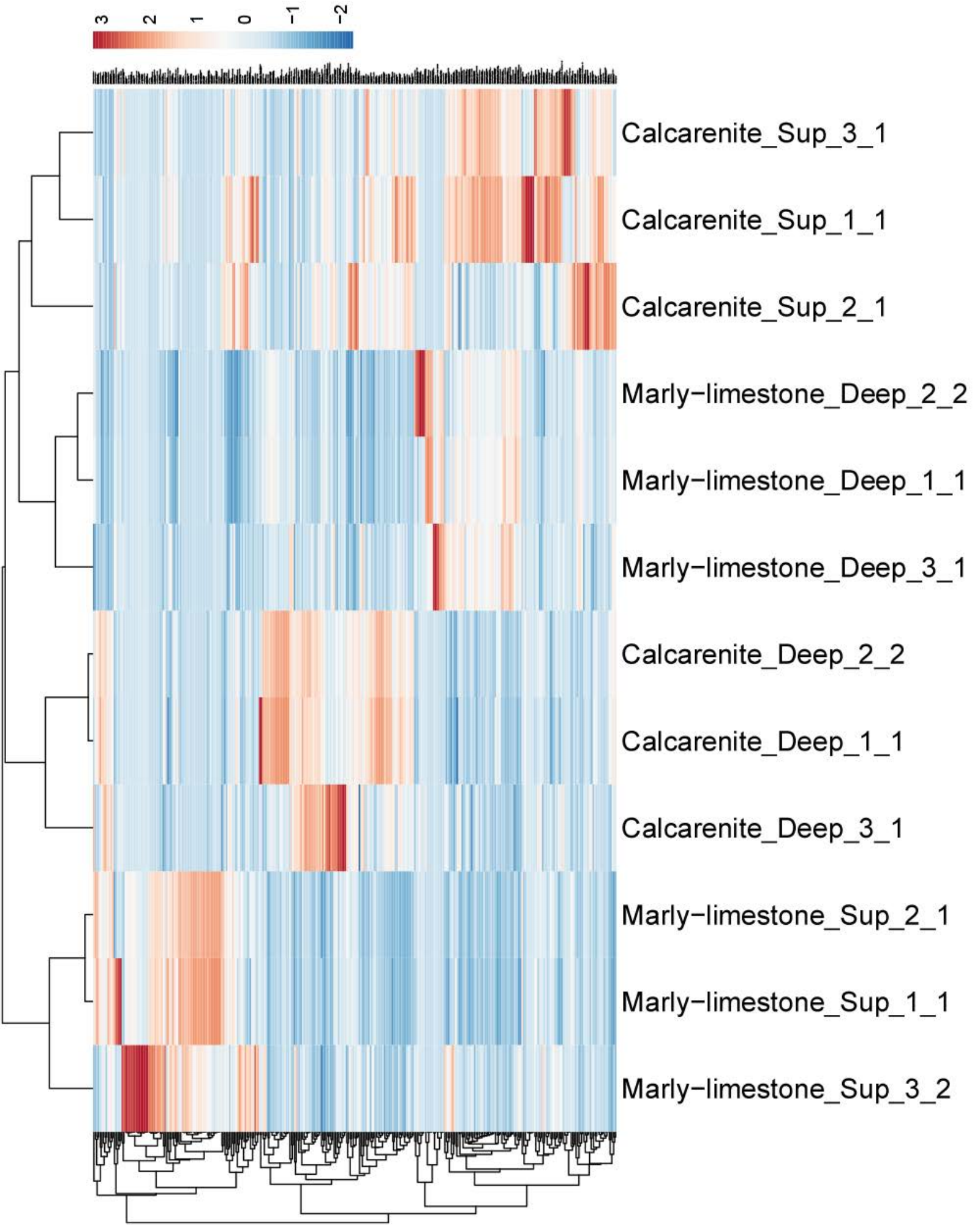
A


55
50
45
40
35
30
25
VWC (%)
03/04/2017
10/04/2017
17/04/2017
24/04/2017
01/05/2017
08/05/2017
15/05/2017
22/05/2017
29/05/2017
05/06/2017
12/06/2017
19/06/2017
26/06/2017
03/07/2017
10/07/2017
17/07/2017
24/07/2017
31/07/2017
07/08/2017
14/08/2017
21/08/2017
28/08/2017
04/09/2017
11/09/2017
18/09/2017
25/09/2017
Marly-limestone
Calcarenitic

B


55
50
45
40
35
30
25
VWC (%)
03/04/2017
10/04/2017
17/04/2017
24/04/2017
01/05/2017
08/05/2017
15/05/2017
22/05/2017
29/05/2017
05/06/2017
12/06/2017
19/06/2017
26/06/2017
03/07/2017
10/07/2017
17/07/2017
24/07/2017
31/07/2017
07/08/2017
14/08/2017
21/08/2017
28/08/2017
04/09/2017
11/09/2017
18/09/2017
25/09/2017
Calcarenitic
Marly-limestone


Marly-limestone Superficial
Marly-limestone Deep
Calcarenite Superficial
Calcarenite Deep
Coordinate 2 10.3%
Coordinate 1 75.9%
0.4
0.3
0.2
0.1
-0.1
-0.2
-0.3
-0.4
