

CAPÍTULO 3: Land-use modulates soil bacterial and fungal diversity after drought-induced decline in Holm-oak Mediterranean ecosystems

Summary

The magnitude of the effects of climate-change induced tree decline over diversity and structure of soil microbial communities is still poorly understood. In this sense, how resilient these communities are will determine the capacity of ecosystems to recover after episodes of tree decline. Although not taken so much into account, the use to which a forest has been subjected not only affects its ability to respond to climatic perturbations but also its ability to recover from the climate-induced mortality episodes. Here, we wanted to test how resilient soil microbial communities are to drought-induced tree decline and how forest management (land-use) can modulate the response of these communities after a perturbation triggered by tree mortality. For that, we studied soil microbial (bacteria and fungi) diversity and composition in natural forests, man-made intensively managed savannahs called *dehesas*, and open woodlands, formed from the abandonment of *dehesas*. In these three land-use scenarios, we investigated the effect of holm oak decline in soil microbial communities by sampling under healthy, affected and dead holm oaks, as well as from bulk soils away from tree influence. We used Illumina technology to estimate the alpha and beta diversity of the bacterial and fungal soil communities. We found out that soil microbial communities from forests and *dehesas* were taxonomically different and presented different responses to tree decline. For instance, in forests, structure and diversity of fungal communities remained highly unaltered, while bacterial communities experience strong changes during decline. In *dehesas*, the trend was the opposite of that found in forests, with a more resilient bacterial community and highly altered fungal community. On the other hand, both bacteria and fungi experienced changes during decline in open woodlands, lying between that under forests and that under *dehesas*. Our results, therefore, suggest that land-use determine the degree of soil bacterial/fungal resilience after climate-induced tree decline, demonstrating how the suppression of anthropic activities (abandonment of *dehesas*) reverts in a process of recovery of microbial communities towards values of diversity and taxonomic identity found in forests (long-term resilience).

Introduction

Given the importance of soil microbial communities for the overall functioning of ecosystems (e.g. Van Der Heijden et al. (2008)), understanding how climate change and land-use affect soil microbial communities has become a relevant issue (Jangid et al., 2011; Barba et al., 2016a; de Carvalho et al., 2016; Bastida et al., 2019). Climate change-induced forest decline has been related to the increase on quantity and duration of drought episodes in the last decades (Adams et al., 2010; Allen et al., 2010; Anderegg et al., 2012; Hereş et al., 2018). Tree defoliation and dead (tree decline) induced by the increase in the length and intensity of water-limiting periods (droughts), triggers cascading effects that ultimately affects the structure and functioning of soil microbial communities (Curiel Yuste et al., 2012; de Sampaio e Paiva Camilo-Alves, 2013; Avila et al., 2016; Curiel Yuste et al., 2019). This is because trees transform the soil abiotic (microclimate, pH) and biotic (e.g. fine root production, secretion of exudates and enzymes, etc...) environment (e.g. Curiel Yuste et al. (2019)) of their surrounding soil, so changes in tree health and subsequent death greatly affects the microbial communities that inhabit it. In this regard, tree decline may exacerbate climate-induced changes in soil microbial communities, ultimately affecting soil stoichiometry and soil nutrient cycling (García-Angulo et al., submitted).

The impact of tree decline on soil microbial communities is not linear. In the early stages, tree decline generally results in an increase in soil carbon (C) due to the increment in litter (specially leaves and fine roots) deposition, and in a subsequent increase in soil CO₂ emissions (Kaňa et al., 2013). However, in the last stages of decline, when the trees dies, carbon labile forms are drastically reduced as the supply of radical exudates stops (Štursová et al., 2014). These decrease in labile C supply affects the structure of soil microbial communities, favoring soil microorganisms capable of decomposing more recalcitrant carbon (C) sources, as Verrucomicrobia (Martinez-Garcia et al., 2012) or saprotrophic fungi (Ma et al., 2013; Voříšková and Baldrian, 2013) over more copiotrophic microorganisms such as Proteobacteria (Pascault et al., 2013; Goberna et al., 2016; Yan et al., 2018). This decrease in the labile C source provided by trees has been further associated with a decrease in soil heterotrophic respiration and soil CO₂ emissions (Avila et al., 2016). On the other hand, the content of nitrogen (N) in soils will increase with tree decline due to an increment in N litter, altering soil stoichiometry and microbial communities (Griffin et al., 2011; Rodríguez et al., 2017).

However, it is expected that impacts of tree decline over the structure, taxonomic composition and functioning of microbial communities will be also regulated by how forests have been managed and which land-use have been given. For instance, it is expected that in dense and less managed forest, diversity and structure of microbial communities will be more resilient to an event of tree decline, since the system has more capacity to absorb perturbation and/or recover (Nave et al., 2011; Barba et al., 2013; Barba et al., 2016a). This is because depending on the scale of the mortality event, there is a fast-subterranean colonization of the microorganisms and roots associated with surrounding living trees and surrounding regenerated seedlings (Curiel Yuste et al., 2012; Curiel Yuste et al., 2019; Rodríguez-Calcerrada et al., 2019). However, it has been observed that changes in bacterial community structure may be fast after an event of tree mortality, preceding changes in soil carbon quality (Curiel Yuste et al., 2012). On the contrary, in open woodlands, where adult trees are sparse, and grasses dominate the understory vegetation, it is expected that tree decline will exert a strong change over the diversity and functioning of soil microbial communities (Rodríguez et al., 2017; Rodríguez et al., 2019). In the Iberian Peninsula, for instance, it is very common to find open forests, especially those dominated by *Quercus* species such as *Quercus ilex* or *Quercus suber*, for livestock use (Ayánz, 1994; Pulido et al., 2010). These open woodlands, called “dehesas”, have been greatly affected by climate change and pathogen attacks during the last decades (Lloret et al., 2004; Corcobado et al., 2013b; Corcobado et al., 2014; Rodríguez et al., 2017; Hereş et al., 2018), having experienced very pronounced dieback processes in the southwestern part of the peninsula (Avila et al., 2016; Gómez-Aparicio et al., 2017; Gallardo et al., 2019). In these open woodlands once the tree dies, it would be expected that the demography and biomass of certain fungal guilds, e.g. ectomycorrhiza from the tree or the population of saprotrophs specialized in digestion of woody tissue, would be negatively affected, also because cattle are actively preventing the natural regeneration of the trees (Zamora et al., 2001; López-Sánchez et al., 2014). On the other hand, in a system mainly dominated by annual and perennial grasses, it is expected that diversity and structure of bacterial communities will be strongly linked to the grass compartment, and hence less affected by tree mortality. Understanding how forest management may modulate the capacity of functional and structural recovery of soil microbial communities to an event of tree mortality may help us also sizing the magnitude and the severity of the potential perturbation of the system associated with climate-change induced tree mortality.

