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When drought meets forest management: effects on the soil microbial community of a Holm oak forest ecosystem

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***Responses to Reviewers Comments**

Dear Dr. Ortega Calvo and Prof. Jay Gan,

Thank you very much for handling our manuscript. We are very grateful for the positive and constructive feedbacks of the Reviewers. We have addressed all comments of this Minor revision and believe that our manuscript has been consequently improved. Genomic sequences have been deposited.

Please, find below a point-by-point response to each comment.

Sincerely,

Felipe Bastida
CEBAS-CSIC

Reviewers/Editor comments:

Reviewer #1: This study explored how the draught, forest management (thinning) and their interactions influenced soil microbial community and soil functions in a Holm oak forest. The results seem good and it could be published in STOTEN. Though, there are numbers of problem throughout the manuscript. The results and discussion sections are not properly written. Several sentences are not comprehensible. The English version of manuscript needs critical revision. I have pointed out few issues but not all.

Dear Reviewer,
Thank you very much for your revision and constructive points. We have addressed all of them and revised the English of the manuscript.
Please, find below a detailed point-by-point response to each of your comments.
Best wishes

Line by line comment:
L-50-52: Revise English in this sentence.
Ok, modified

L70-71: Rewrite this sentence.
Ok, modified

L70-71: Give here at least 3 citations to emphasize this statement.
Ok. We have added the following references: Crowther et al., 2014; Bastida et al., 2015; Lladó et al., 2017.

L99-101: review English in this sentence.
Ok, modified

L104-105: Are you sure, microbes are producers? Please explain how.
Ok, sentence revised.

L109-113: Review English in this sentence.
Ok, done

L117: Replace climate change with climatic factor
Ok, done

L141: In materials and methods section, it will be very nice if you include the information of topography and slope of the study site.
As requested, this info has been added in the revised version.

L187: use above ground biomass rather than aerial biomass.
As requested, this term has been replaced through the manuscript.

L227: correct the style of citation across the manuscript. The et al should not be in italic.
Ok, checked and corrected.

L268-269: use abbreviation SOC for soil organic carbon.
Ok, done

L268-271: please see how to explain the results from 3way interactions (see Singh et al., 2017 (Forest ecology and management 391, 458-468)). For instance, you did not have included the differences between DT and DU or CT and CU.

In order to clarify a bit more the results, we have included a few lines for explanning the differences you have mentioned. However, we do not want to satúrate in excess the text with 3-way ANOVA results (Ln 285-288, revised manuscript).

L278: Delete that of. Just use phenol oxidase rather than p-phenol oxidase.
Ok, done

L281: all enzymes (except phenol oxidase)
Ok, done

L283-287: Simply use DT, CT, DU and CU. Do not repeat their full name.
Ok, done

L422-424: Rewrite
Ok, rewritten

L446-446: rewrite
Ok, rewritten

L454-457: it would be nice if you compare it from tropical forest (Singh et al., 2018; where Phenol oxidase activity was higher under the rainy season owing to increase of persistent organic compared to easily hydrolysable organic matter.
Ok, reference added (Ln 494-496 revised manuscript).

L518-520: Revise English in this sentence.
Ok, revised

Fig.1. Author should correct figure legend
Fig.2: revise your figure captions. Some seems not good.
Fig. S1: You must include y-axis title.

Overall, Table and Fig.captions, legends and units have been checked and corrected when neccesary. Fig S1, y-axis is unitless 8this has been clarified in the Figure caption). Further, we have included a new table (Table 2) about the diversity index.

Reviewer #2: The paper "When drought meets forest management: effects on the soil microbial community of a Holm oak forest ecosystem" by F. Bastida et al.The paper reports the impacts of induced drought and thinning, determining if the thinning of Holm oak modulates the resistance of the soil microbial community to drought.
The paper is well written and organized, original and has a good technical quality.
It requires only few improvements
In particular the Graphical abstract is very simple, not attractive

Dear Reviewer,
Thank you very much for your revision and constructive points. Graphical abstract has been revised and improved. Please, find below a detailed point-by-point response to each of your comments.
Best wishes

Specific comments
Line 245: in the MG-RAST public database (XXXXX) ?
Sequences have been deposited and the numbers appear in the revised manuscript.

