CAPÍTULO 2: Land-use intensity modulate the sensitivity and recovery of soil core microbial communities in Mediterranean Holm oaks

Summary

Land-use is, together with climate change, a major driver of habitat degradation, affecting woodland ecosystems worldwide. Effects may be direct, such as soil disturbance or fragmentation, or indirect, through changes in soil biodiversity and the functions performed by the soil microbiota. However, in depth regional studies exploring how the intensity of land-use may alter soil microbiota and its vulnerability to environmental perturbations are still missing, hindering our ability to understand the relationships between above and below ground communities. Here, we investigated the structure of the co-occurrence networks of soil microbiota under holm oaks (*Quercus ilex* subsp. *ballota*) along a gradient of land-use intensity ranging from heavily managed dehesas to relatively intact forests. Our study covers the whole area of distribution of holm oaks in the Iberian Peninsula, and include gradients in climate, pH and tree health. We used a filter-step criteria based on abundance and presence to define and study the structure and composition the core community of soil bacteria and fungi, which represent the most abundant taxa from each soil microbial community. Our results show a strong effect of land-use intensity over the composition and diversity of the core soil microbiota, resulting in a higher proportion of climate-sensitive microorganisms towards the most managed end of our land-use intensity gradient. Keystone taxa, identified through co-occurrence networks, showed similar responses to environmental and soil variables as the rest of the core community. The transformation of dehesas into open woodlands due to a natural process of ecosystem restoration seemed to alleviate the effect of land-use in soil microbiota, returning the structure and composition of the soil core community to levels similar to unperturbed forests.

Introduction

Anthropogenic activities on natural systems are, together with climate change, major drivers of the ongoing global change. These factors exert effects on the health and functioning of woodland ecosystems worldwide (Allen et al., 2010; Smith et al., 2016), affecting directly above-ground diversity by removing (e.g. deforestation) or replacing tree species with more profitable or adaptive species (Hereş et al., 2012). Furthermore, these factors also have indirect effects through the belowground microbiota associated to tree species, which has been further linked to changes in soil stoichiometry and nutrient storage (Barba et al., 2013; Gazol et al., 2018a). These changes are, in turn, also known to affect aboveground plant growth and resilience to climate change (Barba *et al.*, 2013, Gazol *et al.*, 2018, Garcia-Angulo et al. *submitted*), resulting in a feedback between the below and above ground communities. Several studies show that the transformation of forest into croplands or pastures results in shifts in the abundance and diversity of soil microbial communities (de Carvalho et al., 2016; Petersen et al., 2019), which may decrease soil organic carbon (SOC) and soil nitrogen (N) (Murty et al., 2002; Wei et al., 2014). Other anthropogenic activities, such as thinning (removal of selective trees to increase nutrients, water and light availability) or tillage (mechanical manipulation of the soil to increase crop production), also have the capacity to alter soil microbial communities and nutrients (Anderson et al., 2017; Wu et al., 2019). Furthermore, climate change may modify the structure and composition of aboveground vegetation by affecting tree health directly (e.g. tree dieback) (Allen et al., 2010; Carnicer et al., 2011; Anderegg et al., 2012), triggering a cascade of causal-effects that can end up also affecting soil microbiota and biogeochemistry (Rodríguez et al., 2017; Curiel Yuste et al., 2019). Likewise, changes in land-use intensity and anthropogenic activities have the capacity to, at least in some cases, mitigate the effects of climate change (Flores-Rentería et al. 2015). Thus, an understanding of the link between land-use intensity, belowground diversity and plant resistance to climate change is of foremost importance and can open unexplored venues for the amelioration and restoration of global change perturbations on natural ecosystems worldwide.

Understanding these dynamics is a pressing matter given current projections on climate change (IPCC, 2014), biodiversity loss and the increasing pressure by anthropogenic activities (IPBES, 2019). This is particularly relevant for areas that are both heavily managed and suffer strong effects of ongoing climate change. One of such

cases is the Mediterranean basin. Human presence and intensive land management along the Mediterranean spans thousands of years. This ecosystem is nowadays facing longer periods of warmer temperatures and lack of precipitation, hindering the correct functioning of soil microbiota (Sardans and Peñuelas, 2005). For instance, the development of activities such as agriculture, grazing, logging, and hunting in the Iberian Peninsula has been, and still is, highly widespread (Joffre et al., 1999; Guzmán Álvarez, 2016). Intensively managed grasslands with a very sparse canopy of evergreen oaks, are iconic agroecosystems called "dehesas" in Spain and "montados" in Portugal. Dehesas have a strong historical socioeconomic component, as they support livestock, agricultural production and forestry (Campos-Palacín, 1986; Joffre et al., 1999; Linares, 2007). However, many of these dehesas have been recently abandoned because of migrations of human populations towards urban areas, resulting in two contrasting scenarios: on one side, if the dehesa is not degraded and the soil maintains its proper characteristics, regeneration is highly possible, leading to open woodlands ecosystems (Pulido et al., 2013); on the other side, if the nutritional condition of the soil is deficient and/or the climatic conditions are adverse (e.g. increasing temperatures, drought episodes…), the regeneration of tree communities is not possible, resulting in grasslands (Corcobado et al., 2013b).

The aim of this study was to assess how soil microbiota (bacteria and fungi) differed along a gradient in land-use intensity, ranging from heavily managed dehesas, open woodlands (abandoned dehesas), to intact forests. We used a battery of complementary analysis to study the composition and structure of the core soil microbiota community and their sensitivity to abiotic variability (climate and pH) in a regional gradient and their relation to soil nutrient cycling (Carbon, Nitrogen and Phosphorus). We used co-occurrence networks to obtain an overview of the response of the core soil microbial community to land-use changes and environmental variables (Fuhrman, 2009; Ramirez et al., 2018), and to identify key taxa whose removal may cause drastic shifts in the structure and/or functioning of the community (Martín González et al., 2010; Fisher and Mehta, 2014; Herren and McMahon, 2018). Based on previous bibliography, we hypothesize that i) the structure and composition of the microbial core community (and the identity of the keystone taxa) will differ along land-use categories, ii) that the abandonment of the dehesas will revert soil microbial communities to previous stages (intact forests).

Material and methods

Study area

We selected 13 locations across the Iberian Peninsula covering the large distribution range of *Quercus ilex* subs *ballota*, representing all different land-use intensities at which holm oaks are subject to in the Mediterranean basin. In this regard, the Iberian Peninsula is a great laboratory, because all the spectra of land-use intensities are well represented, from protected forests with little or no management, to highly managed dehesas, used intensively for livestock grazing. And, in between, woodland formations (open woodlands) that generally result from the abandonment of anthropogenic activities in the dehesas. We categorized our sampling locations based on these three types of land-use intensities according to *Q. ilex* crown coverage. The climate of our sampling locations had a predominance of continental Mediterranean, characterized by rainfalls concentrated in spring and autumn, and summers with high temperatures and water scarcity. Mean annual temperatures (MAT) and mean annual precipitation (MAP) ranged from 10.62 - 16.15 º C and 326 - 919 mm, respectively (Felicísimo et al., 2011). Detailed information about the study area can be found on pages 31 - 33.

Experimental design and soil sampling

We took soil samples from soils under the influence of holm oaks (defined a 0.5 m from the trunk) and from soils away from the tree influence (defined as more than 0.5 m distance from the trunk). As, in our locations, holm oaks were affected by some degree of dieback, we took soil samples from trees with different health status. Tree selection and soil sampling can be found on pages 34 – 38 from “Material y métodos” section.

Soil biogeochemical analysis

We characterized the chemical environment of each of the 156 soil samples. We measured soil pH, SOC, $_{av}P$, NH_4^+ - N, NO_3^- - N, following the methodology describes on page 38 from “Material y métodos”.

DNA extraction and bioinformatic analysis of 16S and ITS sequencing

DNA extraction and bioinformatic analysis is described on pages 43 - 51. As a brief summary, we extracted soil DNA using PowerSoil DNA Isolation kit (MoBio, Laboratories, Inc) and we submitted the resulting DNA to the Research Technology Support Facility at Michigan State University, where they create the necessary libraries to carried out the metabarcoding of soil bacterial and fungal communities. After that, we performed the bioinformatic analysis of the sequences (pre-processing, overlapping, clustering, etc.) using Qiime 1.9.1 (Caporaso et al., 2010).

Core community analysis and co-occurrence networks

The analysis of soil microbial core community and co-occurrence networks is explained in depth on pages 56 - 58. In these analyses, we studied the core community of soil microbiota for each of our land-use categories. We calculated co-occurrence networks for bacteria and fungi separately from each land-use category using the R package "spieceasi" (v. 1.0.2; Kurtz et al. (2015)). In these co-occurrence networks, nodes are bacteria or fungi OTUs, and links between nodes represent associations between the microorganisms (global network descriptors in Table 13). We estimated the importance of individual nodes with two complementary centrality metrics: Normalized Degree and Betweenness Centrality. Normalized Degree (ND) measures the proportion of connections a focal node has out of the total possible connections in its network. Nodes with high ND are local hubs, as they have direct links to a greater proportion of the community (Martín González et al., 2010). The Betweenness Centrality (BC) of a node quantifies the proportion of shortest paths between any two nodes in the network pass through the focal node. Nodes with high BC are connectors, as they link areas of the network that would otherwise be poorly connected (Martín González et al., 2010). Both metrics are normalized within each network, allowing direct comparisons across networks. Topological keystone species are local hubs (OTUs with high ND), connectors (OTUs with high BC) or network hubs (OTUs with both high ND and BC), and maintain the overall network structure and dynamics through their linkage architecture (Martín González et al., 2010; Banerjee et al., 2016b). To identify keystone taxa, we established thresholds in these centrality metrics based on the distribution of centralities among species: a threshold of ND=0.8 for bacterial and fungal networks and all land-use categories, whereas the threshold for BC ranged

between 0.5-0.8 in bacteria and fungi and among the land-use categories (Tables 9 & 10, Figures 18 & 19).