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The aim of this study was to assess how forest management/use may modulate the response of soil microbial communities (diversity and composition) to episodes of climate-change induced tree mortality. We conducted a study to compare these responses between sites with high density of overstory trees (forests) versus sites where forest have been transformed into open woodlands for livestock use (dehesas). We also included a third category that includes sites previously used as dehesas that has been abandoned (open woodland) that are characterized by having an understory dominated by shrubs and seedlings. We used high-throughput sequencing technologies to make DNA metabarcoding from soil bacterial and fungal communities. To understand the effect of *Quercus ilex* decline in each ecosystem, at each studied stand, sampling was conducted under trees with different health status (healthy, affected and dead holm oaks), as well as in soils under no tree influence. Based on previous bibliography, we hypothesize that i) the diversity and composition of soil bacteria will change in natural (forests) with tree defoliation while in managed (dehesas) and open woodland ecosystems would remained more stable, ii) soil fungal communities would be more affected by Holm oak decline in managed ecosystems due to cattle and nutrient availability.

Material and methods

Study area

We selected 13 locations across the Iberian Peninsula following two criteria: (1) locations where Holm oaks (*Quercus ilex* subs. *ballota*) have experienced symptoms of decline (e.g. defoliation) in the last decade (Lloret et al., 2004; MAGRAMA, 2007; Corcobado et al., 2013a; Camarero et al., 2014); and (2) locations where Holm-oaks are subjected to a land-use representative of the Iberian landscape. We selected three categories of land-use: two contrasting ecosystems (forest and dehesas), and a transitional woodland (open woodland) resulting from the abandonment and/or the change of use from dehesas (e.g. hunting or logging). To extend the information about the study area used in this chapter, please go to pages 31- 33.

Experimental design and soil sampling

The information related to the experimental design and soil samples can be found on pages 34 - 38. As a brief summary, for each location, we took soil samples under, and away (out the crown projection over soil) from, *Q. ilex*'s influence. To study the

effect of Holm oak's decline in soil microbial communities, we selected trees with three different health status based on the defoliation level of their canopies: healthy trees (defoliation level up to 25%), affected trees (defoliation level from 25 % to 99 %) and dead trees (defoliation level of 100 %) (Rodríguez et al., 2017; Hereş et al., 2018).

Soil biogeochemical variables

For this chapter, we used only the information related to pH, SOC and mineral N. The methodology can be found on page 38.

DNA extraction and Illumina data processing

We extracted soil DNA from each soil sample using PowerSoil DNA Isolation kit (MoBio, Laboratories, Inc) (Pages 43 - 45). After that, we submitted our samples to the Research Technology Support Facility (RTSF) at Michigan State University (MSU) to analyze the metabarcoding of bacterial and fungal communities (extended information can be found on pages 45 - 46). Once the DNA samples were analyzed, we performed a bioinformatic analysis (pages 46 - 51) using the platform Qiime 1.9.1 (Caporaso et al., 2010).

Statistical analyses

We used R v.3.5.1 (R Core Team, 2016) to perform the statistical analysis of bacterial and fungal communities among each land-use, and within them, for each defoliation degree and bulk soil. We imported and cleaned the microbial data with the package phyloseq [v. 1.24.2; McMurdie and Holmes (2013)]. We filtered the data using the conservative threshold (0.005% of total sequences) recommended by Bokulich et al. (2013). Furthermore, we used rarefaction curves to check the quality of our sampling method, removing all samples that did not reach asymptote. Once we filtered the datasets, we calculated the alpha-diversity with Shannon (H') and Simpson's reciprocal (1/D) indices as a measure of diversity and Pielous' index (J') as evenness. We rarefied the data to calculate the beta-diversity, using phyloseq package to estimate the weighted UniFrac and Bray distances for bacteria and fungi, respectively. To visualize the beta-diversity, we plotted a Non-metric Multidimensional Scaling (NMDS) with the *metaMDS* function and performed a permutational test to check the homogeneity of multivariate dispersion with *betadisper* function, both from vegan package [v. 2.5-4; Oksanen et al. (2019)]. As the permutational test didn't show any difference in the dispersion of the samples, we performed a permutational ANOVA

(PERMANOVA) using the *adonis* function from the *vegan* package. Since our study presented a nested experimental design (13 different sites and several treatments), we used the option *strata = site* included in the *adonis* function, which allows the analysis of this type of nestedness. To explain the differences showed in the PERMANOVA analyses, we calculated the relative abundance to phylum level for each defoliation degree and bulk soil of our land-uses. Moreover, we complemented the relative abundance graphics with a mixed effect model for each phylum, using site as a random parameter. For the detection of indicator species, we used the indicator value [IndVal, Dufrêne and Legendre (1997)] using the *indval* function from the *labsv* package [v. 1.8-0; (Roberts, 2007)].

Results

Land-use effects over soil environment

We obtained the average soil pH, SOC and soil mineral nitrogen for each land-use category (Table 14). Forests and open woodlands showed similar soil pHs, ranging from 6.70 in forest to 7.01 units in open woodlands, and mineral N. However, in dehesas, we observed a significantly lower soil pH, with more acidic soils (5.58 units). Values of mineral N were, on the other hand, higher in dehesas than in forests and open woodlands. On the other hand, SOC remained highly unaltered in both extremes of our land-use gradient, increasing their values in open woodlands.