Line 683 (references): IPCC 2013. http://www.ipcc.ch/ please provide the reference in the right way
Reference added.

Please report in the Supporting Tables F and P acronyms
Ok, done.

Reviewer #4: This study employed a drought treatment (25% reduction of throughfall) in thinned and unthinned Holm oak Mediterranean forests to examine the impacts of drought, thinning and their interactions on soil microbial community composition and function (mainly enzyme activities). The experiment is well designed and the test of thinning effects on soil microbial communities is novel in such subtropical forests. They found that drought and thinning did not affect the total PLFA biomass, but affected the composition of both bacterial and fungal communities, as indicated by the alterations in the G+/G- and F/B ratios, beta-diversity, and relative abundance of particular phylum. The alpha diversity of bacterial communities was influenced by both thinning, drought and their interactions; while fungal community composition was solely affected by thinning but not drought, which is in line with the general recognition that fungi are more resistant to drought than bacteria. The
drought and thinning effects were also confoundedly shaped by the season of sampling (winter and summer). Overall, substrate availability (SOC, WSC and WSN) altered by drought and thinning imposed critical indirect control on the soil microbial communities in this semiarid forest ecosystem. The manuscript is clearly-written and the results have critical implications for the management of the Holm oak forests in the Mediterranean climate that is projected to be drier in the future.

Dear Reviewer,
Thank you very much for your constructive suggestions and positive feedback. We have addressed all your comments. Please, find below a point-by-point response to each of them.
Best wishes

My main suggestions to the improvement of this manuscript are: i) the results showed that Holm oak biomass increment was greater in unthinned than thinned plots with the drought treatment, and SOC accumulation was higher in thinned-nodrought than in thinned-drought treatments. These results indicate that thinning is not a suitable forest management practice for such ecosystems under drought conditions in terms of both aboveground and belowground carbon sequestration. I could be wrong on this, but this has important practical implications and should be emphasized in the discussions; 2) Season is treated as an important factor in the analysis. In such Mediterranean ecosystems, not only soil moisture but also temperature differ distinctly

between the winter and summer seasons. How the differences in soil temperature confoundedly affect the effects of drought and thinning on soil microbial communities was barely mentioned in the discussions.

Thanks for highlighting this important point. First, we agree that thinning does not seem a suitable practice for increasing SOC content (at least in this study). Hence, we have highlighted this finging, including a sentence in the Abstract (Ln 46-47) and Conclusions sections (ln 598-599) of the revised manuscript. Second, at respect of the soil temperature, this parameter was not measured, but we agree on its importance. Hence, we have added a new sentence in the Discussion (Ln 406-411, revised manuscript) highlighting how thinning may interplay with light penetration and soil microclimate (including temperature).

Below are some specific comments:
Line 146, since season is one of the three factors to be tested, providing seasonal mean temperature and precipitation data here would be helpful for readers to better understand the climatic context of the study system.

This climate data have been added in the revised versión (Ln 154-156, revised manuscript).

Line 162, Delete the sentence. The information has been given in line 154.
Ok, done.

Line 182-184, these treatment abbreviations can be merged into lines 167-170 that describe the treatments.
This information has been moved above for clarity.

Line 303, which ratio, G+/G- or F/B?
We meant F/B ratio. The sentence has been modified for a better clarity.

Line 176, A justification should be provided for the 25% reduction of throughfall. How severe is this drought treatment with respect to historical rainfall?

The total and seasonal precipitation is now included in the manuscript. The rainfall reduction (25%) is within the range predicted by climate models (15-30% for 2080, IPCC 2013). A sentence has been added for justification in the revised manuscript (Ln 187-188, revised manuscript).

Lines 187-188, the aerial biomass was greater in unthinned plots than in thinned plots. How would this affect light penetration and subsequently soil climates?

Probably light penetration will be more intense in thinned plots and this may affect soil microclimate. Accordingly, we have added a sentence in section 4.1 (Ln 406-411, revised manuscript).

Lines 191-192, this is unexpected to me. Holm oak actually grew better in unthinned than in thinned plots under drought conditions, which means that thinning is not a good practical strategy to promote Holm oak growth in drought conditions.

