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Mediterranean biomes are biodiversity hotspots and also have been historically related to wine production. During the last decades, land occupied by vineyards has increased considerably threatening these Mediterranean ecosystems. Land use change and agricultural management affect soil biodiversity, changing physical and chemical properties of soil. These changes may have consequences on wine production, especially because soil is a key component of wine identity or *terroir*. Here, we characterized the taxonomic and functional diversity of bacterial and fungal communities present in soil from vineyards in Central Chile. To accomplish this goal we collected soil samples from organic vineyards from Central Chile and employed a shotgun metagenomic approach. Additionally, we also studied the surrounding native forest as a picture of the soil conditions prior to the establishment of the vineyard. Our metagenomic analyses revealed that both habitats shared most of the soil microbial species. In general, bacteria were more abundant than fungi in both types of habitats, including soil-living genera such as *Candidatus Solibacter*, *Bradyrhizobium* and *Gibberella*. Interestingly, we found presence of lactic bacteria and fermenting yeasts in soil, which are key during wine production. However, their abundances were extremely low, suggesting unlikeness of soil as a potential reservoir in Chilean vineyards. Regarding functional diversity, we found that genes for metabolism of amino acids, fatty acids, nucleotides and secondary metabolism were enriched in forest soils, whereas genes for metabolism of potassium, proteins and miscellaneous functions were more abundant in vineyard soils. Our results suggest that organic vineyards have similar soil community composition than forest habitats. Additionally, we suggest that native forests surrounding vineyards may be acting as microbial reservoir buffering the land conversion. We conclude that the implementation of environmentally friendly practices by the wine industry may help to maintain the microbial diversity and ecosystem functions related to natural habitats.

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Metagenomic analysis exploring taxonomic and functional diversity of soil microbial communities in Chilean vineyards and surrounding native forests

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Running title: Soil metagenomics from Chilean vineyards

Abstract

Mediterranean biomes are biodiversity hotspots and also have been historically related to wine production. During the last decades, land occupied by vineyards has increased considerably threatening these Mediterranean ecosystems. Land use change and agricultural management affect soil biodiversity, changing physical and chemical properties of soil. These changes may have consequences on wine production, especially because soil is a key component of wine identity or *terroir*. Here, we characterized the taxonomic and functional diversity of bacterial and fungal communities present in soil from vineyards in Central Chile. To accomplish this goal we collected soil samples from organic vineyards from Central Chile and employed a shotgun metagenomic approach. Additionally, we also studied the surrounding native forest as a picture of the soil conditions prior to the establishment of the vineyard. Our metagenomic analyses revealed that both habitats shared most of the soil microbial species. In general, bacteria were more abundant than fungi in both types of habitats, including soil-living genera such as *Candidatus Solibacter*, *Bradyrhizobium* and *Gibberella*. Interestingly, we found presence of lactic bacteria and fermenting yeasts in soil, which are key during wine production. However, their abundances were extremely low, suggesting unlikeness of soil as a potential reservoir in Chilean vineyards. Regarding functional diversity, we found that genes for metabolism of amino acids, fatty acids, nucleotides and secondary metabolism were enriched in forest soils, whereas genes for metabolism of potassium, proteins and miscellaneous functions were more abundant in vineyard soils. Our results suggest that organic vineyards have similar soil community composition than forest habitats. Additionally, we suggest that native forests surrounding vineyards may be acting as microbial reservoir buffering the land conversion. We conclude that

the implementation of environmentally friendly practices by the wine industry may help to maintain the microbial diversity and ecosystem functions related to natural habitats.

Keywords: bacterial diversity; conservation; ecosystem services; fungal diversity; pyrosequencing; shotgun sequencing; wine.

Introduction

Land use change affects many important ecosystem properties and functions and is one of the main drivers of global change (Vitousek et al., 1997). Land conversion is also responsible for the decrease of native habitats, which can have consequences at the ecosystem level because some ecological function may be lost during conversion (Griffiths & Philippot, 2013). Particularly, land conversion has occurred at a very fast rate during the last decades in Mediterranean biomes (Cincotta, Wisnewski & Engelman, 2000; Lauber et al. 2008; Underwood et al. 2008). This is especially important because Mediterranean ecosystems are biodiversity hotspots containing a high number of endemic plant species that are increasingly threatened (Cowling et al. 1996; Myers et al. 2000). Therefore, conservation programs are necessary to preserve the biodiversity contained in these ecosystems.

Mediterranean climate is suitable for viticulture, which historically has thrived in these areas (Hannah et al. 2013; Viers et al. 2013). During the last decades, land occupied by vineyards has increased by 70% between 1988 and 2010 in New World Mediterranean zones (Chile, the Californias, Australia, and South Africa) (Viers et al. 2013). Land use change and agricultural management (e.g. tillage, pesticide and fertilizer applications) affect soil biodiversity, changing physical and chemical properties of soil (Pampulha & Oliveira, 2006; Jangid et al 2008). For

instance, vineyards under organic management have higher soil microbial biomass and nematode densities than conventional vineyards (Coll et al. 2012). On the other hand, Bevivino et al. (2014) reported that undisturbed soils showed more stable bacterial communities through seasons than vineyards, suggesting that natural habitats are more resilient to environmental or human perturbations. Furthermore, soil biodiversity is very significant to wine production, which relies on the importance of soil and climate as key components of wine identity or *terroir* (van Leeuwen et al. 2004; Gilbert, van der Lelie & Zarraonaindia, 2014).

Soil is one of most diverse environments on the Earth and current information estimates the presence of 2,000 to 18,000 microbial genomes in one gram of soil (Delmont et al. 2011; Xu et al. 2014). There is abundant evidence confirming the important role played by soil microorganisms in several ecosystem services such as erosion control, soil formation, nutrient cycling, and plant health (Tiedje et al. 1999; Nanniepieri et al. 2003; Garbeva, van Veen & van Elsas, 2004; Gardi et al. 2009). However, soil microbial communities change across agricultural practices and environmental gradients (Bevivino et al. 2014; García-Orenes et al. 2013). For instance, addition of organic matter increases the fungal abundance in managed soils, and also microbial community structures were more similar to those found in forest soil (García-Orenes et al. 2013). In addition, Corneo et al. (2013) reported that microbial communities changed across an altitudinal gradient, where physical (e.g. soil moisture, clay content) and chemical (e.g. Al, Mg, Mb, B) properties explained most of the altitudinal variation in the communities.