Table 14. Environmental variables for each land-use. The letter represents significant differences among land-use for each soil variable. pH = Soil pH; SOC = Soil Organic Carbon; Mineral N = Soil Mineral Nitrogen

Land-use	pH	SOC	Mineral N
Forest	6.70 (0.14) A	2.47 (0.11) A	254.80 (14.99) A
Open Woodland	7.01 (0.08) A	3.32 (0.19) B	274.21 (13.99) A
Dehesa	5.58 (0.07) B	2.39 (0.10) A	323.06 (10.45) B

General characteristics of Illumina datasets and alpha-diversity indices

We obtained a total of 3,607,958 and 4,968,402 raw reads for bacterial and fungal sequences, respectively; which after the filtering and removal of low represented OTUs (see above), it resulted in 2,063,572 and 3,953,106 sequences for each bacterial and fungal community, respectively. From theses trimmed sequences, we obtained 2,203 OTUs for bacteria and 1,661 OTUs for fungi. Good's coverage values ranged from 80.1%

to 93.6% for bacterial samples and 96.7% to 99.9% for fungal samples, showing that both communities were well characterized. Alpha-diversity measured by Shannon, inverse Simpson and Pielous' indices showed no differences in richness and diversity of soil microbiota among land-uses (Table 15), or defoliation degree (including also soils under no tree influence; Table 19). Alpha-diversity of soil bacterial communities was significantly higher in dehesas soils than in soils from the other two land-use categories ($H'_{forest} = 6.05$, $H'_{dehesa} = 6.23$, $H'_{open\ woodland} = 6.08$, $P < 0.001$), whilst fungal communities were no significantly different among land-use categories ($H'_{forest} = 3.79$, $H'_{dehesa} = 3.64$, $H'_{open\ woodland} = 3.89$, $P < 0.10$). Within these land-uses, soil microbial communities under dehesas were the only one affected by defoliation, at some extent (Table 19).

Table 15. Alpha-diversity of bacterial and fungal communities among land-uses. H' = Shannon index, $1/D$: Simpson index, J' = Pielou's index. Different letters mean statistically (p -value < 0.05) significant differences between different land-uses.

Soil microbial community	Index	Land-use		
		Forest	Open woodland	Dehesa
Bacterias	H'	6.05 (A)	6.08 (A)	6.23 (B)
	$1/D$	149.87 (A)	164.10 (B)	187.71 (C)
	J'	0.85 (A)	0.85 (A)	0.86 (B)
Fungi	H'	3.79 (A)	3.89 (A)	3.64 (A)
	$1/D$	25.29 (A)	20.73 (A)	23.34 (A)
	J'	0.71 (A)	0.68 (A)	0.69 (A)

Beta-diversity analyses

We did not observe any difference in the multivariate dispersion of the weighted UniFrac (wUniFrac) and Bray distance from bacteria and fungi. However, NMDS separating the data in land-use categories showed different soil microbial composition in dehesas with respect to forest and open woodland (Figure 20). The analysis of beta-diversity within each land-use showed a homogeneous dispersion of the samples too, but different centroids among defoliation degrees, including soils collected under no tree influence. PERMANOVA analyses showed that bacterial community composition was affected the most by defoliation degree under forest and open woodlands. In forest, bacterial communities under healthy trees were different that those under defoliated and dead trees while in open woodlands, bacterial communities under dead trees were different that those found under "living trees" (healthy and defoliated) (Table 16 & 17). On the other hand, bacterial community composition did not show any sensitivity to tree defoliation under dehesas, whatsoever. Nevertheless, in all three

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land-uses, the presence of the tree influenced soil bacterial and fungal community structure, as evidenced in the significant differences found between soils under trees and out of the tree influence (Tables 16 & 17).

Regarding fungal communities, the PERMANOVA analyses yielded opposite results to those obtained for bacteria (Tables 16 & 17). Under forests and open woodlands, the composition of the fungal communities was not sensitive to the tree defoliation degree, while under dehesas fungal communities suffered strong changes in composition when tree start to defoliate (Tables 16 & 17).

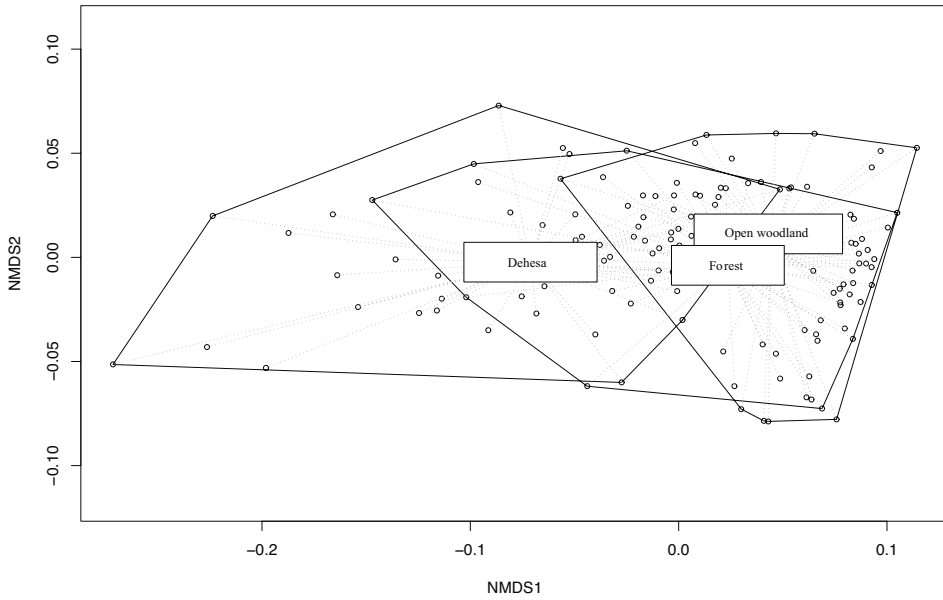
Table 16. PERMANOVA analyses of soil microbial communities (bacteria and fungi) comparing the composition from the different defoliation degrees and bulk soil within each land-use. Asterisks (*) represents statistically significant differences with p-values < 0.05, and dots (.) represent marginal differences.