-0.5
-0.1
0.1
0.2
0.3
0.4
0.5
0.6


3
2
1
0
−1
−2
Calcarenite_Sup_3_1
Calcarenite_Sup_1_1
Calcarenite_Sup_2_1
Marly−limestone_Deep_2_2
Marly−limestone_Deep_1_1
Marly−limestone_Deep_3_1
Calcarenite_Deep_2_2
Calcarenite_Deep_1_1
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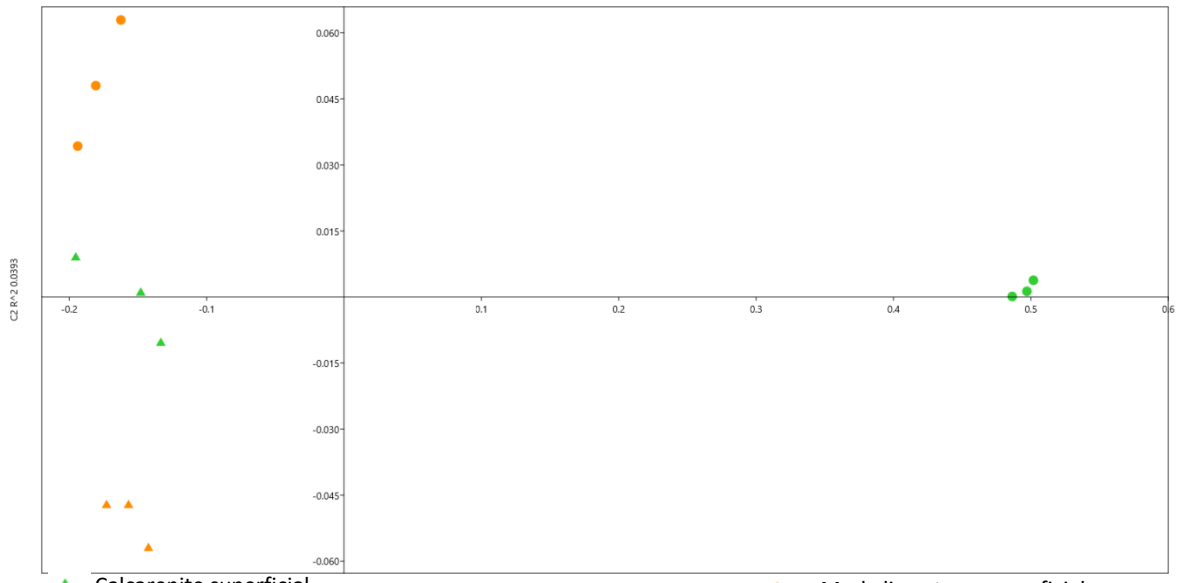
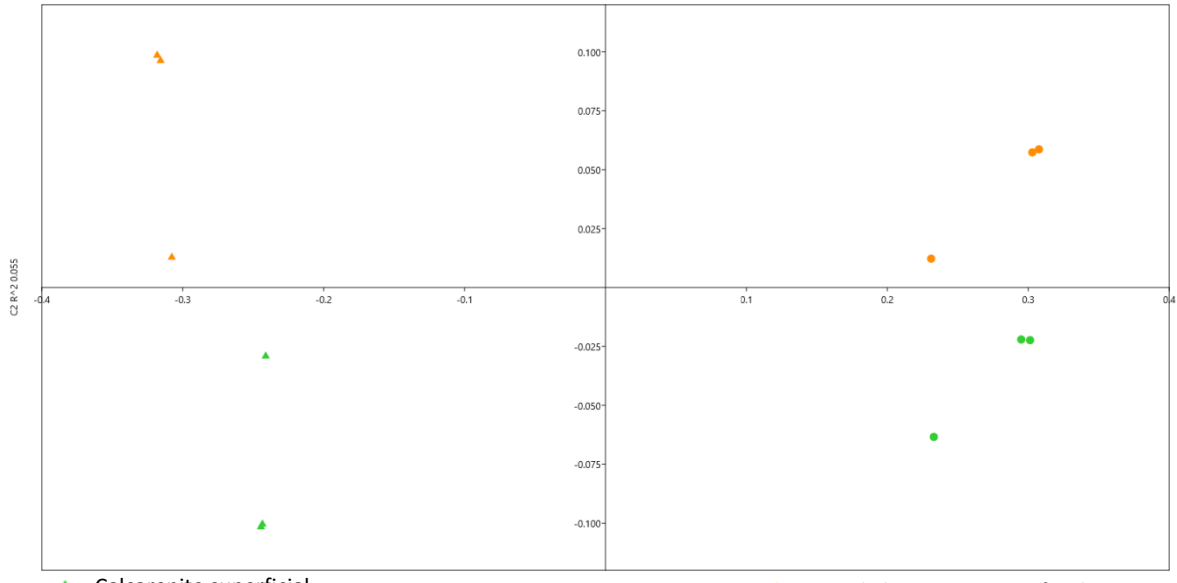
Marly−limestone_Sup_3_2

A



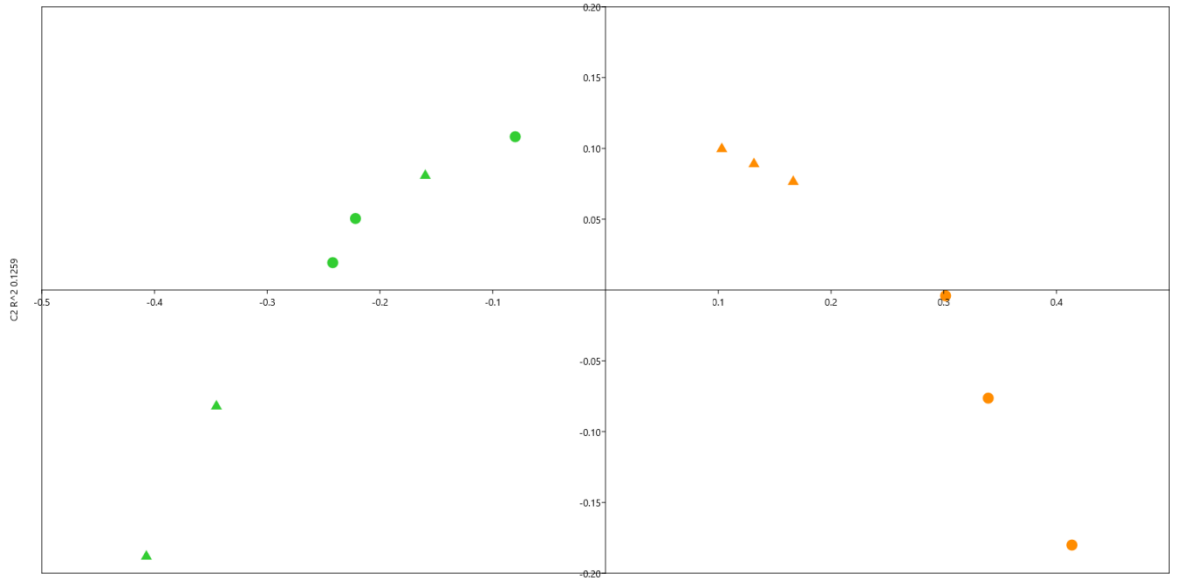
Calcarenite superficial
Calcarenite deep
Marly limestone superficial
Marly limestone deep

B

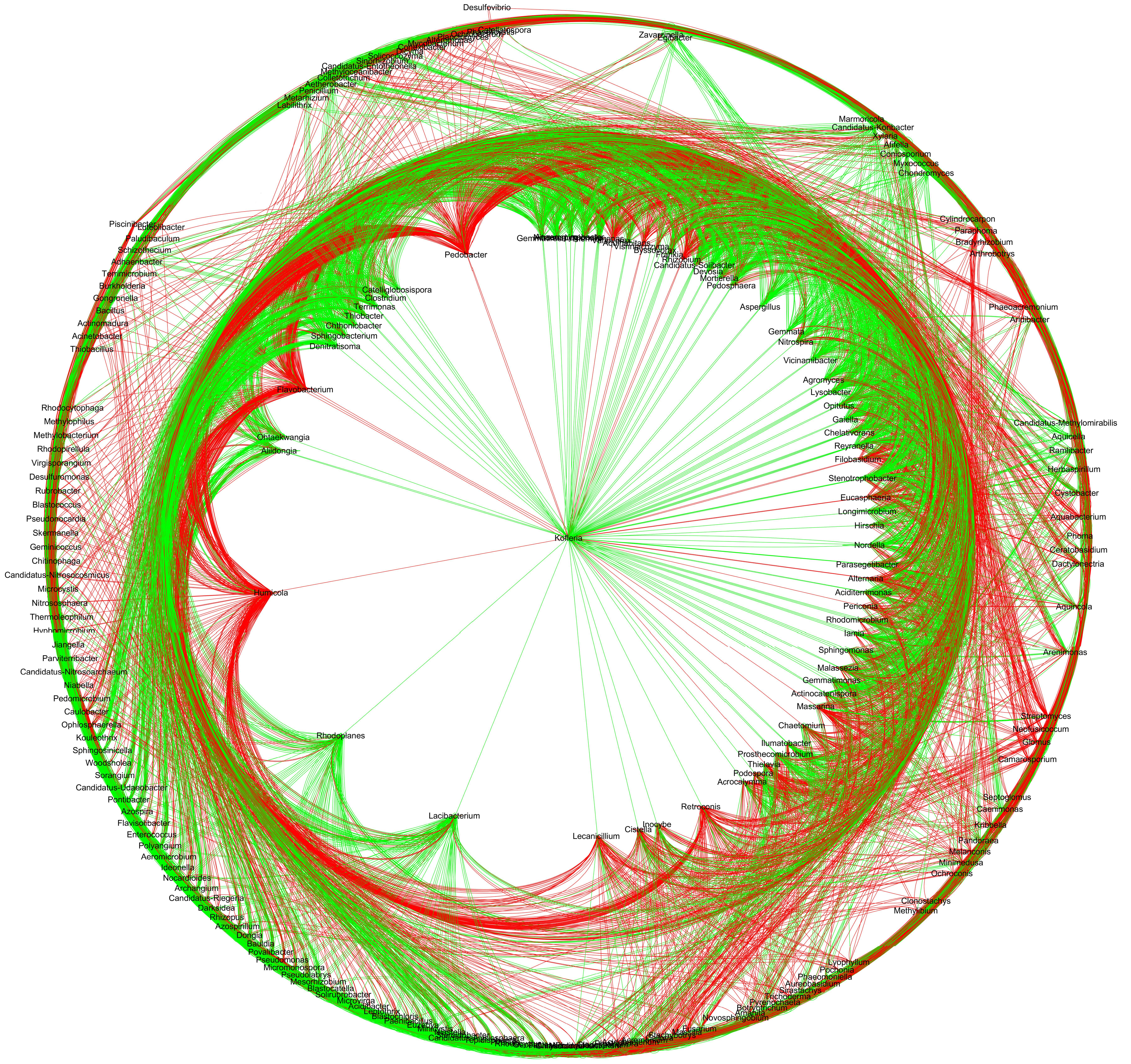


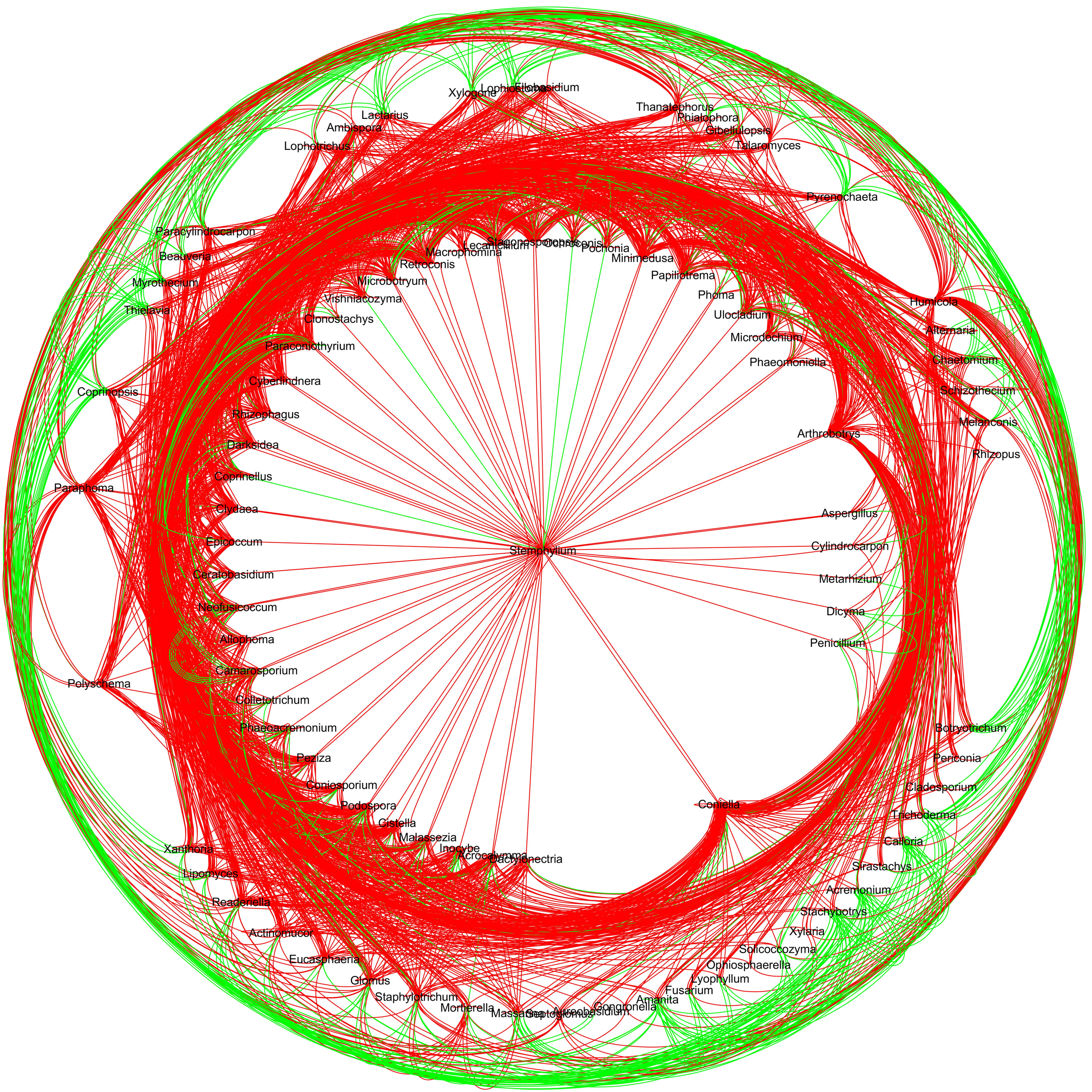
Calcarenite superficial
Calcarenite deep
Marly limestone superficial
Marly limestone deep

C



Calcarenite superficial
Calcarenite deep
Marly limestone superficial
Marly limestone deep


Desulfovibrio
Zavarzinella
Marmoricola
Candidatus-Koribacter
Xylaria
Afifella
Coniosporium
Myxococcus