Recent development of high-throughput sequencing techniques has allowed a deep understanding of the microbial diversity in vineyard soils in different winery regions around the world (Corneo et al. 2013; Fujita et al. 2010; Zarraonaindia et al. 2015). Although Chilean Mediterranean is one of the most important regions for wine production and vineyard area has

exhibited rapid expansion (Viers et al. 2013), there are very few studies exploring the microbial diversity in soil vineyards (Aballay et al. 2011; Castañeda et al. 2015). Recently, Castañeda et al. (2015) explored the soil microbial communities inhabiting native forests and vineyards in Chile employing a T-RFLP approach. While T-RFLPs is a reliable technique, it does not provide a deep taxonomic resolution or information about ecological functions present in the microbial community. Therefore, the main goal of the present study was to characterize the taxonomic and functional diversity of bacterial and fungal communities present in soil from vineyards in Central Chile. To accomplish this goal, we collected soil samples from three organic vineyards from Central Chile and assessed taxonomical and functional diversity employing a shotgun metagenomic approach. These organic vineyards are relatively young (< 10 years-old) and surrounded by natural landscapes. The surrounding natural landscapes are dominated by native sclerophyllous forest and shrubs, which likely represent soil conditions prior to the establishment of the vineyard. Therefore, we also characterized the taxonomic and functional diversity of soil microbial communities present in sclerophyllous native forests adjacent to vineyards. Knowledge of the soil microbial communities of native habitats could provide a starting point for the conservation of microbial diversity and preservation of ecosystem functions provided by natural habitats (Gardi et al. 2009). This is important for conservation priority areas of high ecological value such as the Central Chilean biodiversity hotspot (Mittermeier et al. 2011; Hannah et al. 2013; Viers et al. 2013), where the hotspot status is mainly based on the number of endemic plant species. However, the knowledge of microbial communities living in this biome is scarce and metagenomic studies could provide valuable information about bacterial and fungal species for their consideration in conservation areas (Heilmann-Clausen et al. 2014)

Materials and Methods

Sampling

Soil samples were collected in three different organic vineyards and the neighboring sclerophyllous forest patch in Central Chile located in Ocoa (32º52'S – 71º7'W), Leyda (33º34'S – 71º22'W) and Apalta (34º36'S – 71º7'W), respectively. The owners of vineyards and surrounding native forest patches granted all necessary permits to access the sampling sites: Seña Vineyards in Ocoa (Chile), Cono Sur Vineyards in Leyda (Chile), and Emiliana Vineyards in Apalta (Chile; Table 1).

In each vineyard and adjacent forest, we collected five soil samples at a depth of 15 cm using soil cores and at a distance of 5 cm from five randomly selected vines (vineyard) or from five randomly selected native trees (forest). Vines and native trees were within 3.5 m from each other. This depth was chosen because the most microbial activity occurs within 15cm (O'Brien et al. 2005). Collected samples were stored in a sterile bag and placed in a cooler with ice packs. During the same day, the 30 soil samples were transported to the laboratory where they were individually homogenized, sieved and stored at -80 ºC until DNA extraction.

Metagenomic sequencing

For a total of 30 soil samples (3 vineyard areas × 2 habitats × 5 soil samples), DNA was extracted using the Power Soil DNA isolation kit (MoBio Laboratories Inc., Carlsbad, CA) following the manufacturer's instructions. DNA quality of each extraction was determined by electrophoresis using a 0.8% agarose gel and also by DNA quantification using a nanospectrophotometer (NanoDrop Technologies Inc., Wilmington, DE).

For sequencing, the DNA extractions from each habitat (5 samples) were pooled into one sample. Thus, we sequenced one pooled vineyard sample and one polled forest sample per vineyard areaThe amount of DNA was assessed by fluorescence using the Quant-iT kit PicoGreen dsDNA (Invitrogen, Carlsbad, CA) on a DQ 300 fluorometer (Hoefer Scientific Instruments, San Francisco, CA). Then, each metagenomic library was prepared using the 454 GS Junior Titanium Rapid DNA library preparations according to the manufacturer's instructions. Emulsion PCR (emPCR) was performed according the Amplification Method Manual using a Lib-L kit. All steps involved in massive DNA sequencing were performed in AUSTRAL-omics Core-Facility (Facultad de Ciencias, Universidad Austral de Chile) in 454 GS Junior Titanium Series (Roche, Branford, CT) following the standard protocol of Roche.

Data analysis

Raw sequences of each one of the six metagenomes were uploaded to the MG-RAST server at http://metagenomics.anl.gov (Meyer et al. 2008). Number of uploaded sequences ranged between 141,694 and 195,138 for forest soil samples and between 189,372 and 208,095 for vineyard soil samples. After quality control was performed through MG-RAST, the number of retained sequences for forest soil samples ranged between 114,120 and 131,618 with an average length of 442.7 bp, whereas vineyard soil samples passed between 108,385 and 138,101 sequences with an average length of 445.3 bp (see Table S1 for more detailed information). For the taxonomical assignments, the sequences were compared using the SEED database, whereas functional assignments were performed comparing the Subsystems database. For both assignments, we employed a maximum e-value of 1e-5, a minimum identity of 60%, and a maximum alignment length of 15 bp. After that, taxonomical and functional profiles were downloaded and analyzed

using the STAMP software (Parks & Beiko, 2010). For analysis, we pooled samples and compared relative abundances between forest (n = 3) and vineyard (n = 3) soils performing a White's non-parametric t-test (White, Nagarajan & Pop, 2009) given the non-normal distribution of our data. The accession numbers for the metagenomes in the MG-RAST server (http://metagenomics.anl.gov) were: 4565458.3, 4565459.3, 4565460.3, 4565461.3, 4565462.3, and 4565463.3. Rarefaction curves for each samples reached a good taxonomic depth as can be seen in Fig. S1.

Results

Taxonomical analysis

Metagenomic analyses based on the SEED database showed that Bacteria dominated forest as well as vineyard soil samples (mean = 95.97 % and 95.97 %, respectively), followed by Eukaryota (mean = 0.53 % and 0.41 %, respectively) and Archaea (mean = 0.74 % and 0.82 %, respectively). The other sequences correspond to Viruses and unassigned sequences (Table 1). Among Bacteria, Proteobacteria was the most abundant phylum both in forest soil as well as in vineyard soil, followed by Actinobacteria, Acidobacteria, Bacteriodetes, Firmicutes and Planctomycetes (Table 1). However, we did not find significant differences in the abundances of these phyla (Table 1).