Land-use	Level	Bacteria		Fungi	
		F	P	F	P
Forest	Healthy vs Affected	0.81	0.015*	0.70	0.53
	Healthy vs Dead	0.74	0.037*	0.72	0.47
	Affected vs Dead	0.26	0.89	0.71	0.49
	Bulk soil vs Healthy	2.18	0.001*	1.54	0.001*
	Bulk soil vs Affected	1.26	0.004*	1.36	0.006*
Open woodland	Bulk soil vs Dead	1.18	0.001*	1.55	0.001*
	Healthy vs Affected	0.36	0.77	0.68	0.88
	Healthy vs Dead	0.84	0.019*	0.97	0.06
	Affected vs Dead	0.65	0.039*	0.81	0.28
	Bulk soil vs Healthy	3.21	0.001*	2.01	0.001*
Dehesa	Bulk soil vs Affected	2.79	0.001*	1.84	0.001*
	Bulk soil vs Dead	2.08	0.001*	1.90	0.001*
	Healthy vs Affected	0.62	0.63	1.03	0.022*
	Healthy vs Dead	0.96	0.14	1.08	0.018*
	Affected vs Dead	0.41	0.85	0.61	0.97
	Bulk soil vs Healthy	9.21	0.001*	3.10	0.001*
	Bulk soil vs Affected	7.80	0.001*	3.22	0.001*
	Bulk soil vs Dead	7.27	0.001*	2.99	0.001*

Table 17. PERMANOVA results of soil microbial communities (bacteria and fungi) comparing the composition from different defoliation degrees and bulk soil within each land-use. Different letters mean statistically (p -value < 0.05) significant differences between tree health and/or bulk soil.

Soil microbial community	Land-use	Tree health			Bulk soil
		Healthy	Affected	Dead	
Bacteria	Forest	A	B	B	C
Bacteria	Open woodland	A	A	B	C
Bacteria	Dehesa	A	A	A	B
Fungi	Forest	A	A	A	B
Fungi	Open woodland	A	AB	B	C
Fungi	Dehesa	A	B	B	C

A)



B)

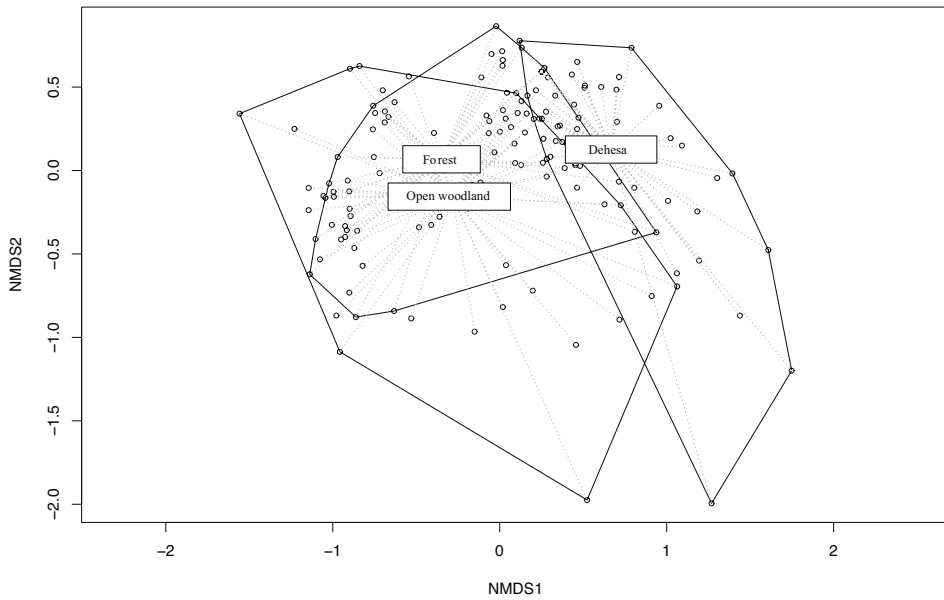


Figure 20. Non-metric multidimensional scaling (NMDS) of; A) bacteria with the wUniFrac distance; B) fungi with Bray distance; among land-uses.

Soil bacterial and fungal composition

The changes in the relative abundance at phylum level with defoliation degree, together with the mixed effect model obtained for each phylum (Figures 21 & 22), showed the alterations in the microbial composition associated with defoliation also observed by the NMDS (Figure 23). In all land-uses, the most representatives' phyla for bacteria, with more than 90% of their relative abundance, were Acidobacteria, Actinobacteria, Bacteroidetes, Planctomycetes, Proteobacteria and Verrucomicrobia; while in fungi, were Ascomycota and Basidiomycota. Mixed effect models showed the significant differences of these phyla based on its defoliation degree (Figure 21 & 22). Acidobacteria and Actinobacteria significantly increased and decreased, respectively, with defoliation degree in forest ecosystems (Figures 21a & 21b); while in dehesa and open woodland, Actinobacteria and Proteobacteria significantly decreased in their relative abundance with defoliation (Figures 21b & 21e). Besides, Verrucomicrobia did not show any whatsoever, though it showed not-significant positive trends with tree defoliation in forest and open woodland (Figures 21f). The relative abundance from the different phyla in soils under no tree influence was in general following the trend observed with Holm oak decline, i.e. values of abundances were closer to those found under dead trees, with Acidobacteria and Verrucomicrobia increasing, and Actinobacteria and Proteobacteria decreasing with respect to living trees (Figure 23).

The relative abundance of the most abundant fungal phyla did not show any sensitivity to defoliation under forest ecosystems (Figure 22). Under dehesas and open woodlands, however, Ascomycota and Basidiomycota presented a significant (p -value < 0.05) increased and decreased with defoliation, respectively, being this trend specially marked under dehesas (Figures 22a & 22b). As in bacteria, soils under no-tree influence followed the defoliation trend, with abundances more similar to that under dead than under living trees (Figure 23).