Statistical analyses

We performed all statistical analyses on R v. 3.5.1 (R Core Team, 2016) and the packages "phyloseq" (v. 1.24.2; McMurdie and Holmes (2013)) and "vegan" (v. 2.5-4; Oksanen et al. (2019)). We used the Shannon, Inverse Simpson and Pielou's evenness indexes to calculate the alpha diversity and evenness of the core microbiota community and examined potential differences between land-use categories with an ANOVA with Tukey's HSD correction. We visualized dissimilarities in the core community composition among different land-use categories through a NMDS of the beta diversity for each microbial community, using the Bray distance as a dissimilarity index and 500 tries to achieve data convergence (Oksanen et al., 2019). We thereafter compared the relative abundance of phyla among land-uses with an ANOVA with Tukey's HSD correction and studied the relationship between phyla abundance and environmental variables with a correlation heatmap using the function "taxa.env.correlation" ("microbiomeSeq"; Lahti and Shetty (2017)). Environmental variables included climate (MAT and MAP), pH, tree defoliation, tree influence and soil nutrients [soil organic carbon (SOC), ammonium (NH4- - N), nitrate (NO3+- N) and available phosphorus ($_{av}$P)]. We duplicated the previous analyses related to relative abundance of phyla and the correlation heatmap for the study of our keystone OTUs.

Results

Core community analysis

Our complementary analyses on different aspects describing the core community of soil microbiota showed how the composition, structure and sensibility of soil microbiota differed along the gradient in land-use intensity. Furthermore, although the relative abundance of bacterial and fungal communities in dehesas differed from that of open woodlands and forests (Table 8), these responded differently to other variables associated with land-use intensity. Specifically, alpha and beta diversity of the core bacterial communities differed significantly across land-use categories (Table 7, Figure 12a). Co-occurrence networks confirmed these differences, and further showed that the structure of the core community was more similar between forest and open woodlands (Tables 9 & 10). Contrastingly to bacteria, fungal alpha diversity remained almost unaltered among the land-use categories (Table 7), and there was a complete overlap in the beta diversity between forest and open woodlands core communities (Figure 12b).

Regarding environmental and soil variables (Figure 13), core bacterial communities showed the strongest association with NH_4^--N, pH, and $_{av}$P in forests; with MAT and pH in open woodlands; and with $_{av}$P, climate (MAT and MAP), pH and tree influence in dehesas. Contrastingly, only one fungal phylum, Mortierellomycota, was affected in the forests, while in open woodlands, pH was the only relevant variable, having significant correlations with all phyla except Mortierellomycota, which here was solely affected by MAP. Finally, Ascomycota and Basidiomycota were highly affected by climate and soil nutrients (SOC, NH_4^- - N, NO_3^+- N) in dehesas.

Table 7. Means (SE) of alpha diversity from the core community of soil bacteria and fungi in the different land-use categories. Different letters denote statistically significant differences ($P < 0.05$).

		Land-use category		
	Index	**Forest**	**Open Woodland**	**Dehesa**
Bacteria	Shannon	4.77 (0.03) A	4.93 (0.02) B	5.16 (0.02) C
	InvSimpson	64.34 (2.42) A	77.34 (2.90) B	88.29 (2.51) C
	Evenness	0.86 (0.00) A	0.87 (0.00) A	0.88 (0.00) B
Fungi	Shannon	3.40 (0.06) A	3.37 (0.07) A	3.57 (0.09) A
	InvSimpson	17.70 (1.30) A	17.63 (1.26) A	20.17 (1.57) A
	Evenness	0.73 (0.00) A	0.71 (0.01) A	0.71 (0.02) A

Table 8. Means (SE) of the relative abundance of the core community of soil bacteria and fungi in the different land-use categories. Different letters denote statistically significant differences ($P < 0.05$).

		Land-use category		
Soil community	**Phylum**	**Forest**	**Open woodland**	**Dehesa**
Bacteria	Acidobacteria	26.82 (0.61) A	27.93 (0.85) A	28.37 (0.79) A
Bacteria	Actinobacteria	10.49 (0.65) A	8.97 (0.63) A	5.62 (0.26) B
Bacteria	AD3	0.00 (0.00) A	0.00 (0.00) A	0.32 (0.07) B
Bacteria	Armatimonadetes	0.00 (0.00) A	0.00 (0.00) A	0.08 (0.00) B
Bacteria	Bacteroidetes	12.06 (0.65) A	11.13 (0.62) A	11.89 (0.61) A
Bacteria	Chloroflexi	0.98 (0.15) A	0.95 (0.08) A	3.62 (0.68) B
Bacteria	Crenarchaeota	1.07 (0.10) A	0.47 (0.06) B	1.05 (0.14) A
Bacteria	Cyanobacteria	0.00 (0.00) A	0.00 (0.00) A	0.29 (0.11) B
Bacteria	Elusimicrobia	0.00 (0.00) A	0.00 (0.00) A	0.09 (0.00) B
Bacteria	Firmicutes	0.96 (0.18) A	0.97 (0.13) A	2.11 (0.33) B
Bacteria	Gemmatimonadetes	1.16 (0.14) A	1.43 (0.17) B	1.09 (0.07) C
Bacteria	Nitrospirae	0.00 (0.00) A	0.00 (0.00) A	0.09 (0.01) B
Bacteria	Planctomycetes	2.82 (0.11) A	3.37 (0.09) B	2.47 (0.10) C
Bacteria	Proteobacteria	33.45 (0.58) A	37.01 (0.68) B	32.35 (0.76) A
Bacteria	Tenericutes	0.00 (0.00) A	0.00 (0.00) A	0.15 (0.02) B
Bacteria	Verrucomicrobia	10.20 (1.03) A	7.78 (0.58) B	10.31 (0.52) A
Bacteria	WPS-2	0.00 (0.00) A	0.00 (0.00) A	0.11 (0.02) B
Fungi	Ascomycota	64.37 (2.10) A	54.24 (2.83) B	58.40 (03.01) AB
Fungi	Basidiomycota	29.59 (2.19) A	37.47 (3.02) A	36.92 (3.07) A
Fungi	Entomophthoromycota	0.00 (0.00) A	0.00 (0.00) A	0.05 (0.02) B
Fungi	Glomeromycota	0.00 (0.00) A	0.00 (0.00) A	0.15 (0.04) B
Fungi	Mortierellomycota	3.99 (0.44) A	4.83 (0.49) A	1.28 (0.39) B
Fungi	Mucoromycota	1.95 (0.32) A	3.04 (0.41) A	2.94 (0.40) A
Fungi	Olpidiomycota	0.00 (0.00) A	0.00 (0.00) A	0.04 (0.03) B
Fungi	Oomycota	0.09 (0.02) A	0.37 (0.07) B	0.21 (0.04) C
Fungi	Rozellomycota	0.00 (0.00) A	0.05 (0.02) B	0.00 (0.00) A

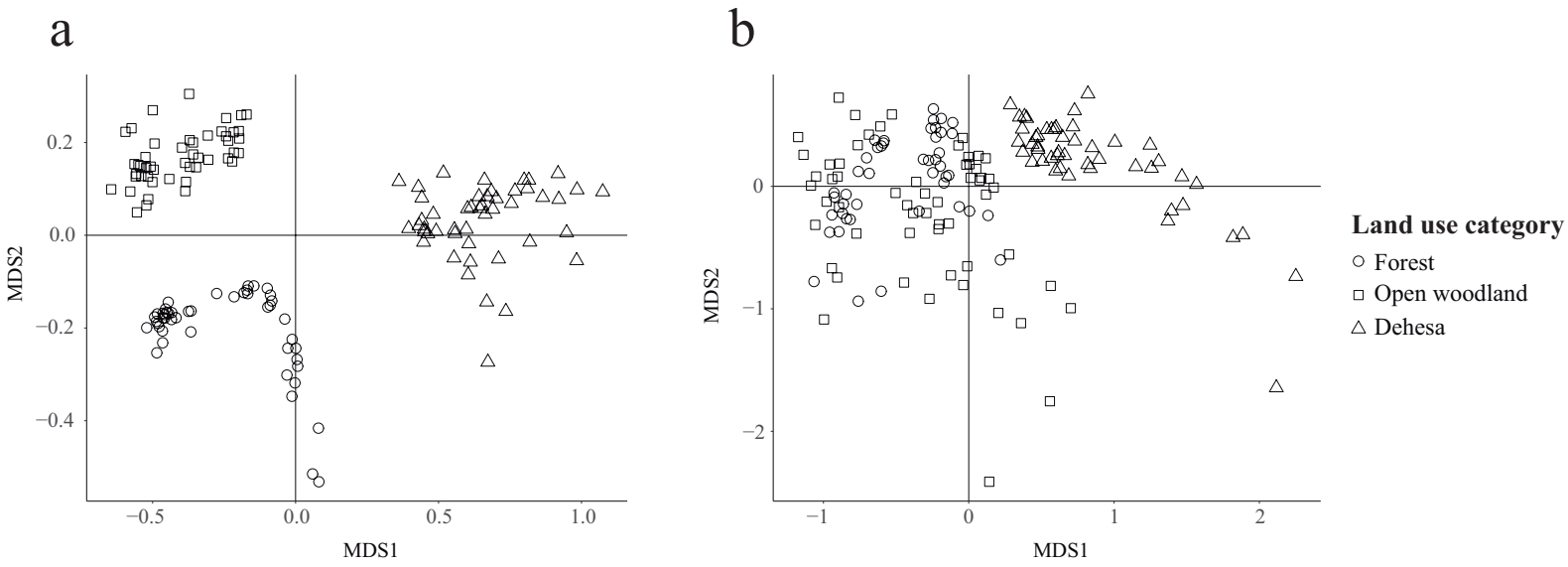

Figure 12. Non-metric multidimensional scaling of beta diversity from soil a) bacterial and b) fungal core communities according to land-use category.