Exploring the SEED database, we found 333 genera of which *Prosthecochloris* and *Flouribacter* were only found in forest soils, whereas *Erwinia* and *Neorickettsia* were only found in vineyard soils. We found presence of lactic bacteria, which are relevant for wine production, but with extremely low relative abundances for the case of *Lactobacillus* (maximum of 50 reads, equivalent to 0.05%), *Oenococcus* (maximum of 6 reads, equivalent to 0.003%), *Pediococcus*

(maximum of 5 reads, equivalent to 0.006%). In the case of species, we found 636 operational taxonomic units (OTUs): 18 and 17 exclusive OTUs in forest and vineyard soils, respectively. Among the most abundant species were *Candidatus Solibacter usisatus* (overall mean = 6.1%), *Bradyrhizobium japonicum* (overall mean = 3.7%), *Conexibacter woesei* (overall mean = 3.5%), *Rhodopseudomonas palustris* (overall mean = 3.2%), *Candidatus Koribacter versatilis* (overall mean = 2.9%), *Sorangium cellulosum* (overall mean = 1.8%), *Myxococcus xanthus* (overall mean = 1.6%), *Spingomonas wittichii* (overall mean = 1.4%) and *Mesorhizobium loti* (overall mean = 1.3%). Nevertheless, none of these dominant species exhibited significant differences in their abundances in forest and vineyard soils. Conversely, significantly different abundances ($P < 0.05$) were found for 17 species, of which seven exhibited higher abundances in forest soils and nine showed higher abundance in vineyard soils (Fig. 2). However, most of these OTUs exhibited a very low abundance in each habitat with the exception of *Bordetella bronchiseptica* (forest mean = 0.28% and vineyard mean = 0.26%; $P = 0.036$), *Pseudomonas stutzeri* (forest mean = 0.11% and vineyard mean = 0.14%; $P = 0.037$) and *Pseudomonas entomophila* (forest mean = 0.08% and vineyard mean = 0.07%; $P = 0.016$).

Among Eukaryota domain we focused on fungal OTUs, which were mainly related to the Ascomycota and Basidiomycota classes (Table 1). Exploring the complete fungal taxonomy, we did not find significant differences for the relative abundance of fungal-related OTUs. At species level, we only found 11 Ascomycota species and 2 Basidiomycota species, while the most abundant fungal-related OTU was the Ascomycota *Gibberella zeae* (maximum of 116 reads, equivalent to 0.13%). Interestingly, we found some OTUs related to *Saccharomyces cerevisiae*, a wine-fermenting yeast, but with an extremely low abundance both in forest and vineyard soils (maximum of 5 reads, equivalent to 0.004%).

Another important group found in both habitats was the domain Archaea represented by its five phyla: Crenarchaeota, Euryarchaeota, Korarchaeota, Nanoarchaeota and Thaumarchaeota. Of them, the phylum Euryarchaeota was the most abundant (forest mean = 0.58% and vineyard mean = 0.62%) but not significantly different between forest and vineyard soils (Table 1). At the species level, we found 54 OTUs with relative abundances lower than 0.05%. *Halobrum lacusprofundi*, *Pyrobaculum calidifontis* and *Nanoarchaeum equitans* were only found in forest soils, while no OTUs were exclusively found in vineyard soils.

Functional analysis

Functional categories found in forest and vineyard soils are represented in Figure 1. The most abundant functional categories were sequences related to carbohydrate metabolism (forest mean = 14.4% and vineyard mean = 14.6%), clustering-based on subsystems (forest mean = 14.0% and vineyard mean = 14.2%) and metabolism of amino acids and their derivatives (forest mean = 10.8% and vineyard mean = 10.6%). Genes for metabolism of amino acid and their derivatives ($P = 0.007$), fatty acids and lipid metabolism ($P = 0.024$), nucleosides and nucleotides ($P = 0.045$) and secondary metabolism ($P = 0.011$) were significantly enriched in forest soils (Fig. 1). On the other hand, genes for potassium metabolism ($P = 0.083$), protein metabolism ($P = 0.089$), and miscellaneous functions ($P = 0.033$) were more abundant in vineyard soils (Fig. 2).

According to functional categories associated to nutrient cycling, we recorded sequences related to sulfur metabolism (forest mean = 1.18% and vineyard mean = 1.15%), phosphorous metabolism (forest mean = 1.04% and vineyard mean = 1.05%), nitrogen metabolism (forest mean = 0.82% and vineyard mean = 0.80%) and potassium metabolism (forest mean = 0.30% and vineyard mean = 0.33%). All these functions showed similar relative abundances in forest

and vineyard soils ($P > 0.1$). Additionally, we explored the SEED level-3 hierarchical gene annotation. In general, assimilation of inorganic sulfur (overall mean = 0.37%), phosphate metabolism (overall mean = 0.54%), phosphorous uptake (overall mean = 0.20%), ammonia assimilation (overall mean = 0.38%), nitrate and nitrite assimilation (overall mean = 0.15%), and potassium homeostasis (overall mean = 0.28%) were the most abundant level-3 functions related to nutrient cycling. However, the relative abundances of these functions were not significantly different between forest and vineyard soils.

Exploring the annotated sequences in the SEED subsystems we found 5,215 genes present in the soil samples. From these, 511 and 599 genes were exclusively found in forest and vineyard soils, respectively. Additionally, we found that 148 out of 4,105 (~ 0.036%) exhibited significantly different abundance between habits: 70 genes were enriched in forest soils, whereas 78 genes were enriched in vineyard soils.

Discussion

With metagenomic analyses, we determined the taxonomic and functional diversity of microbial communities inhabiting forest and vineyard soils from Mediterranean ecosystems in Central Chile. Our metagenomic analyses revealed that both habitats shared most of the soil microbial species, whereas some functional categories showed significant differential enrichment between forest and vineyard soils.