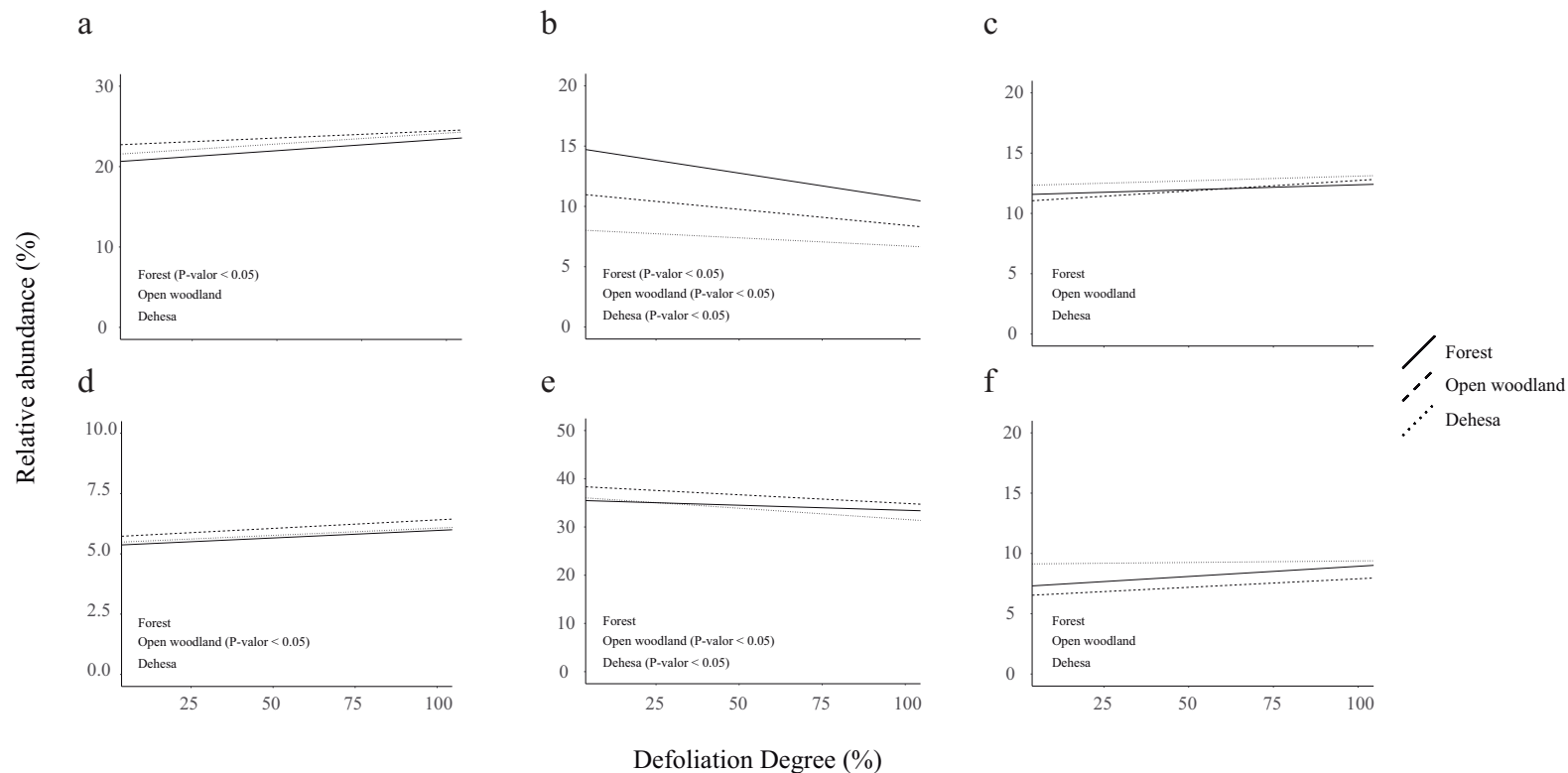


Figure 21. Mixed effect models of bacterial relative abundance according to holm oak defoliation among land-uses (Forests: solid line; Open woodlands: dashed line; Dehesas: dotted line). Locations were used as random effect. a) Acidobacteria; b) Actinobacteria; c) Bacteroidetes; d) Planctomycetes; e) Proteobacteria; f) Verrucomicrobia.