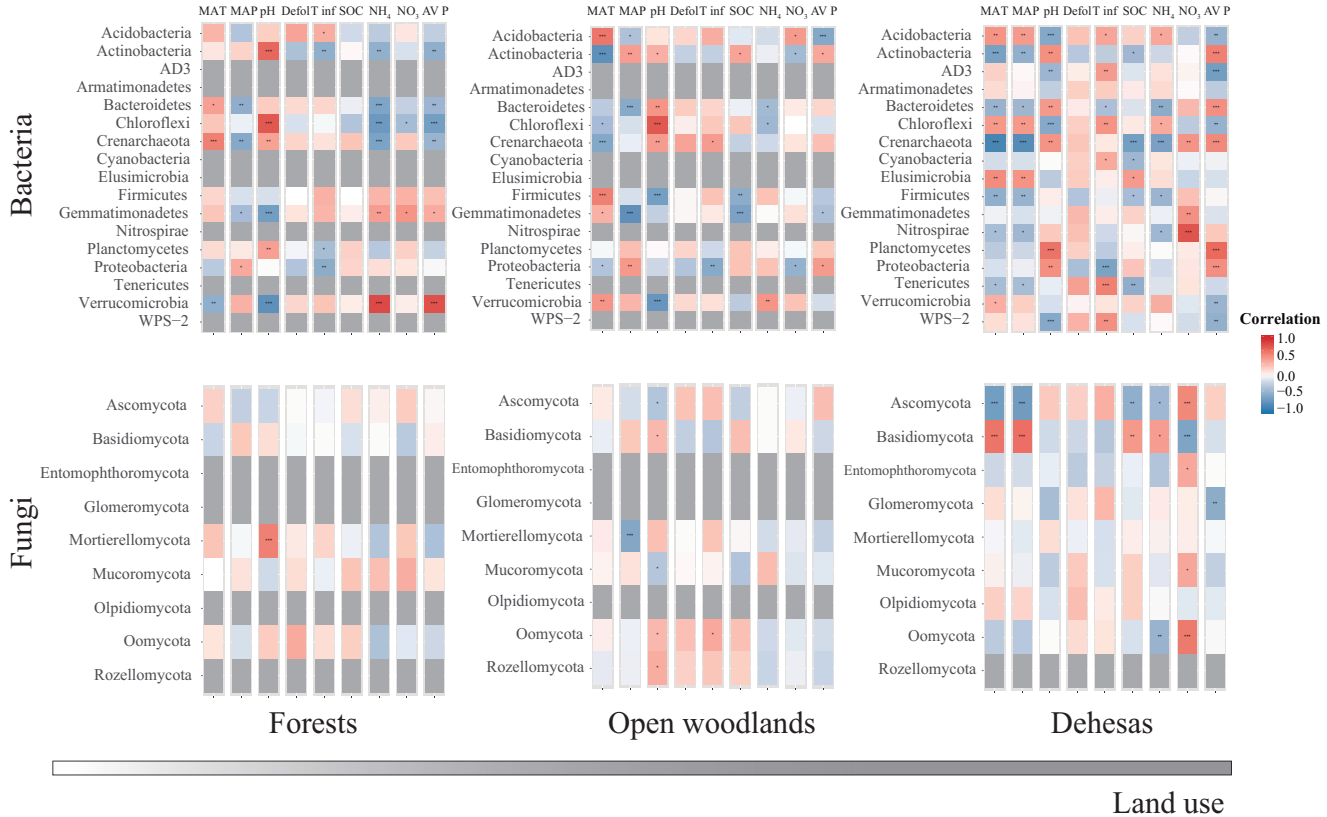

Figure 13. Correlation heatmap of the bacterial and fungal core communities with abiotic variables (climate and pH), tree influence and soil nutrients pools for each land-use. Grey colors denote the absence of that phylum in that land-use category. MAT = Mean annual temperature, MAP = annual precipitation, Defol = holm oaks' defoliation degree, T inf = tree influence, SOC = Soil Organic Carbon, NH_4^+-N = soil Ammonium, NO_3^--N = soil Nitrate and $_{av}$P = Available Phosphorus.

Keystone taxa analysis

The different land-use categories differed in the number and taxonomical composition of their keystone taxa (Figure 14, Table 9 & 10). Only nine of these bacterial and fungal keystones were found in more than one land-use category (Figure 14), with forests showing the highest number of shared OTUs (Tables 9 & 10). Regarding the relative abundance of keystone bacterial and fungal phyla, most keystone species varied in relative abundance between the land-use categories, but there was no clear pattern on this variation (Table 11).

Examining the bacterial keystone networks (Figures 15 & 16), local hubs were only present in forests, where they formed a highly linked area. This structure is lacking in the open woodland and dehesas networks, which were on the contrary more modular. Accordingly, the keystone type profile (network hubs-connectors-local hubs) differs along the gradient, with forest having 2-6-13, compared to 3-16-0 from open woodlands, and 7-5-0 in dehesas (Figure 15). Fungal keystone networks showed an opposite pattern, where forests had the sparsest and the only network without hubs (Figures 15 & 16). The proportion of connectors increased along the gradient in land-use intensity, with keystone type profiles (network hubs-connectors-local hubs) as follows: 5-14-0, 6-17-1 and 9-7-1. Most keystones belonged to Ascomycota (81.7 %), the phylum with the highest relative abundance across all land-use categories. Ascomycota keystones acted as network hubs, connectors, and local hubs. Contrastingly, keystone species belonging to other phyla only acted as connectors and only in some land-use categories — Basidiomycota in all land-uses, and Mortierellomycota (1.7%) and Mucoromycota (3.3%) saprotrophs, only in open woodlands — despite their relative abundance did not vary significantly from that of forests and dehesas (Table 8; only Mortierellomycota, having one keystone connector in open woodland, had a significantly lower relative abundance in dehesas). We were able to retrieve guild information for ~60% of the keystone fungal species but could not identify any pattern in terms of guilds and keystone type or land-use category (Table 10).

The analysis of abiotic variables and soil biogeochemistry for keystone taxa showed different sensibilities in the different land-use categories that resemble that of the core community (Figure 17). In general, keystone bacteria were highly affected by pH in all land-use categories. Apart from pH, in forests, the concentration of limiting

nutrients, like $_{av}P$ and NH_4^--N, had also a large effect, whereas in open woodlands, keystone bacteria were mostly affected by SOC and MAT, and in dehesas by climate (MAT and MAP) and tree presence (Figure 17). Fungal keystone species were also highly sensible to the effect of pH, but also to limiting nutrients like NH_4^--N and $_{av}P$ in forests, and SOC and climate in dehesas. No clear patterns were found in the open woodlands, where two more additional phyla were also identified as keystone connectors (Mortierellomycota and Mucoromycota).

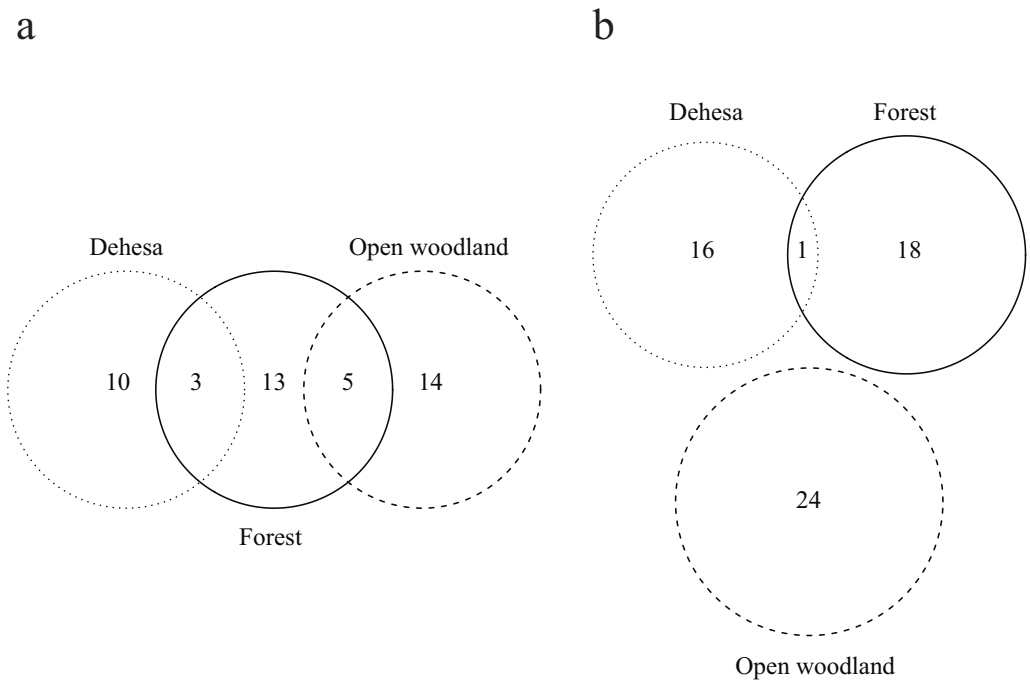

Figure 14. Venn diagrams showing number of OTUs in common between the different land-use categories for soil a) bacteria and b) fungi.