Our analysis showed that bacterial-related OTUs exhibited the highest relative abundance in both habitats. For soil environments, Uroz et al. (2013) reported similar bacterial abundances between organic and mineral soils, which reached *ca*. 94% of the sequences. Proteobacteria are very common in soil environments and are related to a wide variety of functions involved in

carbon, nitrogen and sulfur cycling (Spain, Krumholz & Elshahed, 2009). The relative abundances found in the present study are similar to those previously reported in other soil habitats (*ca*. 40% according to Janssen [2006]). Actinobacteria also are a dominant phylum in soils, participating in carbon cycling and producing secondary metabolites (Jenkins et al. 2010). In our study, the most abundant bacterial genera on soil were *Candidatus Solibacter*, *Bradyrhizobium*, *Conexibacter* and *Rhodopseudomonas*, which have been previously reported as dominant genera in several types of soil (Delmont et al. 2011; Pearce et al. 2012). Comparing from bacterial phyla to genera, we did not find differential abundance between forest and vineyard soils. Previous evidence suggests that bacterial communities differ between forest and managed soils (García-Orenes et al. 2013). However, the relationship between microbial diversity and habitat disturbance is very complex and some disturbed habitats exhibit higher diversity than forest systems (Montecchia et al. 2015). Employing a T-RFLP approach, we previously showed that bacterial communities are similar between forest and vineyard habitats (Castañeda et al. 2015). However this molecular technique provides a limited taxonomic resolution of microbial communities compared to metagenomic analysis employed in the present work. Indeed, we found differential abundances of soil bacteria such as *K. radiotolerans* and the denitrifying bacteria *P. stutzeri* (Lalucat et al. 2006), which was more abundant in vineyard soils and likely related to higher nitrogen supply in managed systems.

Our metagenomic analyses revealed that sequences assigned to eukaryotic organisms only represented 0.5% of the total sequences. This finding was in agreement with previous studies employing shotgun sequencing for describing soil microbial communities (Pearce et al. 2012; Uroz et al. 2013). We found that most of the fungi-related sequences were assigned to Ascomycota, whereas Basidiomycota only represented a small fraction of the total sequences. At

species level, the most abundant fungal species was *Gibberella zeae/Fusarium graminearum* a well-known plant pathogen that attacks cereals (Bai & Shaner, 2004). From a comparative point-of-view, we found similar fungal abundance between forest and vineyard soils. Whereas our previous work employing T-RFLPs showed that fungal community structure changed between forest and vineyard soils (Castañeda et al. 2015), which coincide with changes in fungal diversity composition between *Eucalyptus* forest and *Pinus* plantation in Australia (Kasel, Bennett & Tibbits, 2008). However, the lack of differences in fungal abundances in the present study may be related to the small representation of fungal sequences in soil samples. Uroz et al. (2013) suggested that shotgun metagenomic approaches underestimate fungal diversity and complementary approaches, such as metatranscriptomic, should be employed to study soil eukaryotic communities. In addition, it should be considered that changes in taxonomic abundance are limited to taxonomic groups that changed functionally because taxonomic assignment is based on a nonredundant protein database such as SEED (Carrino-Kyker, Smeno & Burke, 2013).

Microbial contribution is very important during several stages of wine production (Mills et al. 2008). For instance, fermenting yeasts are involved in the alcoholic fermentation (i.e. the sugar conversion into ethanol and carbon dioxide) and lactic bacteria perform the malolactic fermentation (i.e. the conversion of malate into lactate) (Fleet 2003; Mills et al. 2008). Our data show the presence of lactic bacteria such as *Lactobacillus*, *Oenococcus* and *Pediococcus* and the fermenting-yeast *S. cerevisiae* in soil samples. However, their abundances are relatively low compared to dominant taxa, suggesting that soil may not be a suitable ecological niche or reservoir for important microorganisms for the wine production as has been previously suggested (Bester, 2005; Chen, Yanagida & Shinohara, 2005; Zarraonaindia et al. 2015). Differences in the

295 methodology may explain these contrasting results. Some studies have employed enrichment
296 methods (Bester, 2005; Chen, Yanagida & Shinohara, 2005) or amplicon sequencing
297 (Zarraonaindia et al. 2015), while shotgun sequencing (technique employed in the present study)
298 could underestimate abundance of fungal sequences. Future research requires evaluating the
299 presence of enologically important microorganisms of surrounding native flora (i.e. leaves and
300 fruits) to determine if these habitats are potential sources and/or reservoirs of microbial diversity
301 relevant for wine production.

302 Most sequences obtained from forest and vineyard soils were related to metabolism of
303 carbohydrates and amino acids. This finding suggests that soil microbial communities are
304 capable of degrading carbohydrates and playing an important role in the carbon cycle, through
305 organic matter and litter decomposition. These results confirm the high relative abundance (*ca*.
306 12%) of genes related to carbohydrate metabolism in organic soils (Uroz et al. 2013; Paula et al.
307 2014). Land-use change may alter the community structure of soil microorganisms, which can
308 have profound effects on functional traits and ecosystem processes (Griffiths & Philippot, 2013;
309 Paula et al. 2014). Higher abundances of genes related to ecological function such as metabolism
310 of secondary metabolism and potassium metabolism were found in forest and vineyard soils,
311 respectively. Additionally, it has been reported that land conversion from primary forest to long-
312 term pastures might change microbial functional diversity of important functional genes related
313 to carbon and nitrogen cycling in Amazon soils (Paula et al. 2014). However, nitrogen-related
314 genes represented 0.8% of the total functional reads and their abundances did not differ between
315 forest and vineyard soils. These abundance values are in concordance with previous studies,
316 including enriched environments with nitrogen-fixing bacteria such as soybean crops (Mendes et
317 al. 2014). A plausible explanation for the lack of differences between habitats is that organic

agriculture supplies nitrogen in its organic form (e.g. compost and manure) similarly to what occurs in forest, thus nitrogen could be available in similar chemical form for both habitats but in higher quantities in vineyards (NH_4 vineyard = 9.2 mg/kg and NH_4 forest = 4.2 mg/kg; NO_3 vineyard = 11.1 mg/kg and NO_3 forest = 7.2 mg/kg).

Conclusions

We identified the taxonomic and functional diversity of microbial communities in Chilean vineyard and forest soils by shotgun sequencing. We also assessed the same information in the soil of the native sclerophyllous forest in the Chilean Mediterranean, one of the most threatened biodiversity hotspots in the world (Myers et al. 2000; Viers et al. 2013). Our metagenomic analyses revealed some functional categories changed between forest and vineyard soils. Conversely, the taxonomic composition does not change between habitats, suggesting that organic vineyards have a similar soil microbial community than native forests. This can be explained because organic management has little impact on microbial communities. Another plausible explanation is native forest surrounding vineyards may be acting as microbial reservoir buffering the effect of land conversion. Therefore, additional research is needed to explore the role of landscape complexity and agriculture management on microbial communities in forest-vineyard habitats. Finally, cumulative evidence suggests the implementation of environmentally friendly practices by the wine industry may help to maintain the microbial diversity and ecosystem functions related to natural habitats.