Chondromyces
Cylindrocarpon
Paraphoma
Bradyrhizobium
Arthrobotrys
Phaeoacremonium
Aridibacter
Candidatus-Methylomirabilis
Aquicella
Ramlibacter
Herbaspirillum
Cystobacter
Aquabacterium
Phoma
Ceratobasidium
Dactylonectria
Aquincola
Arenimonas
Streptomyces
Neofusicoccum
Glomus
Camarosporium
Septoglomus
Caenimonas
Kribbella
Pandoraea
Melanconis
Minimedusa
Ochroconis
Clonostachys
Methylibium
Lyophyllum
Pochonia
Phaeomoniella
Aureobasidium
Sirastachys
Trichoderma
Pyrenochaeta
Botryotrichum
Amanita
Novosphingobium
Fusarium
Sinomonas
Solicoccozyma
Sinorhizobium
Candidatus-Entotheonella
Methyloceanibacter
Colletotrichum
Aetherobacter
Penicillium
Metarhizium
Labilithrix
Piscinibacter
Luteolibacter
Paludibaculum
Schizothecium
Adhaeribacter
Terrimicrobium
Burkholderia
Gongronella
Bacillus
Actinomadura
Acinetobacter
Thiobacillus
Rhodocytophaga
Methylophilus
Methylobacterium
Rhodopirellula
Virgisporangium