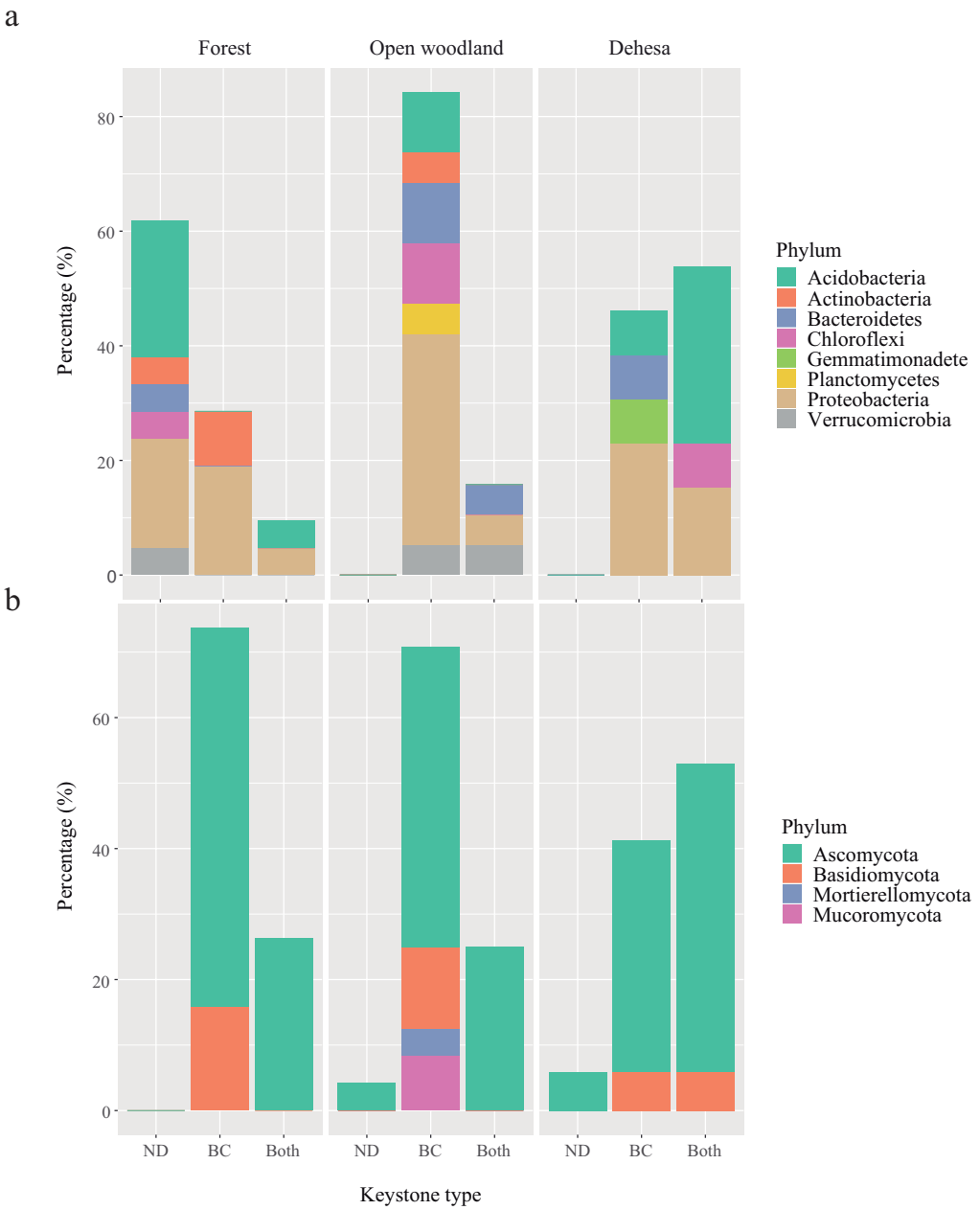

Figure 15. Summary of keystone species. Normalized Degree (ND) measures the number of connections of a node. Keystone OTUs with high ND are hubs, as they are directly linked to a greater proportion of the community. Betweenness centrality (BC) measures how many shortest paths between any two nodes in the network contain the focal node. Keystones with high BC are connectors, as they link areas of the network that are otherwise poorly connected. Percentage of keystone according to their type in a) soil bacteria and b) soil fungi for each land-use category.

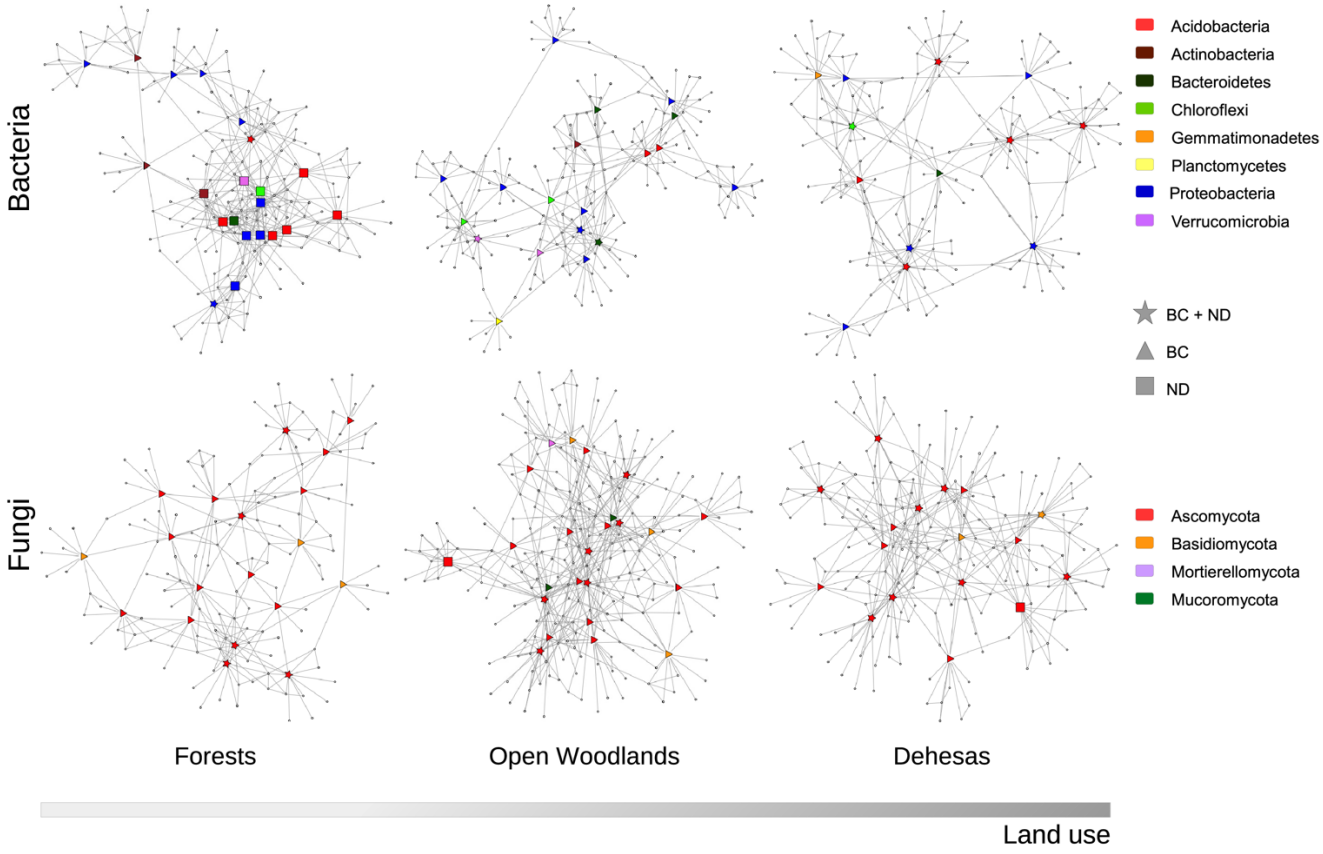

Figure 16. Soil microbial networks representing the structure shaped by bacterial and fungal keystones among land-use categories. Networks include only keystone nodes and their immediate neighbors. BC: keystones with high betweenness centrality (connectors); ND: keystones with high normalized degree (local hubs); BC + ND: keystones with high betweenness centrality and normalized degree (network hubs).

Discussion

Our study provides evidences about changes in composition and structure of the co-occurrence network of soil microbial communities associated with land-use intensity. Results obtained indicates that bacterial co-occurrence network was especially sensitive to changes in land-use, both in the general core community and among keystone OTUs. Also, the structure of the co-occurrence network of the core bacterial communities and the identity and abundance of bacterial keystone species was more sensitive to the gradient of climate, pH and nutrient availability of the study than that of the fungal communities. Hence, it is clear that fungal and bacterial communities from Holm-oak forest show very different sensitivity to the broad gradients of land-use intensity and environmental conditions of this study. On the other hand, abandonment of intense use (open woodlands) suggest that this trend can be to some extent naturally reverted with the abandonment of the anthropogenic activities. Our results further show how patterns found in core communities were also highly replicated in keystones species, suggesting that keystone species, as defined in our study, are species with a strong functional impact over the behavior of the whole microbial community. In this hyper-diverse communities, defining a reduced number of the keystone species, may be very useful to improve our understanding of the functioning and sensitivity of soil microbial communities to the environment.

Abandonment of intensively managed ecosystems facilitates recovery of soil microbiota

As in previous studies (de Carvalho et al., 2016; Szoboszlay et al., 2017; Petersen et al., 2019), the transformation of forest to agroecosystems resulted in an increase in soil bacterial taxonomic diversity, possibly as a consequence of an increase in the abundance and diversity of the herbaceous layer (Solly et al., 2014). Nevertheless, while soil bacteria showed a clear shift in their diversity, the fungal community remained highly unaltered along the axis of land-use, showing a higher resilience to transformations of the landscape associated with human activities (Urcelay et al., 2009; Griffiths and Philippot, 2013). This resilience might be associated to a better stablished plant-fungi interactions (Furze et al., 2017), since mycorrhizal networks conferred them the capacity to better tolerate the decrease in soil C and nutrients (Van Der Heijden and Horton, 2009; Barto et al., 2012) and/or to the capacity that some

fungi possess to the formation of spores, giving them the possibility to persist to environmental and/or human perturbations (Picone, 2000; Violi et al., 2008).