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Competing interests

The authors declare there are no competing interests.

Author Contribution

Luis E. Castañeda analyzed the data, wrote the paper.

Olga Barbosa conceived the idea, designed the experiments, reviewed drafts of the paper.

References

Aballay E, Maternsson A, Persson P. Screenign of rhizosphere bacteria from grapevine for their suppressive effect on *Xiphinema index* Thorne & Allen on in vitro grape plants. Plant Soil. 2011;347: 313–325.

362 Bai G, Shaner G. Management and resistance in wheat and barley to *Fusarium* head blight. Annu
363 Rev Phypathol. 2004;42: 135–161.
364 Bester R. Growth and survival of *Saccharomyces cerevisiae* in soil. MSc Thesis, University of
365 Stellenbosch, South Africa. 2005. Available: http://scholar.sun.ac.za/handle/10019.1/16597
366 Bevivino A, Paganini P, Bacci G, Florio A, Pellicer MS, Papaleo MC, Mengoni A, Ledda L,
367 Fani R, Benedetti A, Dalmastri C. Soil bacterial community response to differences in
368 agricultural management along with seasonal changes in a Mediterranean region. PLoS
369 One 2014;9: e105515.
370 Carrino-Kyker SR, Smeno KA, Burke DJ. Shotgun metagenomic analysis of metabolic diversity
371 and microbial community structure in experimental vernal pools subjected to nitrate pulse.
372 BMC Micriobiol. 2013;13: 78.
373 Castañeda LE, Manzano M, Godoy K, Marquet PA, Barbosa O. Comparative study between soil
374 microbial structure communities from vineyards and sclerophyllous forest in Central Chile.
375 Ecol Evol. 2015. doi:10.1002/ece3.1652.
376 Chen YS, Yanagida F, Shinohara T. Isolation and identification of lactic acid bacteria from soil
377 using enrichment procedure. Lett Appl Micriobiol. 2005;40: 195–200.
378 Cincotta RP, Wisnewski J, Engelman R. Human population in biodiversity hotspots. Nature.
379 2000;404: 990–992.
380 Coll P, Le Cadre E, Blanchart E, Hinsinger P, Villenave C. Organic viticulture and soil quality: a
381 long-term study in Southern France. Appl Soil Ecol. 2011;50: 37–44.
382 Corneo PE, Pellegrini A, Cappellin L, Roncador M, Chierici M, Gessler C, Pertot I. Microbial
383 community structure in vineyard soils across altitudinal gradients and in different seasons.
384 FEMS Microbiol Ecol. 2013;84: 588–602.

385 Cowling RM, Rundel PW, Lamont BB, Arroyo MK, Arianoutsou M. Plant diversity in
386 Mediterranean climate regions. Trends Ecol Evol. 1996;11: 362–366.
387 Delmont TO, Prestat E, Keegan KP, Faubladier M, Robe P, Clark IM, Pelletier E, Hirsch PR,
388 Meyer F, Gilbert JA, Le Paslier D, Simonet P, Vogel TM. Structure, fluctuation and
389 magnitude of a natural grassland soil metagenome. ISME J. 2011;6: 1677–1687.
390 Fernández-Calviño D, Martín A, Arias-Estévez M, Bååth E, Díaz-Raviña M. Microbial
391 community structure of vineyard soils with different pH and cooper content. Appl Soil
392 Ecol. 2010;46: 276–282.
393 Fleet GH. Yeat interactions and wine flavour. Int J Food Microbiol. 2003;86: 11–22.
394 Fujita K, Furuya S, Kohno M, Suzuki S, Takayanagi T. Analysis of microbial community in
395 Japanise vineyard soils by culture-independent molecular approach. Int J Wine Res.
396 2010;2: 75–104.
397 Garbeva P, van Veen JA, van Elsas JD. Microbial diversity in soil: selection microbial
398 populations by plant and soil type and implications for disease suppressiveness. Ann Rev
399 Phytopathol. 2004;42: 243–270.
400 García-Orenes F, Morugán-Coronado A, Zornoza R, Scow K. Changes in soil microbial
401 community structure influences by agricultural management practices in a Mediterranean
402 agro-ecosystem. PLoS One 2013;8: e80522.
403 Gardi C, Montanarella L, Arrouays D, Bispo A, Lemanceau P, Jovilet C, Mulder C, Ranjard L,
404 Römbke J, Rutgers M, Menta C. Soil biodiversity monitoring in Europe: ongoing
405 activities and challenges. Eur J Soil Sci. 2009;60: 807–819.
406 Gilbert, J.A., van der Lelie, D., Zarraonaindia, I. Microbial *terroir* for wine grapes. Proc Natl
407 Acad Sci USA. 2014;111: 5–6.

408 Griffiths BS, Philippot L. Insights into the resistance and resilience of the soil microbial
409 community. FEMS Micriobiol Rev. 2013:37; 112–139.
410 Hannah L, Roehrdanz PR, Ikegami M, Shepard AV, Shaw MR, Tabor G, Zhi L, Marquet PA,
411 Hijmans RJ. Climate change, wine, and conservation. Proc Natl Acad Sci USA. 2013;110:
412 6907–6912.
413 Heilmann-Clausen J, Barron ES, Boddy L, Dahlber A, Griffith GW, Nordén J, Ovaskainen O,
414 Perini C, Senn-Irlet B, Halme P. Conserv Biol 2014;29: 61-68.
415 Jangid K, Williams MA, Franzluebbers AJ, Sanderlin JS, Reeves JH, Jenkins MB, Endale DM,
416 Coleman DC, Whitman WB. Relative impacts of land-use, management intensity and
417 fertilization upon soil microbial community structure in agricultural systems. Soil Biol
418 Biochem. 2008;40: 2843.2853.
419 Janssen PH. Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA
420 genes. Appl Environ Microbiol. 2006;72: 1719–1728.
421 Jenkins SN, Waite IS, Blackburn A, Husbund R, Rushton SP, Manning DC, O'Donnell AG. A
422 Van Leeuw J Microb. 2010;95: 319–334.
423 Kasel S, Bennett LT, Tibbits J. Land use influences soil fungal community composition across
424 central Victoria, south-eastern Australia. Soil Biol Biotechnol. 2008;40: 1724–1732.
425 Lalucat J, Bennasar A, Bosch R, García-Valdés E, Palleroni NJ. Biology of Pseudomonas
426 stutzeri. Microbiol Mol Biol Rev. 2006;70: 510–547.
427 Lauber CL, Strickland MS, Bradford MA, Fierer N. The influence of soil properties on the
428 structure of bacterial and fungal communities across land-use types. Soil Biol Biochem.
429 2008;40: 2407–2415.