Desulfuromonas
Rubrobacter
Blastococcus
Pseudonocardia
Skermanella
Geminicoccus
Chitinophaga
Candidatus-Nitrosocosmicus
Microcystis
Nitrososphaera
Thermoleophilum
Hyphomicrobium
Jiangella
Parviterribacter
Candidatus-Nitrosoarchaeum
Niabella
Pedomicrobium
Caulobacter
Ophiosphaerella
Kouleothrix
Sphingosinicella
Woodsholea
Sorangium
Candidatus-Udaeobacter
Pontibacter
Azospira
Flavisolibacter
Enterococcus
Polyangium
Aeromicrobium
Ideonella
Nocardioides
Archangium
Candidatus-Riegeria
Darksidea
Rhizopus
Azospirillum
Dongia
Bauldia
Povalibacter
Pseudomonas
Micromonospora
Pseudolabrys
Mesorhizobium
Blastocatella
Solirubrobacter
Microvirga
Acidibacter
Leptothrix
Blastochloris
Paenibacillus
Pedobacter
Catelliglobosispora
Clostridium
Terrimonas
Thiobacter
Chthoniobacter
Sphingobacterium
Denitratisoma
Flavobacterium
Ohtaekwangia
Aliidongia
Humicola
Rhodoplanes
Lacibacterium
Lecanicillium
Cistella