On the other hand, although soil bacterial abundance was different in natural (forests) and highly managed (dehesas) ecosystems, their dominant phyla remained highly unaltered, showing how ubiquitous bacterial phylum are among different locations and soils (Fierer, 2017). However, some of these phyla showed clear differences between forests and dehesas. In dehesas, where the distribution of trees is scatter and the herbaceous substrate prevailed (Diaz et al., 1997; Guzmán Álvarez, 2016), we found that phyla related to C availability, like Actinobacteria and Planctomycetes, decreased their abundances in comparison to forests (Wang et al., 2015; Banerjee et al., 2016b). Furthermore, dehesas ecosystems showed also an increase in the abundance of Firmicutes and Chloroflexi, which have been reported before by being associated with animal grazing in agroecosystems (Jangid et al., 2008; Petersen et al., 2019). This increase might be related with animal deposition since Firmicutes is a well-known rumen microbiota phylum (Guo et al., 2008; Min et al., 2019)

As we can see from these results, the contribution of trees can modulate the effect of soil microbial communities, demonstrating the importance of plant-soil interactions in the structure and diversity of soil microorganism. In this regard, we here showed that this process of aboveground spontaneous forest establishment following the abandonment of dehesas was aligned with a parallel recovery of the forest soils belowground structure and taxonomic diversity of the microbiota, i.e. the process of tree establishment after abandonment of dehesas was associated with a parallel convergence process of soil microbial diversities and compositions. This may support our hypothesis of soil microbial recovery to previous, and more natural, stages with secondary succession. Some studies have observed how land-use abandonment benefit the re-establishment of natural vegetation (trees and shrubs) (Rocha et al., 2016; Rolim et al., 2017) and the improvement of the soil nutrient quality and quantity (Zhao et al., 2005; Romero-Díaz et al., 2017). However, the processes that transform soil microbiota, affecting their diversity, composition and organization, after the abandonment of an intensive use are still unknown. We here speculate that tree recovery (seedlings) after abandonment increases labile forms of C from above (leaves) and belowground (root exudates), explaining the rapid increment of copiotrophic-like phylum such as Actinobacteria and Proteobacteria as well as the

decrease observed under open woodlands in the presence of phyla more associated with the decomposition of a more recalcitrant form of C such as Verrucomicrobia (Fierer et al., 2013; Banerjee et al., 2016a). The study of bacterial keystone species confirmed these results: forest and open woodlands shared 5 common keystone species, in contrast with the lack of common keystone species found between open woodland and dehesas. Furthermore, the *Rhizobiales* order, characterized by its capacity to capture N and by promoting its exchange to aboveground vegetation (Jones, 2015), were only found in forest and open woodland, suggesting a possible functional convergence among both ecosystems. The diversity of functions of the keystone species on the co-occurrence network also showed different pattern along the land-use gradient. While under forest the three different keystone types were represented (local hubs, connectors or network hubs), under open woodland and dehesas only two types of keystone species were observed (connectors and network hubs), which might suggest a network community more compartmentalized (Martín González et al., 2010). The complex aboveground structure found in these systems, where scattered trees creates patches with different microenvironmental conditions (grassland, shrubland or tree-dominated patches), may have favored this compartmentalization of the bacterial co-occurrence networks under open woodlands and dehesas (Joly et al., 2017). On the other hand, and in contrast to bacteria, the diversity and abundance of fungal core community remained highly unaltered along the land-use gradient, where 91 - 95% of the total fungal abundances belonged to Ascomycota and Basidiomycota (Fierer, 2017). These similarities found in all land-uses could be related to the formation of spores (Picone, 2000; Violi et al., 2008), giving them the ability to survive and proliferate under the regimes of environmental and anthropogenic perturbations at which they are exposed in this highly transformed systems. In spite of this, transformations of forest into dehesas presented some keystone species belonging to lower abundant fungal phyla (e.g. Mortiellomycota and Mucoromycota), apart from this the similarity in the diversity and structure of the soil fungal keystone species under forests and open woodlands with respect to dehesas (greater number of connectors under forests and open woodlands than under dehesas) further supported the hypothesis of the recovery of soil microbial structure associated with land abandonment.

Sensitivity to environmental variability and perturbations of soil microbial core communities from different land-use categories

The observed lower sensitivity to geographical abiotic and nutrient variability of fungi with respect to bacteria may be related to the capacity of fungi to generate resistance forms (Picone, 2000; Violi et al., 2008), or to their capacity to produce hyphal network, which conferred them the capacity to tolerate soil nutrient scarcity or even less favor moisture conditions (drought) (Curiel Yuste et al., 2011; Barto et al., 2012; Simard et al., 2012). In this sense, bacterial communities were highly sensitive to regional fluctuations in soil pH, independently to land-use intensity, while fungi remained highly unaffected. The effect of pH in shaping the structure and diversity of soil microbiota has been thoroughly studied (Fierer and Jackson, 2006; Lauber et al., 2009; Rousk et al., 2010; Banerjee et al., 2016a; Delgado-Baquerizo et al., 2018). It is well known that soil fungi have the capacity to grow in environments with a wide pH range (between 5-9 units) without present any significant decrease (Rousk et al., 2010) or even increased in acidic soils (Grosso et al., 2016), which could be modulating the persistence of these phyla. Fierer and Jackson (2006) explained how soil pH could determine bacterial diversity and richness, showing their maximum peak in neutral soils. Nonetheless, the bacterial evaluated in this study did not present their highest in this range of pH, since the greatest richness and evenness was shown in the acidic soils from dehesas (5.58 units). This difference in pH peaks might be explained by the higher values of precipitation found in these locations, which may provide a better microenvironmental condition to grow (Delgado-Baquerizo et al., 2018) or even to the higher aboveground diversity found in dehesas ecosystems where herbaceous content is predominant (Pulido et al., 2010).

Our results further show that bacterial communities under the most transformed systems (dehesas) were also the most sensitive to the environmental gradient of the study, whereas microbial communities under forest were the less affected by these environmental gradients. For instance, while soil pH is a soil variable that has the capacity to transform soil bacterial communities in all different land-uses, their sensitivity to climate was strongly dependent on the land-use, being soil microbiota under forest less sensitive to the climatic variability. This resilience could be associated with the fact that trees are a natural buffer against climatic fluctuations, intercepting radiation and controlling temperature and moisture through evapotranspiration, conferring forests the ability to tolerate to some extent changes on MAP and MAT

(Barba et al., 2016a). Therefore, the more scattered distribution of trees in dehesas (Moreno and Pulido, 2009) confer overstorey less buffer capacity to protect soils against climatic variability, which could be related to the sensitivity presented by soil bacteria and the most abundant fungi (Ascomycota and Basidiomycota). Likewise, the bacterial core community and keystone species from dehesas were more sensitive to the presence of trees, while soil fungal communities remained unaltered independently of the land-use intensity. Hence, soil bacterial communities under the most intensively used systems were the most sensitive to the alterations in C and nutrient availability associated with the presence of trees (de Graaff et al., 2010; Lange et al., 2015). In contrast, forests bacteria presented a higher sensitivity to variability in available nutrients, as NH_4^+-N and $_{av}P$, probably because under more dense canopies, competition for mineral resources is also much stronger than under sparser overstories (Sardans et al., 2004; Fichtner et al., 2012; Grossiord et al., 2014). Both nutrients are highly important for microbial cell formation and metabolisms, as N is an essential component of enzymes (Stein and Klotz, 2016), playing also a key role in the aerobic oxidation of SOC (Hobbie et al., 2012) and P is a structural element in nucleic acid (Spohn and Widdig, 2017) which also play a role in soil nitrification by facilitate the release of litter N (Norman and Barrett, 2014; Mori et al., 2017). Hence, our results show that land-use intensity not only transform the structure and organization of microbial communities, but also determines their sensitivity to different limiting factors: under sparser overstoreys microbial communities might be more sensitive to climatic variability and spatial variability of tree influence, whereas in forest, microbial communities might especially sensitive to variability in essential mineral nutrients.

Abandonment of dehesas (open woodland) resulted in microbial communities less sensitive to climate or tree influence, supporting the fact that recovery of the original structure of soil microorganisms after dehesas abandonment was also associated with the recovery of the stability of these communities. In this intermediates state, keystone species were specially sensitivity to SOC variability, probably because the recovery of the original forest structure also increases the presence of labile forms of C in the soil from root exudation and increases in litter deposition (Blagodatskaya and Kuzyakov, 2008) that stimulates the proliferation of copiotroph microorganisms within the community (Pascault et al., 2013; Goberna et al., 2016; Yan et al., 2018). This intermediate state also coincided with a peak in the phylogenetic diversity of the fungal keystone species, with presence of fungi from less abundant phyla as Mucoromycota and Mortierellomycota, not present in forest and dehesas. The presence of these phyla

in this intermediate state, classified as saprotrophic fungi (Lehmann et al., 2020), are probably related to the increase in more complex structural molecules such as lignin and hemicellulose, from the proliferation of seedlings and shrubs. This increase in saprophytic fungi is key to decompose those complex molecules to further provide with labile C to bacteria (Purahong et al., 2016), which also explains why in open woodlands some keystone species are directly associated with the decomposition of both labile and recalcitrant forms of C (belonging to the phyla Actinobacteria and Verrucomicrobia, respectively (Fierer et al., 2013; Banerjee et al., 2016b)).

Conclusions

In conclusion, in our gradient of land-use intensity, natural forests and dehesas showed clear differences in their soil core microbial structure and composition with less copiotroph phyla in the most managed ecosystem. Nonetheless, the abandonment of the most intensive use in dehesas and the subsequent gradual recovery of the aboveground vegetation towards forest-like ecosystems, resulted in a parallel recovery on the structure of the taxonomic composition and co-occurrence networks of the belowground microbiota. In this regard, woodlands ecosystems showed communities dominated by copiotroph bacteria and saprotroph fungi suggesting an increase in soil C sources, probably from the proliferation of seedling and shrubs. Besides, land-use seem to modulate the sensitivity of the soil core community from bacteria and fungi. While dehesas showed a bacterial community more sensitive to climate probably linked to the limited capacity of open woodlands to buffer atmospheric climatic variability, the bacterial core community under forests only exhibited sensitivity to variability in mineral forms of essential nutrients (e.g. ammonium and available P) associated with a higher competition between the different trees found in natural ecosystems. Our results further show how the soil fungal communities were, in general, less sensitive to environmental fluctuations regardless the land-use intensity, which suggest that soil fungi are more resilient to perturbation, probably due to their capacity to produce an hyphal network, which conferred them the capacity to tolerate soil nutrient scarcity or even less favor moisture conditions.

Supplementary material

Table 9. Summary of bacterial keystone taxa found in each land-use category.