Yes, we agree. Of course, this can be dependen ton the intensity of thinning, soil fertility conditions and many other factors. Moreover, the impacts of thinning maybe plat species-dependent. For instance, in a similar study with Pinus halepensis as dominant tree (Bastida et al., 2017 GCB), we observed that thinning benefited tree survival and trunk diameter in drought plots.

Line 203, please be specific if this is acid or alkaline phosphatase.
Alkaline (corrected).

Lines 266-267, did soil moisture differ in thinned vs unthinned plots?
Added info in the revised version: "Soil moisture was generally higher in unthinned plots than in the corresponding thinned plots" (Ln 279-280).

Line 268-270, soil organic C was higher in CT than in DT, indicating that thinning in drought conditions does not favor SOC accumulation. Is it right?
Yes, we agree. Indeed, as mentioned above, we have highlighted this finding by including a sentence in the Abstract (Ln 46-47) and Conclusions sections (ln 598-599) of the revised manuscript. The revised version of the Graphical Abstract also includes this finding.

Reviewer #6: The manuscript "When drought meets forest management: effects on the soil microbial community of a Holm oak forest ecosystem" addresses a timely and important research question in the subject area of "agriculture, forestry, land use and management" and is a multidisciplinary study as it comprises biosphere (microbial data) and lithosphere (soil chemistry). Thus the study fulfills the criteria to be eligible for STotEn.
The manuscript is very well written, structured and organized. The full factorial study design with three factors each at two levels: drought (natural rainfall vs. induced drought), thinning (thinning vs. no thinning), and season (summer vs. winter) is well thought and the experiment has been carried out carefully. The measured parameters complement each other and allow different aspects to be not only qualitatively described but also quantitatively determined.

Dear Reviewer,
Thank you very much for your constructive suggestions and positive feedback. We have addressed all your comments. Please, find below a point-by-point response to each of them.
Best wishes

Major comment:
The only major point of criticism is that the authors could have made even more use of their complementary measurements. Wouldn't it be very interesting to test the link between changes in bacterial and/or fungal community composition and an ecosystem process such as enzyme activities (see Bier et al. 2015 FEMS Microbiology Ecology, 91)? From this point of view, the graphical abstract could take this direction - if there is a possibility to not only connect changes in microbial diversity to the treatment but also to the possible functional changes that are connected to them.

Many thanks for this constructive suggestion. In the revised manuscript, the relationships between community composition/diversity and soil enzymes have been explored. For this purpose, we performed correlation analyses between NMDS coordinates or Shannon index, and soil enzyme activities. Results have been included in a new Table (Table S5) and also in the manuscript text (new section 3.6, Ln 380-387, revised manuscript). In summary, bacterial community was strongly correlated with soil enzyme activities than fungal community. This was also includeed in the Discussion section (Ln 529-530).
Further, Graphical abstract has been modified too.

Minor comments
Line 180: For how long were the samples stored between sampling and sieving and then after sieving before they were frozen for DNA and PLFA analyses or used for chemical and enzyme analyses?
Ok, info added in the revised manuscript (Ln 191-193 of the revised manuscript).

Line 261: I there a reference missing for R-studio package?
Ok, sentence corrected.

Line 310: "those" seems to be incorrect here

Ok, corrected

Line 342: Section 3.5 could benefit from adding more biological/ecological/functional information on those taxa that were identified at the genus level. Instead of a list with names, I would suggest to add their functional assignment as has partly been done in the discussion. So, my suggestion would be to group the genera by function (ectomycorrhizae, saprotrophs, wood decayers…) and to add the functional assignment. This would probably interest most readers more than a list of names and would facilitate non-mycologists to imagine the ecosystem relevant changes in fungal communities that occurred. This could also be tried for Bacteria i.e. adding when possible and meaningful microbial functional information in section 3.4 although I am aware that the genus level in most cases is not suitable for functional assignments in bacteria - but in some it is. For bacteria, I think the issue has been well solved in the discussion line 486 onwards.

Thank you very much for this suggestion. We would prefer to keep the genus description as it is current stated in Results section. We believe that having such genus description is useful for some readers that want to know exactly which genus respond to any treatment (I have acknowledged it when doing literature searches for this classification). Then, the ecological assignment of fungi genera is utilized in the Discussion for a better understanding of their potential role in soil (as has been done with the copio/oligotrophic lifestyle of bacterial populations). In any case, if the Reviewer believes that this must be changed, we are open to make it.