Inocybe
Retroconis
Kofleria
Candidatus-Solibacter
Rhizobium
Frankia
Devosia
Mortierella
Pedosphaera
Aspergillus
Gemmata
Nitrospira
Vicinamibacter
Agromyces
Lysobacter
Opitutus
Gaiella
Chelativorans
Reyranella
Filobasidium
Stenotrophobacter
Eucasphaeria
Longimicrobium
Hirschia
Nordella
Parasegetibacter
Alternaria
Aciditerrimonas
Periconia
Rhodomicrobium
Iamia
Sphingomonas
Malassezia
Gemmatimonas
Actinocatenispora
Massarina
Chaetomium
Ilumatobacter
Prosthecomicrobium
Thielavia
Podospora
Acrocalymma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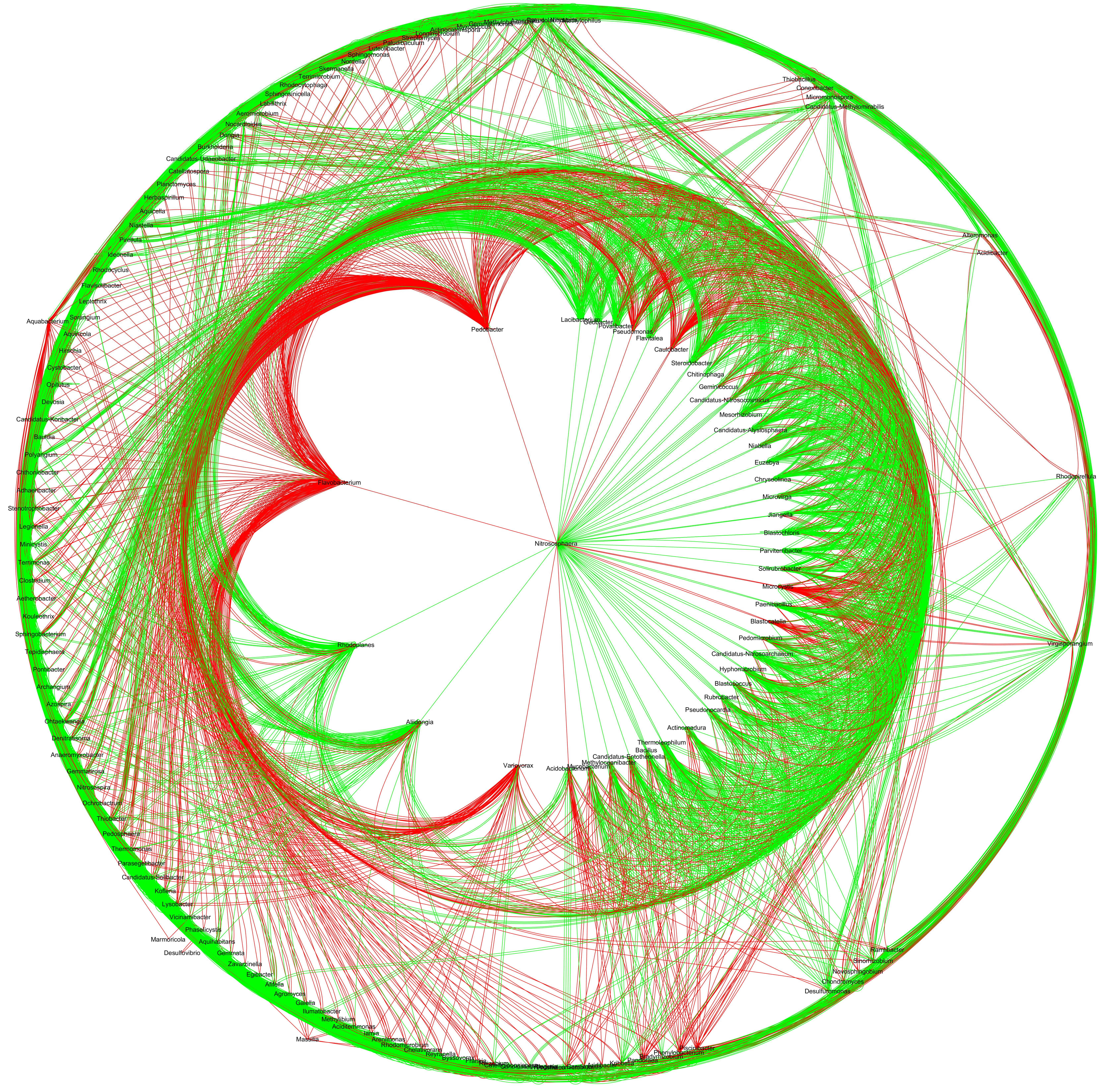
Lophiostoma
Filobasidium
Thanatephorus
Phialophora
Gibelliulopsis
Talaromyces
Lactarius
Ambispora
Lophotrichus
Pyrenochaeta
Paracylindrocarpon
Beauveria
Myrothecium
Thielavia
Macrophomina
Retroconis
Lecanicillium
Ochroconis
Pochonia
Minimedusa
Papiliotrema
Microbotryum
Vishniacozyma
Clonostachys
Phoma