Land-use category	ID	Taxonomy (Phylum, Class, Order, Family)	Keystone Type
Forest	OTU_01	Acidobacteria, Solibacteres, Solibacterales, Solibacteraceae	Local hub
Forest	OTU_02	Acidobacteria, iii1-8, DS-18	Local hub
Forest	OTU_03	Acidobacteria, Acidobacteria-6, iii1-15, mb2424	Network hub
Forest	OTU_04	Acidobacteria, Acidobacteria-6, iii1-15	Local hub
Forest	OTU_05	Acidobacteria, Acidobacteria-6, iii1-15	Local hub
Forest	OTU_06	Acidobacteria, Acidobacteriia, Acidobacteriales, Koribacteraceae	Local hub
Forest	OTU_07	Actinobacteria, Actinobacteria, Actinomycetales, Microbacteriaceae	Local hub
Forest	OTU_08	Actinobacteria, Actinobacteria, Actinomycetales, Nocardioidaceae	Connector
Forest	OTU_09	Actinobacteria, Actinobacteria, Actinomycetales, Streptomycetaceae	Connector
Forest	OTU_10	Bacteroidetes, Cytophagia, Cytophagales, Cytophagaceae	Local hub
Forest	OTU_11	Chloroflexi, Gitt-GS-136	Local hub
Forest	OTU_12	Proteobacteria, Betaproteobacteria, MND1	Network hub
Forest	OTU_13	Proteobacteria, Betaproteobacteria, Burkholderiales, Burkholderiaceae	Connector
Forest	OTU_14	Proteobacteria, Betaproteobacteria, Burkholderiales, Alcaligenaceae	Local hub
Forest	OTU_15	Proteobacteria, Alphaproteobacteria, Sphingomonadales, Sphingomonadaceae	Connector
Forest	OTU_16	Proteobacteria, Alphaproteobacteria, Rhizobiales, Phyllobacteriaceae	Local hub
Forest	OTU_17	Proteobacteria, Alphaproteobacteria, Rhizobiales, Bradyrhizobiaceae	Connector
Forest	OTU_18	Proteobacteria, Alphaproteobacteria, Rhodospirillales, Rhodospirillaceae	Connector
Forest	OTU_19	Proteobacteria, Alphaproteobacteria, Rhodospirillales, Rhodospirillaceae	Local hub
Forest	OTU_20	Proteobacteria, Gammaproteobacteria, Thiotrichales, Piscirickettsiaceae	Local hub
Forest	OTU_21	Verrucomicrobia, [Spartobacteria], [Chthoniobacterales], [Chthoniobacteraceae]	Local hub
Open woodland	OTU_1	Acidobacteria, iii1-8, DS-18	Connector
Open woodland	OTU_2	Acidobacteria, [Chloracidobacteria], RB41	Connector
Open woodland	OTU_3	Actinobacteria, Actinobacteria, Actinomycetales, Nocardioidaceae	Connector
Open woodland	OTU_4	Bacteroidetes, [Saprospirae], [Saprospirales], Chitinophagaceae	Network hub

Open woodland	OTU_5	Bacteroidetes, [Saprospirae], [Saprospirales], Chitinophagaceae	Connector
Open woodland	OTU_6	Bacteroidetes, [Saprospirae], [Saprospirales], Chitinophagaceae	Connector
Open woodland	OTU_7	Chloroflexi, Anaerolineae, envOPS12	Connector
Open woodland	OTU_8	Chloroflexi, Gitt-GS-136	Connector
Open woodland	OTU_9	Planctomycetes, Planctomycetia, Pirellulales, Pirellulaceae	Connector
Open woodland	OTU_10	Proteobacteria, Betaproteobacteria, Burkholderiales, Comamonadaceae	Connector
Open woodland	OTU_11	Proteobacteria, Alphaproteobacteria, Rhizobiales, Methylocystaceae	Connector
Open woodland	OTU_12	Proteobacteria, Alphaproteobacteria, Sphingomonadales, Sphingomonadaceae	Connector
Open woodland	OTU_13	Proteobacteria, Alphaproteobacteria, Rhizobiales, Phyllobacteriaceae	Connector
Open woodland	OTU_14	Proteobacteria, Betaproteobacteria, Burkholderiales, Burkholderiaceae	Connector
Open woodland	OTU_15	Proteobacteria, Betaproteobacteria, MND1	Network hub
Open woodland	OTU_16	Proteobacteria, Betaproteobacteria, MND1	Connector
Open woodland	OTU_17	Proteobacteria, Alphaproteobacteria, Rhizobiales, Hyphomicrobiaceae	Connector
Open woodland	OTU_18	Verrucomicrobia, [Spartobacteria], [Chthoniobacterales], [Chthoniobacteraceae]	Network hub
Open woodland	OTU_19	Verrucomicrobia, [Spartobacteria], [Chthoniobacterales], [Chthoniobacteraceae]	Connector
Dehesa	OTU_1	Acidobacteria, Solibacteres, Solibacterales, Solibacteraceae	Network hub
Dehesa	OTU_2	Acidobacteria, Acidobacteriia, Acidobacteriales, Koribacteraceae	Network hub
Dehesa	OTU_3	Acidobacteria, Acidobacteriia, Acidobacteriales, Koribacteraceae	Network hub
Dehesa	OTU_4	Acidobacteria, Acidobacteriia, Acidobacteriales, Koribacteraceae	Connector
Dehesa	OTU_5	Acidobacteria, Acidobacteriia, Acidobacteriales, Koribacteraceae	Network hub
Dehesa	OTU_6	Bacteroidetes, [Saprospirae], [Saprospirales], Chitinophagaceae	Connector
Dehesa	OTU_7	Chloroflexi, Ktedonobacteria, Thermogemmatisporales, Thermogemmatisporaceae	Network hub
Dehesa	OTU_8	Gemmatimonadetes, Gemm-1	Connector
Dehesa	OTU_9	Proteobacteria, Betaproteobacteria, Burkholderiales, Burkholderiaceae	Network hub
Dehesa	OTU_10	Proteobacteria, Betaproteobacteria	Connector
Dehesa	OTU_11	Proteobacteria, Betaproteobacteria, Burkholderiales, Comamonadaceae	Connector
Dehesa	OTU_12	Proteobacteria, Gammaproteobacteria, Xanthomonadales, Sinobacteraceae	Network hub
Dehesa	OTU_13	Proteobacteria, Gammaproteobacteria, Xanthomonadales, Xanthomonadaceae	Connector

Table 10. Summary of fungal keystone taxa found in each land-use category.

Land-use	ID	Taxonomy (Phylum, Class, Order, Family)	FunGuild	Keystone type
Forest	OTU_1	Ascomycota, Sordariomycetes, Coniochaetales,Coniochaetaceae	Saprotroph	Connector
Forest	OTU_2	Ascomycota, Eurotiomycetes, Chaetothyriales, Herpotrichiellaceae	Saprotroph	Network hub
Forest	OTU_3	Ascomycota, Sordariomycetes, Coniochaetales	Unknown	Network hub
Forest	OTU_4	Ascomycota, Leotiomycetes, Leotiomycetes_ord_Incertae_sedis, Pseudeurotiaceae	Saprotroph	Connector
Forest	OTU_5	Ascomycota, Eurotiomycetes, Chaetothyriales, Herpotrichiellaceae	Saprotroph	Network hub
Forest	OTU_6	Ascomycota	Unknown	Connector
Forest	OTU_7	Ascomycota, Sordariomycetes, Xylariales, Xylariaceae	Endophyte	Network hub
Forest	OTU_8	Ascomycota, Eurotiomycetes, Eurotiales, Trichocomaceae	Saprotroph	Connector
Forest	OTU_9	Ascomycota, Eurotiomycetes, Chaetothyriales, Herpotrichiellaceae	Endophyte	Network hub
Forest	OTU_10	Ascomycota, Eurotiomycetes, Eurotiales, Aspergillaceae	Unknown	Connector
Forest	OTU_11	Ascomycota, Leotiomycetes, Leotiomycetes_ord_Incertae_sedis, Pseudeurotiaceae	Saprotroph	Connector
Forest	OTU_12	Ascomycota, Eurotiomycetes, Chaetothyriales	Unknown	Connector
Forest	OTU_13	Ascomycota, Sordariomycetes, Hypocreales	Unknown	Connector
Forest	OTU_14	Ascomycota, Eurotiomycetes, Eurotiales, Aspergillaceae	Unknown	Connector
Forest	OTU_15	Ascomycota, Eurotiomycetes, Chaetothyriales	Unknown	Connector
Forest	OTU_16	Ascomycota	Unknown	Connector
Forest	OTU_17	Basidiomycota, Agaricomycetes, Thelephorales, Thelephoraceae	Ectomycorrhizal	Connector
Forest	OTU_18	Basidiomycota, Agaricomycetes, Boletales, Melanogastraceae	Ectomycorrhizal	Connector
Forest	OTU_19	Basidiomycota, Agaricomycetes, Polyporales, Ganodermataceae	Plant Pathogen	Connector
Open woodland	OTU_1	Ascomycota, Eurotiomycetes, Eurotiales, Aspergillaceae	Unknown	Connector
Open woodland	OTU_2	Ascomycota, Leotiomycetes, Helotiales, unidentified	Unknown	Network hub
Open woodland	OTU_3	Ascomycota, Eurotiomycetes, Eurotiales, Aspergillaceae	Saprotroph	Network hub
Open woodland	OTU_4	Ascomycota, Sordariomycetes, Hypocreales, Hypocreaceae	Unknown	Connector
Open woodland	OTU_5	Ascomycota, Sordariomycetes, Coniochaetales, Coniochaetaceae	Saprotroph	Connector