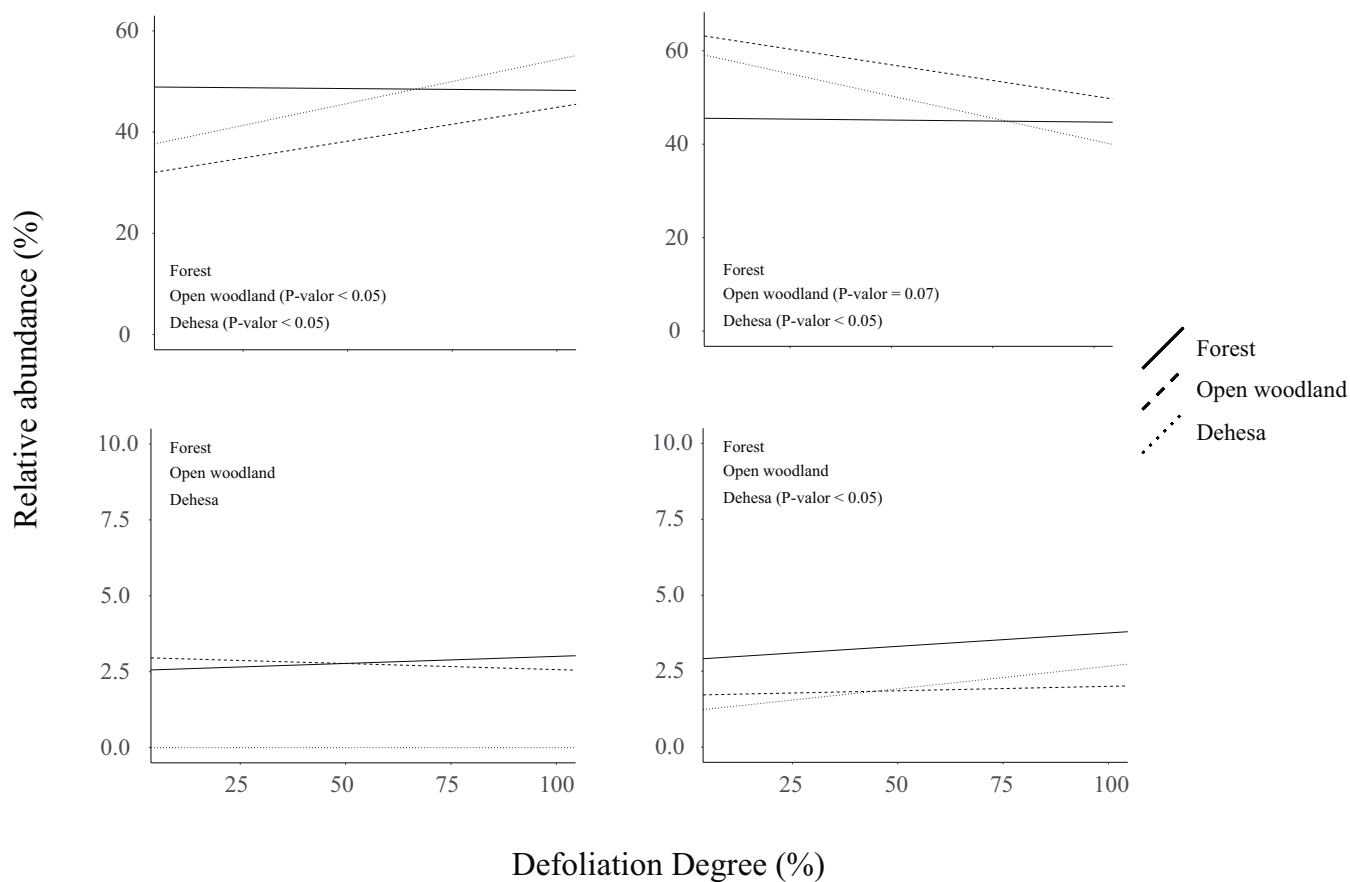


Figure 22. Mixed effect models of fungal relative abundance according to holm oak defoliation among land-uses. Locations were used as random effect. A) Ascomycota; B) Basidiomycota; C) Mortierellomycota; D) Mucoromycota.

Indicator species of each land-use

The values calculated by the IndVal index showed that dehesas presented more bacterial and fungal species than the rest land-uses (Table 18). These results were more evident in bacterial communities, with an IndVal of 27.42%; while in fungi was only a 3.60%. We also calculated the IndVal index associated with tree defoliation within each land-use, observing results similar in all of them for bacteria and fungi (Table 20). In all cases, the absence of tree influence increased the number of indicator/unique species, and this trend was especially marked under dehesas.

Table 18. Indicator species for bacteria and fungi in each land-use, represented by the Indicator value (IndVal).

Soil microbial community	Land-use		
	Forest (%)	Open Woodland (%)	Dehesa (%)
Bacteria	8.12	13.25	27.42
Fungi	1.46	0.73	3.60

Discussion

We investigated here the alpha and beta diversity of soil bacterial communities as a means of understanding the capacity of soil biota under holm-oaks stands to absorb a perturbation and/or their capacity to recover after a perturbation (resilience) such as that caused by anthropic effects (land-use) or climate change induced tree mortality. Intensive transformation of natural forests in the Mediterranean area into open woodlands, intensively used for livestock (dehesas) causes, as expected, large changes in the diversity and composition of soil microbial communities (de Carvalho et al., 2016; Flores-Rentería et al., 2016; Petersen et al., 2019). This anthropic transformation of the forest into savanna-like ecosystems negatively affected especially saprophytic, mycelial-formers phyla, such as Actinobacteria and fungi from the Ascomycota and Basidiomycota phyla, which also represent the majority of the ectomycorrhizal fungi (Landeweert et al., 2001; Tedersoo et al., 2010; Tedersoo and Smith, 2013). On the other hand, it affected positively to prokaryotic phyla more associated with copiotroph environments, such as Proteobacteria (Pascault et al., 2013; Goberna et al., 2016; Yan et al., 2018) and other phyla like Acidobacteria, which further resulted in an increase in alpha diversity of prokaryotes with respect to forests and open woodlands. Hence, this anthropic transformation of forest into dehesa reflected a logical functional change towards a soil community less specialized in oxidation of carbon and tree symbiotic

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relations, but more diverse, probably because the increase in plant alpha and beta diversity (Hiiesalu et al., 2014; Prober et al., 2015) and the input of nutrients from grazing animals (specially N and P) (Herridge et al., 2008; Gómez-Rey et al., 2012) that creates new niches in contrast with under the influence of trees. This new niches favors the predominance of some phyla (de Carvalho et al., 2016; Petersen et al., 2019) since tree coverage alleviates the environmental effect of sun and wind exposure (Pimentel and Kounang, 1998); while serves as a C source (Blagodatskaya and Kuzyakov, 2008). On the other hand, the recovery of the composition and diversity of soil microbial communities observed after abandonment of decades of intensive use in old-dehesas evidences the strong resilience of these systems to a long-term perturbation, evidencing the capacity of soil natural systems to recover. For instance, Zornoza et al. (2009) observed an increase in microbial biomass and richness with the abandonment of agricultural soils supporting their hypotheses of soil recovery, while Holtkamp et al. (2008) found that land abandonment is going to facilitate the slow recovery of above and belowground communities.

It is also possible that differences in pH between dehesas and forest/open woodlands may also explain part of the observed trends, since it is well known that pH is the best predictor of structure and diversity of soil microbial diversity (Fierer and Jackson, 2006; Lauber et al., 2009; Rousk et al., 2009). The observed tendencies, however, indicate the opposite of what it would be expected, since acidic pH is usually affecting negatively the soil bacterial diversity (Fernández-Calviño and Bååth, 2010; Tripathi et al., 2018) and generally favors fungal communities, which are more tolerant to acidic conditions (Rousk et al., 2010).