Ulocladium
Microdochium
Humicola
Alternaria
Chaetomium
Schizothecium
Melanconis
Rhizopus
Paraconiothyrium
Cyberlindnera
Coprinopsis
Rhizophagus
Darksidea
Coprinellus
Paraphoma
Clydaea
Epicoccum
Ceratobasidium
Neofusicoccum
Allophoma
Camarosporium
Polyschema
Colletotrichum
Phaeoacremonium
Peziza
Coniosporium
Podospora
Cistella
Malassezia
Inocybe
Acrocalymma
Dactylonectria
Xanthonia
Liponyces
Readeriella
Actinomucor
Eucasphaeria
Glomus
Staphylotrichum
Mortierella
Massarina
Septoglomus
Aureobasidium
Gongronella
Amanita
Fusarium
Lyophyllum
Ophiosphaerella
Solicoccozyma
Xylaria
Stachybotrys
Acremonium
Sirastachys
Calloria
Trichoderma
Cladosporium
Periconia
Botryotrichum
Coniella
Arthrobotrys
Aspergillus
Cylindrocarpon
Metarhizium
Dicyma
Penicillium
Stemphylium


Nitrososphaera
Pedobacter
Flavobacterium
Rhodoplanes
Aliidongia
Variovorax
Lacibacterium
Geobacter
Povalibacter
Pseudomonas
Flavitalea