Open woodland	OTU_6	Ascomycota, Sordariomycetes, Hypocreales, Clavicipitaceae	Animal Pathogen	Connector
Open woodland	OTU_7	Ascomycota, Eurotiomycetes, Chaetothyriales, unidentified	Unknown	Connector
Open woodland	OTU_8	Ascomycota, Eurotiomycetes, Eurotiales, Aspergillaceae	Unknown	Connector
Open woodland	OTU_9	Ascomycota, Pezizomycetes, Pezizales, Tuberaceae	Ectomycorrhizal	Local hub
Open woodland	OTU_10	Ascomycota, Eurotiomycetes, Chaetothyriales, Herpotrichiellaceae	Saprotroph	Connector
Open woodland	OTU_11	Ascomycota, Pezizomycetes, Pezizales, Pyronemataceae	Ectomycorrhizal	Network hub
Open woodland	OTU_12	Ascomycota, Eurotiomycetes, Chaetothyriales, Herpotrichiellaceae	Saprotroph	Connector
Open woodland	OTU_13	Ascomycota, Sordariomycetes, unidentified, unidentified	Unknown	Network hub
Open woodland	OTU_14	Ascomycota, Lecanoromycetes, Ostropales, unidentified	Unknown	Connector
Open woodland	OTU_15	Ascomycota, Sordariomycetes, Hypocreales, Hypocreaceae	Unknown	Connector
Open woodland	OTU_16	Ascomycota, unidentified, unidentified, unidentified	Unknown	Network hub
Open woodland	OTU_17	Ascomycota, Eurotiomycetes, Chaetothyriales, Herpotrichiellaceae	Saprotroph	Connector
Open woodland	OTU_18	Ascomycota, Eurotiomycetes, Eurotiales, Aspergillaceae	Unknown	Network hub
Open woodland	OTU_19	Basidiomycota, Agaricomycetes, Agaricales, Inocybaceae	Ectomycorrhizal	Connector
Open woodland	OTU_20	Basidiomycota, Microbotryomycetes, unidentified, unidentified	Unknown	Connector
Open woodland	OTU_21	Basidiomycota, Agaricomycetes, Thelephorales, Thelephoraceae	Ectomycorrhizal	Connector
Open woodland	OTU_22	Mortierellomycota, Mortierellomycetes, Mortierellales, Mortierellaceae	Saprotroph	Connector
Open woodland	OTU_23	Mucoromycota, Mucoromycotina_cls_Incertae_sedis, Mucoromycotina_ord_Incertae_sedis, Mucoromycotina_fam_Incertae_sedis	Unknown	Connector
Open woodland	OTU_24	Mucoromycota, Umbelopsidomycetes, Umbelopsidales, Umbelopsidaceae	Saprotroph	Connector
Dehesa	OTU_1	Ascomycota, Eurotiomycetes, Eurotiales, Trichocomaceae	Saprotroph	Connector
Dehesa	OTU_2	Ascomycota, Eurotiomycetes, Eurotiales, Trichocomaceae	Saprotroph	Connector
Dehesa	OTU_3	Ascomycota, Pezizomycetes, Pezizales, Pyronemataceae	Ectomycorrhizal	Network hub
Dehesa	OTU_4	Ascomycota, Sordariomycetes, Hypocreales, Ophiocordycipitaceae	Animal Pathogen	Network hub
Dehesa	OTU_5	Ascomycota, Eurotiomycetes, Chaetothyriales, Herpotrichiellaceae	Saprotroph	Connector
Dehesa	OTU_6	Ascomycota	Unknown	Connector
Dehesa	OTU_7	Ascomycota, Eurotiomycetes, Chaetothyriales, Herpotrichiellaceae	Saprotroph	Network hub

Dehesa	OTU_8	Ascomycota, Dothideomycetes, Pleosporales, Pleosporaceae	Unknown	Network hub
Dehesa	OTU_9	Ascomycota, Eurotiomycetes, Eurotiales, Trichocomaceae	Saprotroph	Local hub
Dehesa	OTU_10	Ascomycota, Sordariomycetes, Branch06	Unknown	Network hub
Dehesa	OTU_11	Ascomycota, Dothideomycetes, Dothideales, Dothioraceae	Saprotroph	Network hub
Dehesa	OTU_12	Ascomycota, Sordariomycetes, Xylariales	Unknown	Network hub
Dehesa	OTU_13	Ascomycota, Leotiomycetes, Helotiales, Helotiales_fam_Incertae_sedis	Endophyte	Connector
Dehesa	OTU_14	Ascomycota, Eurotiomycetes, Chaetothyriales, Herpotrichiellaceae	Saprotroph	Connector
Dehesa	OTU_15	Ascomycota, Leotiomycetes, Helotiales, Helotiales_fam_Incertae_sedis	Endophyte	Network hub
Dehesa	OTU_16	Basidiomycota, Agaricomycetes, Agaricales, Lycoperdaceae	Saprotroph	Connector
Dehesa	OTU_17	Basidiomycota, Agaricomycetes, Sebacinales, Sebacinaceae	Unknown	Network hub

Table 11. Mean (SE) of the relative abundance from keystone taxa of soil bacterial and fungal communities. Different letters denote statistically significant differences among land-use categories ($P < 0.05$).

		Land-use category		
Soil community	**Phylum**	**Forest**	**Open woodland**	**Dehesa**
Bacteria	Acidobacteria	3.39 (0.27) A	0.28 (0.04) B	3.94 (0.25) A
Bacteria	Actinobacteria	0.80 (0.13) A	0.46 (0.46) B	0.00 (0.00) C
Bacteria	Bacteroidetes	0.71 (0.71) A	0.66 (0.06) A	0.56 (0.56) A
Bacteria	Chloroflexi	0.17 (0.17) A	0.41 (0.02) B	0.08 (0.08) A
Bacteria	Gemmatimonadetes	0.00 (0.00) A	0.00 (0.00) A	0.20 (0.20) B
Bacteria	Planctomycetes	0.00 (0.00) A	0.11 (0.11) B	0.00 (0.00) A
Bacteria	Proteobacteria	8.80 (0.52) A	3.29 (0.14) B	1.52 (0.10) C
Bacteria	Verrucomicrobia	0.25 (0.25) A	2.32 (1.05) B	0.00 (0.00) A
Fungi	Ascomycota	6.66 (0.19) A	5.23 (0.09) AB	3.57 (0.08) B
Fungi	Basidiomycota	0.94 (0.15) A	0.28 (0.01) B	0.18 (0.02) B
Fungi	Mortierellomycota	0.00 (0.00) A	0.09 (0.09) B	0.00 (0.00) A
Fungi	Mucoromycota	0.00 (0.00) A	1.60 (0.16) B	0.00 (0.00) A

Table 12. Means (SE) of soil variables according to holm oak land-use category. Different letters represent significant differences among land-use (P < 0.05).

	Land-use category		
Variable	**Forest**	**Open Woodland**	**Dehesa**
MAT	13.38 (0.13) A	13.33 (0.23) A	14.31 (0.22) B
MAP	536.28 (5.34) A	587.22 (25.23) A	706.10 (17.86) B
pH	6.70 (0.14) A	7.01 (0.08) A	5.58 (0.07) B
SOC	2.47 (0.10) A	3.32 (0.19) B	2.39 (0.10) A
NH4+-N	145.75 (8.76) A	174.54 (4.47) B	223.12 (11.73) C
NO3--N	109.11 (12.53) A	99.59 (12.50) A	100.01 (10.34) A
Available P	0.23 (0.03) A	0.23 (0.03) A	0.26 (0.03) A

Table 13. Global topological parameters describing the core community from soil bacterial and fungal co-occurrence networks. Diameter measures the shortest distance, in terms of number of shortest paths, between the two most distant nodes in the network, hence describing network size. Average path length measures the average shortest distance between all pairs of nodes, hence describing the average number of links between any two nodes. Connectance quantifies the proportion of realized links out of all possible, hence describing the link density in the network.

Networks	**Land-use category**	**Number of OTUs**	**Diameter**	**Average path length**	**Connectance**
Bacteria	Forest	285	6	3.282	3.835
Bacteria	Open woodland	320	6	3.310	3.820
Bacteria	Dehesa	398	8	3.330	4.028
Fungi	Forest	265	8	3.654	2.853
Fungi	Open woodland	407	6	3.090	4.610
Fungi	Dehesa	416	6	3.084	4.642