Line 351: This seems to be imprecise wording: "fungal populations" cannot by studied at the "Phylum" level as per definition a population has the capability of interbreeding. Please check also lines 410, 433, and throughout the document if "population" is correctly used.

Ok. Checked and corrected through the revised manuscript.

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Abstract

The growth and survival of plants in semiarid Mediterranean forests can be improved through the benefits conferred by thinning, a forest management practice that removes trees and reduces the competition between the remaining ones. Here, we evaluate the impacts of induced drought (the exclusion of 25% of the natural rainfall for 5 years) and thinning, and their interaction, with the objective of determining whether the thinning of Holm oak (*Quercus ilex* L.) modulates the resistance of the soil microbial community to drought. Sequencing of 16S rRNA and ITS amplicons revealed that drought, thinning, and their interaction influenced the composition of the bacterial community, while the fungal community was exclusively affected by thinning. Thinning consisted of the removal of the ~~aerial~~ aboveground parts of the Holm oak trees, which were thereafter left in forest stand. Thinning contributed to the C and N contents, with parallel increases in microbial biomass, particularly in summer. Drought increased the amounts of total organic C and total N ~~in both summer and winter~~, likely due to the reduced enzyme activities. Indeed, the composition of the bacterial community was modulated primarily by the indirect and long-term effects of drought - the accumulation of soil organic matter - rather than by the direct effect of the lower water content imposed by the drought treatments. Thinning under drought conditions did not increase soil organic C

(SOC) content. However, ~~The~~ the resistance of the soil microbial community to drought was fostered by thinning, particularly at the functional level ~~with respect to the C, N, and P cycles~~, as indicated by the enzyme activities related to C, N and P cycles. These responses were associated to variations in the composition of the ~~bacterial and fungal~~microbial communities in thinned, drought-exposed plots, in comparison to unthinned, drought-exposed plots. In conclusion, the interaction between forest management and drought influenced ~~interplay of forest management and drought influenced, seasonally,~~ the soil microbial community of a Holm oak-dominated Mediterranean ecosystem ~~dominated by Holm oak~~.

Keywords: soil microbial community; semiarid; thinning; drought; enzyme activity; Holm oak

1. Introduction

Forest soils store large amounts of organic matter from abovegrounds ~~are ecosystems vital to the maintenance of this planet´s sustainability and the C cycling in the biosphere~~ (Crowther et al., 2014; Bastida et al., 2015; Lladó et al. 2017). Consequently, practices that affect plant development in forests and soil properties may have crucial impacts on ecosystem functions and C feedbacks between the soil and atmosphere (Crowther et al., 2014). Several forest management practices are designed to improve ecosystem functioning. In this sense, thinning is a common management practice that involves the removal of trees to improve the growth rate and health of the remaining trees, by reducing competition for resources such as water, nutrients, or light (Yang et al., 2017). Thinning has been widely practiced in areas that have suffered wildfires, as a strategy to provide sufficient resources to the regenerated plants (Lopez-Serrano et al., 2016). Hence, given the strong recurrence of wildfires in Mediterranean areas in the last few decades (Certini et al., 2011), thinning practices were demonstrated to have a strong influence on the ecosystem function of these forests (López-Serrano et al., 2015, 2016). Moreover, the limited precipitation and the higher temperatures forecasted by climate change models will increase the chance of wildfires in Mediterranean ecosystems (Lindner et al., 2010; Hedo de Santiago et al., 2015).