Caulobacter
Steroidobacter
Chitinophaga
Geminicoccus
Candidatus-Nitrosocosmicus
Mesorhizobium
Candidatus-Alysiosphaera
Niabella
Euzebya
Chryseolinea
Microvirga
Jiangella
Blastochloris
Parviterribacter
Solirubrobacter
Microcystis
Paenibacillus
Blastocatella
Pedomicrobium
Candidatus-Nitrosoarchaeum
Hyphomicrobium
Blastococcus
Rubrobacter
Pseudonocardia
Actinomadura
Thermoleophilum
Bacillus
Candidatus-Entotheonella
Methyloceanibacter
Acidobacterium
Thiobacillus
Conexibacter
Micromonospora
Candidatus-Methylomirabilis
Alteromonas
Acidibacter
Rhodopirellula
Virgisporangium
Ramlibacter
Sinorhizobium
Novosphingobium
Chondromyces
Desulfuromonas
Phenylobacterium
Massilia
Streptomyces
Luteolibacter
Paludibaculum
Sphingomonas
Nordella
Skermanella
Terrimicrobium
Rhodocytophaga
Sphingosinicella
Lobilithrix
Aeromicrobium
Nocardioides
Dongia
Burkholderia
Candidatus-Udaeobacter
Catellatospora
Planctomyces