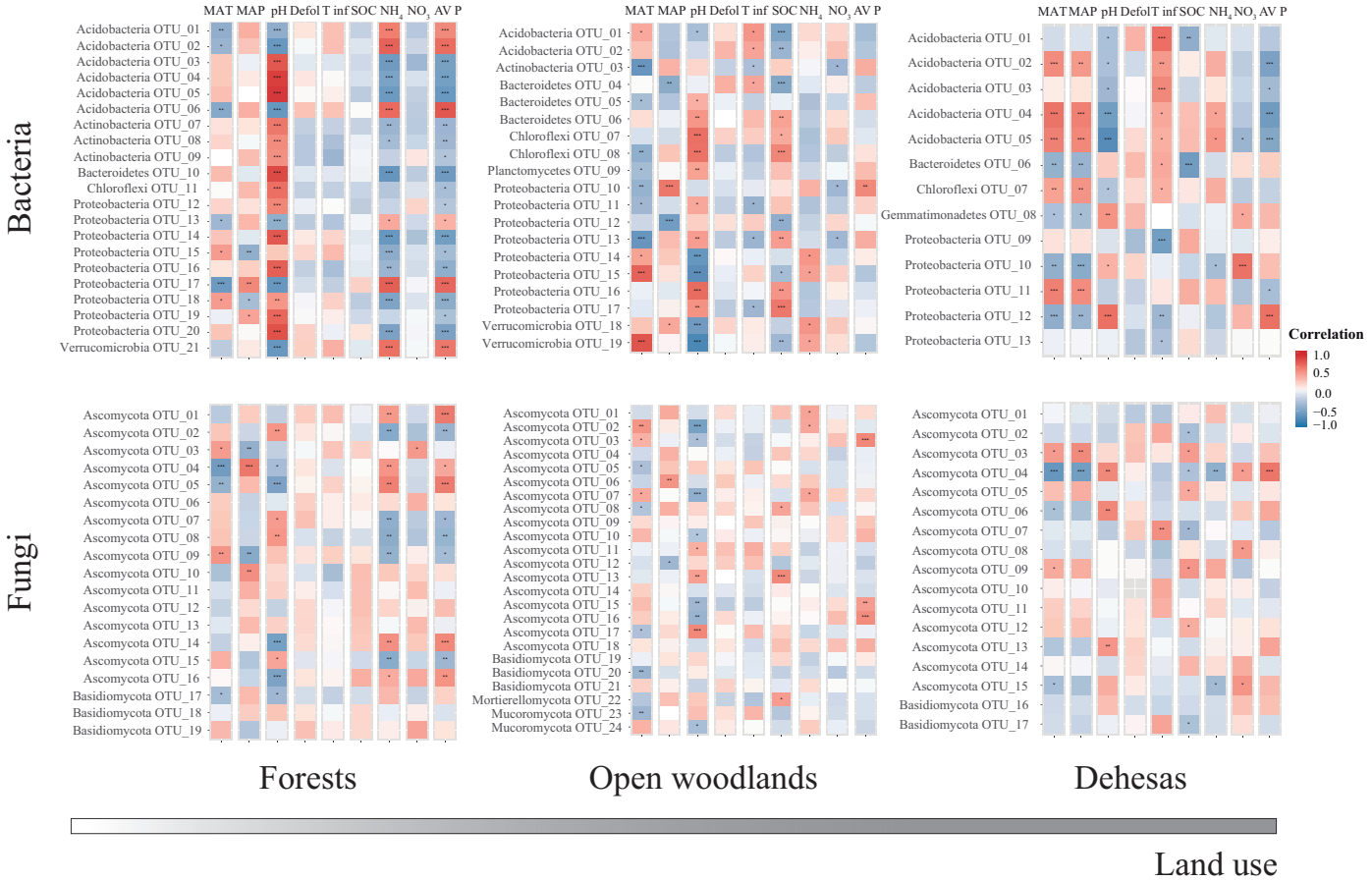

Figure 17. Correlation heatmap of the soil bacterial and fungal keystones with abiotic variables (climate and pH), tree influence and soil nutrients pools for each land-use. Grey colors denote the absence of that phylum in that land-use category. MAT = Mean annual temperature, MAP = annual precipitation, Defol = holm oaks' defoliation degree, T inf = tree influence, SOC = Soil Organic Carbon, NH_4^+-N = soil Ammonium, NO_3^--N = soil Nitrate and $_{av}P$ = available Phosphorus.

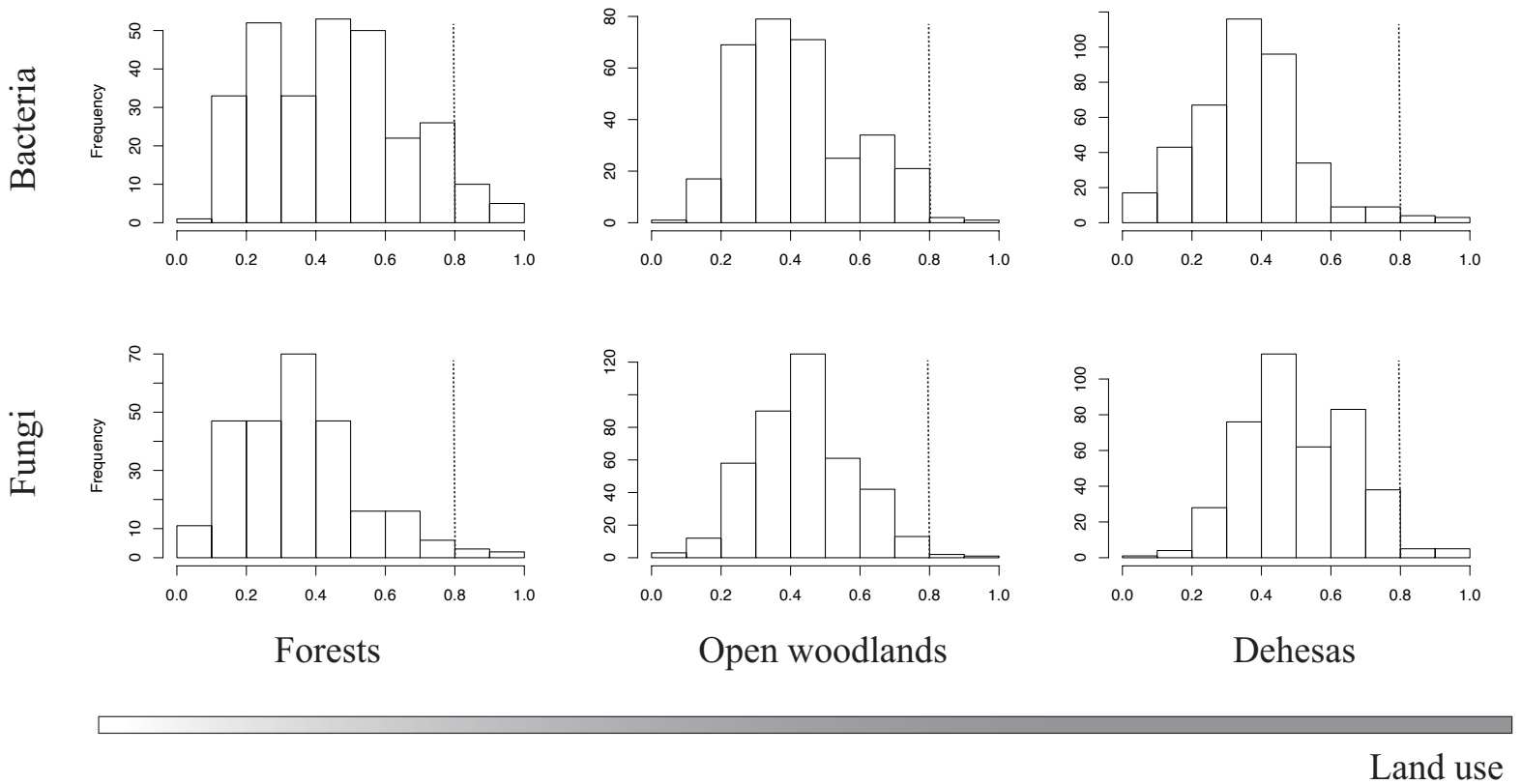

Figure 18. Histograms to determine the thresholds in the degree centrality for the core communities of soil bacteria and fungi.The spotted line represents the threshold selected for each land-use.

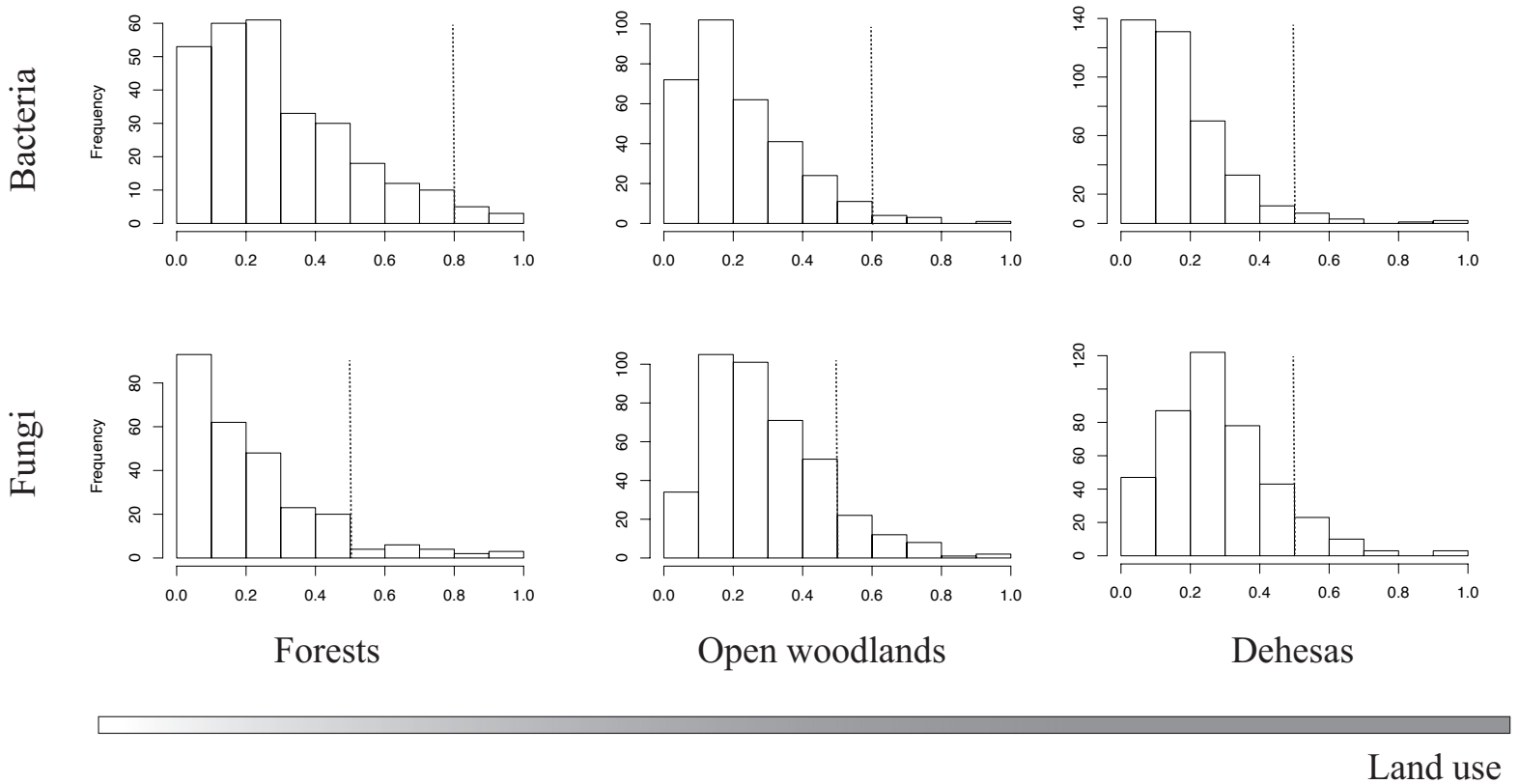

Figure 19. Histograms to determine the thresholds in the betweeness metrics for the core communities of soil bacteria and fungi. The spotted line represents the threshold selected for each land-use.