Land management determines the response and resilience of soil microbial communities after tree decline

The observed shifts in the alpha and beta diversity, as well as in the relative presence of the most abundant prokaryote and fungal phyla, suggest that tree defoliation and mortality have the capacity to transform the belowground environment, triggering a process of ecological succession, as has been observed in the aboveground environment in previous studies (Lloret et al., 2004; Barba et al., 2013; Gazol et al., 2018b). However, we here show how the degree of anthropic transformation of the forest had a very strong impact on the response of the soil microbiota to the environmental perturbations caused by tree decline and subsequent

death (Curiel Yuste et al., 2012; Rodríguez et al., 2017; García-Angulo et al., submitted). Overall, our results support our hypothesis and shows how under denser stands microbial communities are more resilient and able to absorb perturbations associated with tree mortality more efficiently than systems where overstory cover is less dense. This trend was especially reflected in the observed resilience of fungal communities under forest, i.e. tree mortality did not have any effect over abundance or alpha and beta diversity of fungal communities, whatsoever. Under less denser canopies (open woodlands and dehesas) soil fungal communities underwent large changes associated with tree decline: a decrease in relative abundance of Basidiomycota, to which most of the ectomycorrhizal fungi belong (Tedersoo et al., 2010; Sato et al., 2015) and an increase in Ascomycota, which is a phylum dominated by saprophytic organisms specialized in degrading long-chained structural organic compounds (e.g. lignin, celluloses and hemicelluloses) and recalcitrant organic matter in general (Voříšková and Baldrian, 2013; Hudson et al., 2015; Janusz et al., 2017). Hence, under less dense stands tree defoliation and mortality is pushing soil fungal communities towards a more saprophytic and less symbiotic-like functioning. The observed decrease with defoliation in the presence of the most abundant prokaryotic phylum, Proteobacteria, clearly suggest that under less dense systems (open woodlands and dehesas) there is a strong trend towards a less copiotroph soil environment (Pascual et al., 2013; Goberna et al., 2016; Yan et al., 2018), dominated by microbiota specialized in degradation of more recalcitrant SOM (e.g. Ascomycota). This is because tree defoliation and subsequent mortality limits the supply of substrate in the form of labile carbohydrates (exudates or rhizodeposition) demanded by more copiotrophic organisms (e.g. Proteobacteria), symbionts (e.g. mycorrhiza), and other soil rhizospheric communities (e.g. Högberg et al. (2001); Binkley et al. (2006); Barba et al. (2016b); Curiel Yuste et al. (2019)). As expected, therefore, tree defoliation and mortality under less dense overstorey systems was associated with cascading causal-effect relations causing substantial changes in the biological functioning, diversity and taxonomic composition of the soil system (Avila et al., 2016; Rodríguez et al., 2017; Flores-Rentería et al., 2018; Rodríguez et al., 2019) bringing the systems to new equilibriums dominated by microbiota associated with understory vegetation (e.g. grasslands, (Evans et al., 2017; García-Angulo et al., submitted)). This is further supported by the fact that in less dense canopies, soil microbial communities under dead trees resembled those observed in open areas where understory vegetation (grass and shrubs) dominates. Our results coincide with the functional resilience

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generally observed under denser forest stands after episodes of tree mortality (e.g. Nave et al. (2011); Barba et al. (2016a); Curiel Yuste et al. (2019); Rodríguez-Calcerrada et al. (2019)) in contrasts with results obtained in open, more transformed, woodlands (e.g. Gómez-Aparicio et al. (2008); Gómez-Aparicio et al. (2012); Lloret et al. (2015); Rodríguez et al. (2017); Rodríguez-Calcerrada et al. (2019)) where tree mortality results in large soil stoichiometry imbalances also associated with large changes in the ecology and functioning of soil microbial communities.

We, therefore, hypothesize that stand tree density determines the resiliency of the soil system to a climate change driven episode of forest dieback. Density of the tree determines density of root system and rhizospheric related microbiota (e.g. hyphal networks) that maintains the communication and functioning of the system and allows a fast-functional recovery of the system after episodes of tree mortality in forest systems (Van Der Heijden and Horton, 2009; Barto et al., 2012; Simard et al., 2012). Given all these new insights on soil ecology and its role in forest functioning, there are opportunities to improve forest management plans oriented to maintain the health of the soil microbiota and, therefore, of the forest under scenarios of climate change.

Conclusions

In this chapter, we found out that the abandonment of highly managed land-uses (dehesas) and subsequent spontaneous tree establishment forming open woodland structures, was associated with the recovery of states of soil microbial communities similar to those found in forests. Furthermore, in the most managed ecosystems the bacterial community remained highly unaltered, suggesting the presence of different soil niches apart from the ones formed by trees due to the high colonization of the herbaceous layer and/or the presence of grazing animals. On the other hand, in more dense ecosystems (forests) the fungal community showed high resilience to tree defoliation, indicating a better capacity of the system to recovery after this perturbation, since a more established mycorrhizal network will promote the regeneration of trees. Nonetheless, for all our land-use gradient, soil microbial communities, bacteria and fungi, showed a parallelism in the structure and abundance among the soil microorganisms found far from tree influence and the trends followed when tree defoliates and died, suggesting that soil microbial communities may lose their capacity to recovery to these perturbations and change to new equilibriums, like the ones presented in savannah-like ecosystems.

Supplementary material

Table 19. Alpha-diversity of bacteria and fungi at defoliation degree and bulk soil from each land-use. H' = Shannon index, $1/D$: Simpson index, J' = Pielou's index. Different letters mean statistically (p -value < 0.05) significant differences between tree health and/or bulk soil.