Herbaspirillum
Aquicella
Niastella
Pirellula
Ideonella
Rhodocyclus
Flavisolibacter
Leptothrix
Aquabacterium
Sorangium
Aquincola
Hirschia
Cystobacter
Opitutus
Devosia
Candidatus-Koribacter
Bauldia
Polyangium
Chthoniobacter
Adhaeribacter
Stenotrophobacter
Legionella
Minicystis
Terrimonas
Clostridium
Aetherobacter
Kouleothrix
Sphingobacterium
Tepidisphaera
Pontibacter
Archangium
Azospira
Ohtaekwangia
Denitratisoma
Anaeromyxobacter
Gemmatirosa
Nitrosospira
Ochrobactrum
Thiobacter
Pedosphaera
Thermomonas
Parasegetibacter
Candidatus-Solibacter
Kofleria
Lysobacter
Vicinamibacter
Phaselicystis
Marmoricola
Aquihabitans
Desulfovibrio
Gemmata
Zavarzinella
Egibacter
Afifella
Agromyces
Gaiella
Ilumatobacter
Methylibium
Aciditerrimonas
Iamia
Arenimonas
Rhodomicrobium
Chelativorans
Reyranella
Byssovorax
Kribbella
Pandoraea
Bradyrhizobium
Methylophilus
Actinocatenispora
Longimicrobium
Myxococcus
Gemmatimonas