Mendes LW, Kuramae EE, Navarrete AA, van Veen JA, Tsai SM. Taxonomical and functional microbial community selection in soybean rhizosphere. ISME J 2014;8: 1577–1587.

Meyer F, Paarman D, D'Souza M, Olson R, Galss EM, Kubal M, Paczian T, Rodriguez A, Steens R, Wilke A, Wilkening J, Edwards RA. The metagenomics RAST server: a public resource for the automatic phylogenetic and functional analysis of metagenomes. BCM Bioinformatics. 2008;9: 386.

Mills DA, Phister T, Neeley E, Johannsen E. Wine fermantation. In: Cocolin L, Ercolini D, editors. Molecular Techniques in the Microbial Ecology of Fermented Foods. Berlin: Springer-Verlag;2008. pp: 162–192.

Mittermeier RA, Turner WR, Larsen FW, Brooks TM, Gascon C. Global biodiversity conservation: the critical role of hotspots. In: Zachos FE, Hable JC, editors. Biodiversity Hotspots. Berlin: Springer-Verlag; 2011: pp: 3–22.

Montecchia MS, Tosi M, Soria MA, Vogrig JA, Sydorenko O, Correa OS. Pyrosequencing revelas changes in soil bacteria communities after conversion of Yungas forests to agriculture. 2015;10: e0119426.

Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GA, Kent J. Biodiversity hotspots for conservation priorities. Nature. 2000;403: 853–8.

Nannipieri P, Ascher J, Ceccherini MT, Landi L, Pietramellara G, Renella G. Eur J Soil Sci. 2003;54: 655–670.

O'Brien HE, Parrent JL, Jackson JA, Moncalvo J-M, Vilgalys R. Fungal community analysis by large-scale sequencing of environmental samples. Appl Environ Microbiol. 2005;71: 5544–5550.

452 Pampulha ME, Oliveira A. Impact of an herbicide combination of bromoxynil and prosulfuron
453 on soil microorganisms. Curr Microbiol. 2006;53: 238–243.
454 Parks DH, Beiko RG. Identifying biologically relevant differences between metagenomic
455 communities. Bioinformatics. 2010;26: 715–721.
456 Paula FS, Rodrigues JLM, Zhou J, Wu L, Mueller RC, Mirza BS, Bohannan BJM, Nüsslein K,
457 Deng Y, Tiedje JM, Pellizari VH. Land use change alters functional gene diversity,
458 composition and abundance in Amazon forest soil microbial communities. Mol Ecol.
459 2014;23: 2988–2999.
460 Pearce DA, Newsham KK, Thorne MAS, Calvo-Bado L, Krsek M, Laskaris P, Hodson A,
461 Wellington EM. Metagenomic analysis of a southern maritime Antarctic soil. Front
462 Microbiol. 2012;3: doi: 10.3389/fmicb.2012.00403
463 Spain AM, Krumholz LR, Elshahed MS. Abundance, composition, diversity and novelty of soil
464 P*roteobacteria*. ISME J. 2009;3: 992–1000.
465 Tiedje JM, Asuming-Brempong S, Nusslein K, Marsh TL, Flynn SJ. Opening the black box of
466 soil microbial diversity. Appl Soil Ecol. 1999;13: 109–122.
467 Underwood EC, Viers JH, Klausmeyer KR, Cox RL, Shaw MR. Threats and biodiversity in the
468 Mediterranean biome. Divers Distrib. 2008;15: 188–197.
469 Uroz S, Ioannidis P, Lengelle J, Cébron A, Morin E, Buée M, Martin F. Functional assays and
470 metagenomic analysis reveals differences between the microbial communities inhabiting
471 the soil horizons of a Norway spruce plantation. PLoS One. 2013;8: e55929.
472 van Leeuwen C, Friant P, Chone C, Tregoat O, Koundouras S, Dubourdieu D. Influence of
473 climate, soil, and cultivar on terroir. Am J Enol Viticult. 2004;55: 207–217.

474 Viers JH, Willimas JN, Nicholas KA, Barbosa O, Kotzé I, Spence L, Webb LB, Merenlender A,
475 Reynols M. Vinecology: pairing wine with nature. Conserv Let. 2013;6: 287–299.
476 Vitoussek PM, Mooney HA, Lubchenco J, Melillo JM. Human domination of Earth's
477 ecosystems. Science. 1997;227: 194–199.
478 White JR, Nagarajan N, Pop M. Statistical methods for detecting differentially abundant features
479 in clinic metagenomic samples. PLoS Comput Biol. 2009;5: e1000352.
480 Xu Z, Hansen MA, Hansen LH, Jacquiod S, Sorensen S. Bioinformatic approaches reveal
481 metagenomic characterization of soil microbial community. PLoS One. 2014;9: e93445.
482 Zarraonaindia I, Owens SM, Welsenhorn P, West K, Hampton-Marcell J, Lax S, Bokulich NA,
483 Mills DA, Martin G, Taghavi S, van der Lelie D, Gilbert JA. The soil microbiome
484 influences grapevine-associated microbiota. mBio. 2015;6: e02527.
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Table 1(on next page)

Descriptive information of each sampling site

Descriptive information of each sampling site.

	Ocoa, Chile	Leyda, Chile	Apalta, Chile
Latitude	32º 52' S	33º 34' S	34º 36' S
Longitude	71º 7' W	71º 22' W	71º 7' W
Altitude	307 m	216 m	268 m
Mean temperature	14.7 ºC	16.2 ºC	14.6 ºC
Precipitation	354 mm	457 mm	731 mm
pH forest soil[1]	7.87	6.86	6.34
pH vineyards soil[2]	8.1 ± 0.1	7.8 ± 0.5	7.5 ± 0.4
Forest soil content (sand, silt and clay)	73% – 16% – 11%	67% – 22% – 11%	47% – 37% – 15%
Vineyard soil content (sand, silt and clay)	56% – 38% – 16%	61% – 26% – 13%	61% – 27% – 12%
Soil taxonomy	Alfisol	Alfisol	Alfisol
Vine variety	Cabernet Sauvignon	Sauvignon Blanc	Syrah
Planting year (± SD)	2002 ± 3	2006 ± 1	2001 ± 4

[1] pH in forests was determined from a single soil sample, whereas [2] pH in vineyards was determined in each plot and the mean (± standard deviation) is shown.