Soil microbial community	Land-use	Index	Tree influence			Bulk soil
			Healthy	Affected	Dead	
Bacteria	Forest	H'	6.06 (A)	6.06 (A)	6.09 (A)	6.00 (A)
		$1/D$	146.18 (A)	147.56 (A)	164.45 (A)	140.99 (A)
		J'	0.85 (A)	0.85 (A)	0.85 (A)	0.85 (A)
	Open woodland	H'	6.10 (A)	6.07 (A)	6.08 (A)	6.04 (A)
		$1/D$	167.80 (A)	163.52 (A)	166.15 (A)	159.15 (A)
		J'	0.85 (A)	0.85 (A)	0.85 (A)	0.85 (A)
	Dehesa	H'	6.28 (A)	6.31 (A)	6.29 (A)	6.01 (B)
		$1/D$	183.42 (AB)	202.24 (A)	201.86 (A)	163.32 (B)
		J'	0.86 (AB)	0.87 (A)	0.87 (A)	0.85 (B)
Fungi	Forest	H'	3.85 (A)	3.97 (A)	3.83 (A)	3.91 (A)
		$1/D$	23.84 (A)	24.86 (A)	27.81 (A)	24.37 (A)
		J'	0.71 (A)	0.71 (A)	0.69 (A)	0.73 (A)
	Open woodland	H'	3.63 (A)	3.72 (A)	3.56 (A)	3.64 (A)
		$1/D$	21.28 (A)	22.80 (A)	18.85 (A)	19.89 (A)
		J'	0.67 (A)	0.70 (A)	0.67 (A)	0.69 (A)
	Dehesa	H'	3.63 (A)	4.13 (B)	3.77 (AB)	3.62 (A)
		$1/D$	18.87 (A)	31.67 (B)	23.12 (AB)	19.71 (A)
		J'	0.66 (A)	0.74 (B)	0.67 (AB)	0.69 (A)

Table 20. Indicator species for bacteria and fungi at each defoliation degree and bulk soil for each land-use, represented by the Indicator value (IndVal).

Soil microbial community	Land-use	Tree Influence (%)			Bulk soil
		Healthy	Affected	Dead	
Bacteria	Forest	1.51	0.50	0.78	3.71
	Open woodland	2.12	1.15	0.88	5.30
	Dehesa	3.71	2.13	1.57	11.49
Fungi	Forest	0.15	0.31	0.62	2.31
	Open woodland	0.36	0.14	0.29	3.21
	Dehesa	0.84	0.84	0.37	6.70

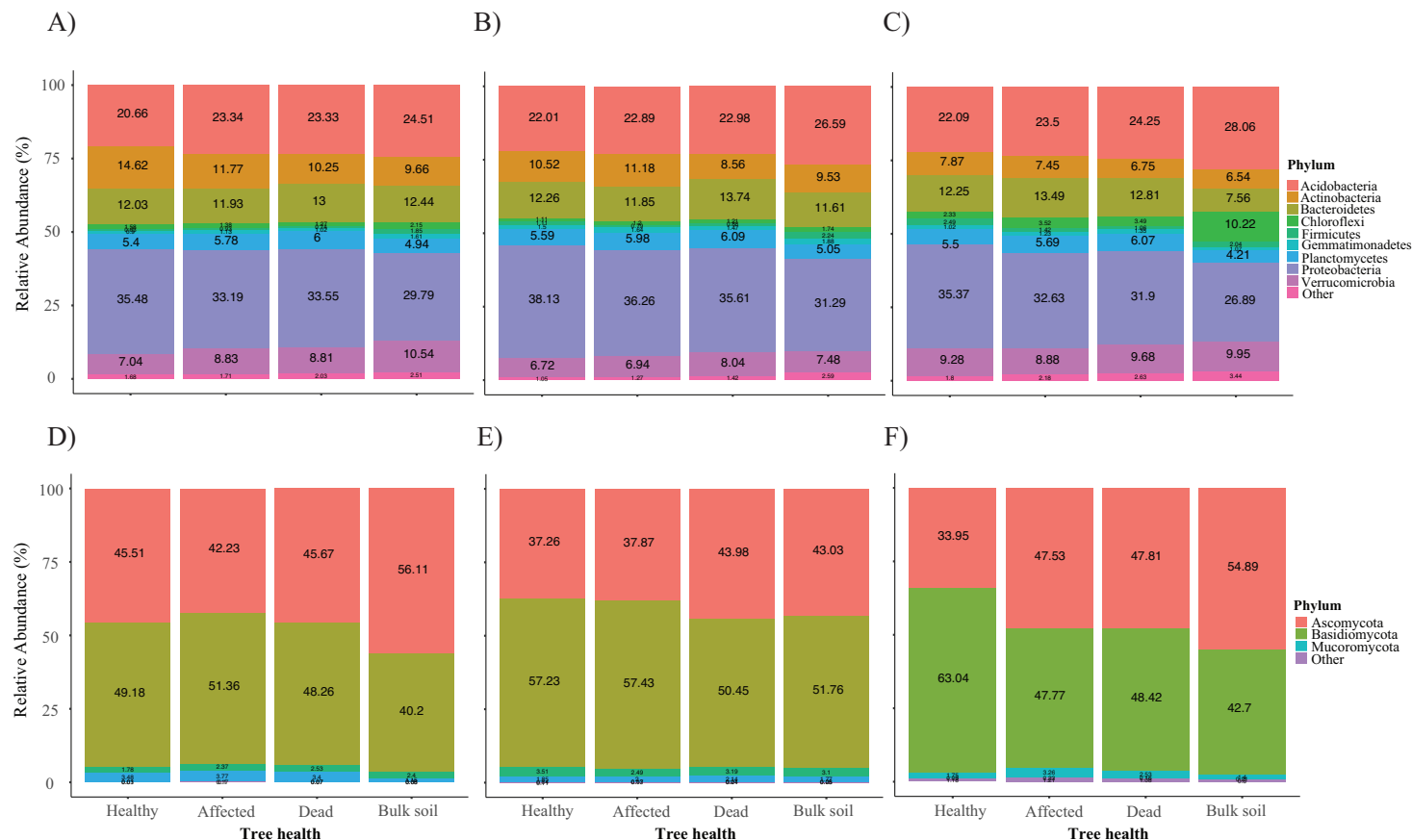


Figure 23. Relative abundance to phylum level for each land-use representing the differences among defoliation degrees and bulk soil. A) Relative abundance of bacterial communities in forests; B) Relative abundance of bacterial communities in open woodlands; C) Relative abundance of bacterial communities in dehesas; D) Relative abundance of fungal communities in forests; D) Relative abundance of fungal communities in open woodlands; E) Relative abundance of fungal communities in dehesas.