Table 2 (on next page)

Abundances of taxonomic groups in forest and vineyard soils

Values are shown as percentage abundance regarding to each habitat (mean ± standard deviation) *P*-values are associated to White's non-parametric t-test (White et al. 2009). Phyla are arranged in a decreasing abundance.

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Abundances of taxonomic groups in forest and vineyard soils. Values are shown as percentage abundance regarding to each habitat (mean ± standard deviation) *P*-values are associated to White's non-parametric t-test (White et al. 2009). Phyla are arranged in a decreasing abundance.

Taxa	Forest	Vineyard	*P*-value
Archaea			
Euryarchaeota	0.5799 ± 0.0370	0.6175 ± 0.0574	0.4936
Crenarchaeota	0.0996 ± 0.0141	0.1142 ± 0.0143	0.3567
Thaumarchaeota	0.0497 ± 0.0265	0.0717 ± 0.0276	0.4742
Korarchaeota	0.0113 ± 0.0026	0.0139 ± 0.0071	0.6789
Nanoarchaeota	0.0005 ± 0.0007	0.0000 ± 0.0000	0.4969
Bacteria			
Proteobacteria	51.0242 ± 0.4965	49.9281 ± 1.0682	0.2436
Actinobacteria	20.6467 ± 1.8879	20.3850 ± 1.5398	0.9150
Acidobacteria	7.5432 ± 0.9247	7.4808 ± 0.9583	0.9692
Bacteroidetes	3.8503 ± 0.4733	4.2730 ± 0.5838	0.4858
Firmicutes	2.8427 ± 0.2245	3.1089 ± 0.2131	0.2786
Planctomycetes	2.4040 ± 0.0465	2.6990 ± 0.4469	0.4300
Chloroflexi	2.0369 ± 0.2347	2.1631 ± 0.2270	0.6178
Cyanobacteria	1.9463 ± 0.1292	2.0760 ± 0.1980	0.4978
Verrucomicrobia	1.5537 ± 0.3182	1.5892 ± 0.1445	0.9242
Deinococcus-Thermus	0.6184 ± 0.0487	0.6346 ± 0.0102	0.6942
Chlorobi	0.5003 ± 0.0332	0.5443 ± 0.0853	0.5456
Unclassified	0.3305 ± 0.0464	0.3645 ± 0.0292	0.4578
Thermotogae	0.1574 ± 0.0089	0.1811 ± 0.0279	0.3036
Spirochaetes	0.0961 ± 0.0023	0.1012 ± 0.0152	0.6906
Aquificae	0.0947 ± 0.0064	0.1042 ± 0.0112	0.3486
Dictyoglomi	0.0820 ± 0.0090	0.0874 ± 0.0088	0.5947
Synergistetes	0.0798 ± 0.0129	0.0855 ± 0.0015	0.5792
Chlamydiae	0.0515 ± 0.0143	0.0429 ± 0.0154	0.6103
Fusobacteria	0.0493 ± 0.0045	0.0433 ± 0.0067	0.3467
Deferribacteres	0.0308 ± 0.0008	0.0380 ± 0.0040	0.0558
Elusimicrobia	0.0205 ± 0.0049	0.0265 ± 0.0065	0.3531
Tenericutes	0.0073 ± 0.0031	0.0125 ± 0.0014	0.0794

Eukaryota						
Ascomycota	0.2680	±	0.0366	0.2430	±	0.0727	0.7153
Streptophyta	0.1096	±	0.0717	0.0651	±	0.0024	0.4544
Chordata	0.0565	±	0.0126	0.0524	±	0.0040	0.7117
Unclassified	0.0520	±	0.0736	0.0123	±	0.0098	0.4997
Arthropoda	0.0190	±	0.0053	0.0221	±	0.0015	0.4889
Nematoda	0.0152	±	0.0027	0.0100	±	0.0043	0.1911
Apicomplexa	0.0040	±	0.0024	0.0032	±	0.0024	0.7717
Basidiomycota	0.0006	±	0.0008	0.0009	±	0.0013	1.0000
Phaeophyceae	0.0005	±	0.0007	0.0000	±	0.0000	0.4969
Cnidaria	0.0000	±	0.0000	0.0005	±	0.0007	1.0000
Viruses	0.0302	±	0.0074	0.0243	±	0.0075	0.4814
Unassigned	2.7352	±	0.0830	2.7795	±	0.2877	0.8572

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Table 3(on next page)

Microbial species that exhibited significantly different abundances (%) between forest and vineyard soils based on the SEED database

Points indicate the differences between forest and vineyard soils (blue and orange bars, respectively), and the values at the right show the p-values obtained with a White's non-parametric t-test (White et al. 2009).

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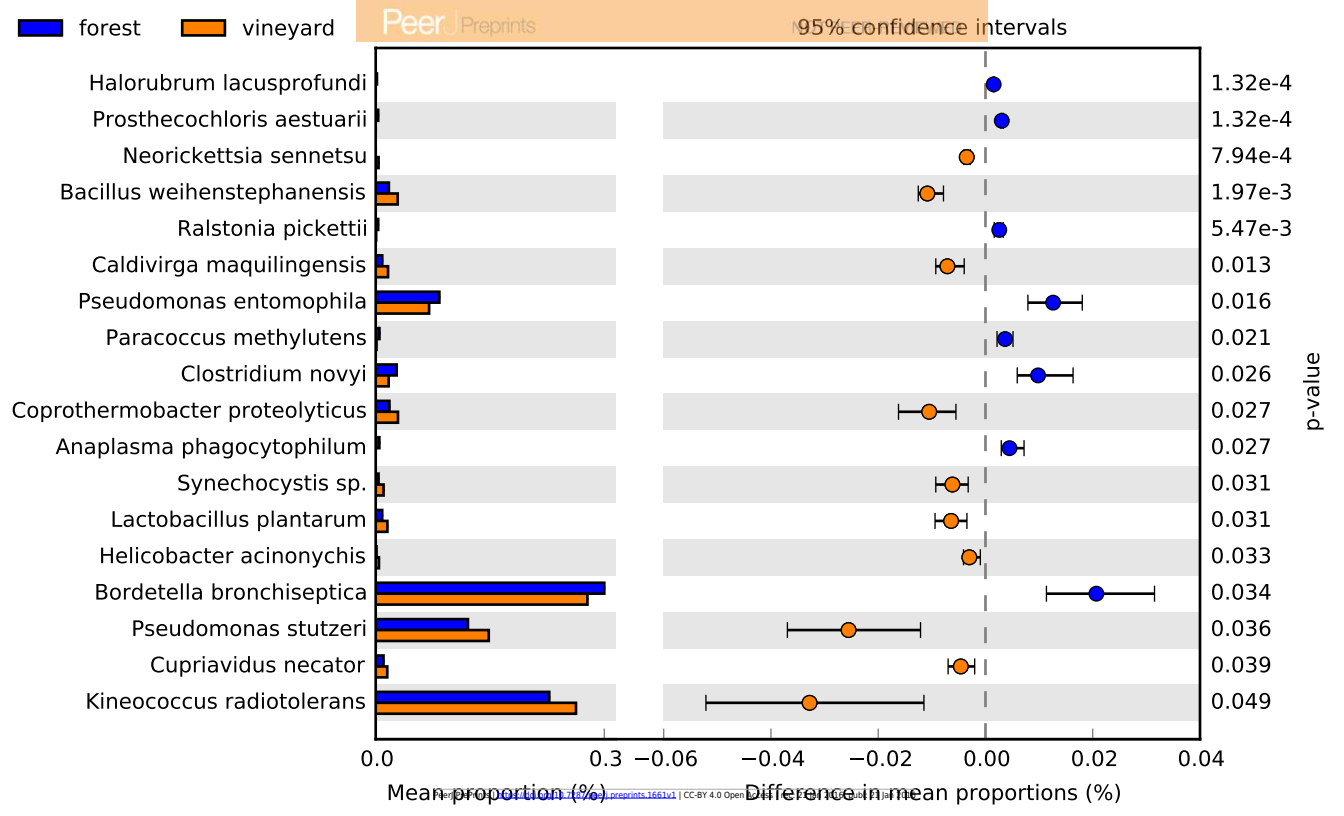
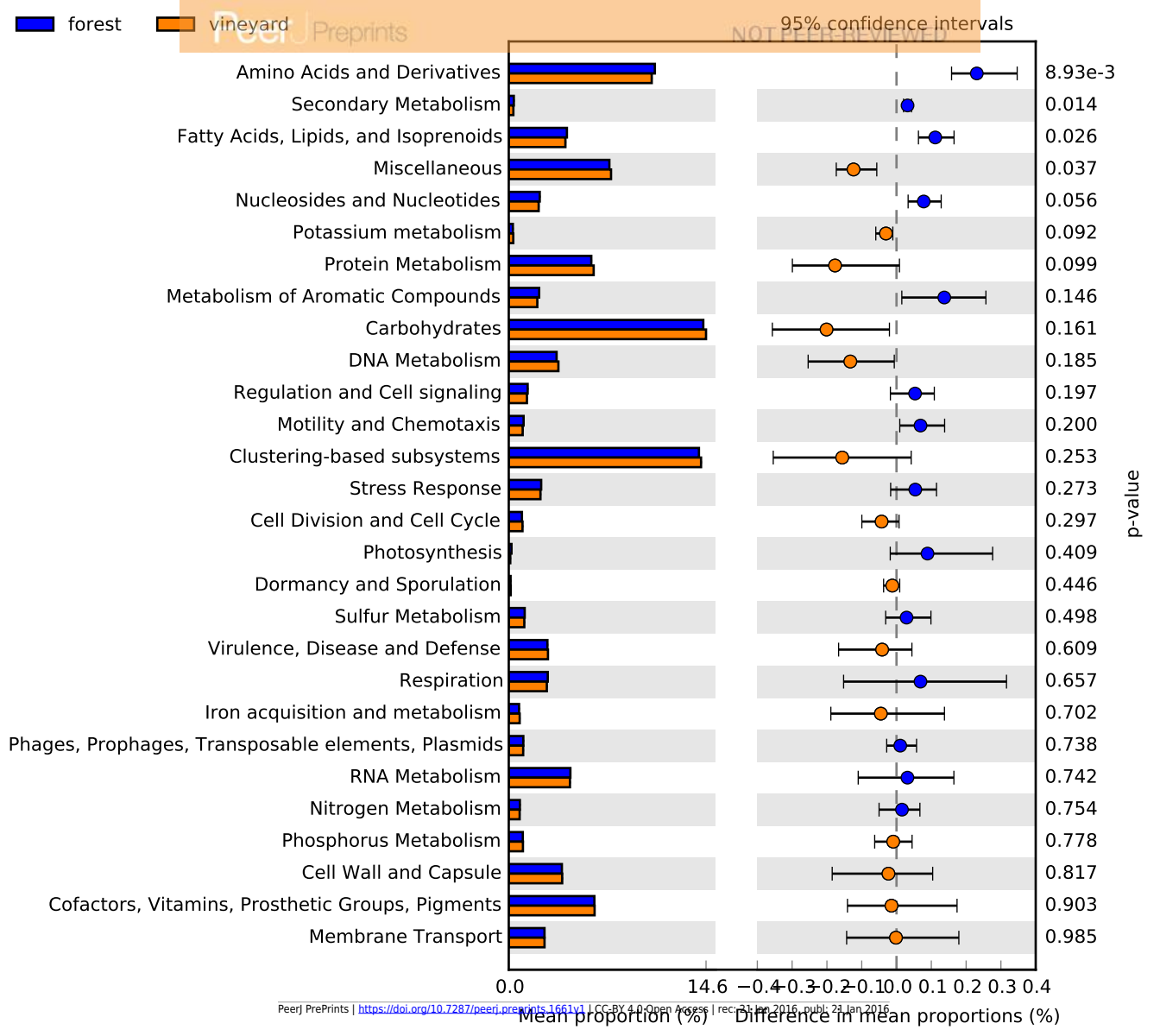


Table 4(on next page)

Mean proportion (%) of functional categories found in soil microbial communities based on the Subsystem database

Points indicate the differences between forest and vineyard soils(blue and orange bars, respectively), and the values at the right show the p-values obtained with a White's non-parametric t-test (White et al. 2009).



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