Although its effects depend on the climate, timing~~,~~ and forest stand characteristics, thinning has an impact on the understory plant community due to the changes in the canopy density, plant community and microclimate (Dang et al., 2018). These changes in plant communities impact the quality and quantity of soil organic matter (SOM) and nutrients (López-Serrano et al., 2016; Bastida et al., 2017a; Dang et al., 2018) as well as the interactions between aboveground and belowground communities (Wardle et al., 2004; Prescott and Grayston, 2013). Although the impacts of thinning on the understory

plant community have been widely studied (Long and Vacchiano, 2014), its consequences for the belowground soil bacterial and fungal communities are less known (Bastida et al., 2017a; Dang et al., 2018). Importantly, the impacts of thinning on the soil microbial community of forest dominated by Holm oak (*Quercus ilex* L.), which is a central and emblematic plant species in the Iberian Peninsula, have not been evaluated so far. Besides the availability of organic C, there is another factor that largely drives the plant and microbial productivity in semiarid areas: the availability of water (Bastida et al., 2017a). This is particularly important when considering the predictions of climate change models~~,~~ that point~~ing~~ to a reduction in precipitation in Southern Europe~~, are taken into account~~ (IPCC 2013). However, there is a lack of knowledge about the interactions between forest management and climate change, and there is no doubt that both factors will contribute to the ecosystem sustainability in Mediterranean forests.

In soil, microbial communities perform key ecosystem functions and biogeochemical processes (i.e., litter decomposition)~~, as they are the primary producers and decomposers~~ (van der Heijden et al., 2008; Baldrian et al., 2011; Bastida et al., 2016; Ochoa-Hueso et al., 2018). Drought may affect the biomass, activity~~,~~ and composition of soil microbial communities (Bastida et al., 2017a; Ochoa-Hueso et al., 2018), fungi being commonly considered as more resistant to drought than bacteria (de Vries et al., 2012; Evans and Wallenstein, 2012). In principle, the impacts of forest management in the soil microbial community can alter soil functioning and the provision ecosystem services, ~~an alteration of soil microbial communities due to forest management may have consequences for the soil functioning and the provision of ecosystem services,~~ although the functional redundancy of soil microbial communities may ~~observed in soil microbial communities frequently~~ minimize these impacts (Allison and Martiny, 2008; Curiel-Yuste et al., 2014). Further, it is known that land use and forest management can

affect the resistance and resilience of soil microbial communities to drought (de Vries et al., 2012; Karlowsky et al., 2017). However, until now, only one study has evaluated the impacts of the interaction between ~~climate change~~climatic factors and thinning on the soil microbial community (Bastida et al., 2017a). This previous study was carried out in a *Pinus halepensis* L. forest, where all the plant remains were completely removed after the thinning activities.

Here, we studied the long-term effects of Holm oak thinning ~~under a drought scenario~~ on the soil microbial community under a drought scenario. Particularly, we determined the impacts of five years of drought on the soil microbial community and evaluate whether thinning influences the responses of the soil fungal and bacterial communities to drought. For these purposes, a multi-parametric study was performed that included the analysis of microbial biomass, enzyme activities, and composition and diversity of microbial communities, as well as chemical soil properties. Given the fact that in Holm oak thinning (this technique is known as 'resalveo' -coppicing with standards- in Spanish, López-Serrano et al. (2010)) the plant remains are left on the ground, we hypothesized that this forest management practice will largely impact the biomass and composition of soil fungal communities rather than the bacterial community, because fungi are strongly connected to the decomposition of cellulose and lignin from plant biomass (López-Mondéjar et al., 2018). Further, we expected that drought affects soil bacterial community more than fungal community, and that this impact will be shaped by thinning. Moreover, given the strong seasonality of Mediterranean forest ecosystems (dry summer vs humid winter), we expect that the effects of rainfall exclusion will be more severe in the humid season. Overall, this study aims to provide new evidence on how plant-microbe interactions are modulated by climate change and forest

144 management, opening the door to the development of applied strategies for improving
145 the sustainability of ecosystems in the predicted climate-change scenario.

146

147 **2.Material and Methods**

148 *2.1.Study site*

149 The study area is an original Mediterranean Maritime pine-Holm oak mixed forest
150 stand, located in the Castilla-La Mancha region (central-eastern Spain) in a forest called
151 "Dehesa de Abajo" (1,000 m.a.s.l., 39° 40′ N, 1° 55′W). This forest is considered a
152 mountain plain, where slopes do not exceed a 3% gradient. The mean annual
153 temperature and precipitation are 15.5 ºC and 510 mm, respectively (López-Serrano et
154 al., 2016). For the period 2008-2010, the mean seasonal temperature and precipitation
155 was 7.5 ºC and 170.3 mm in winter, and 24.0 ºC and 24.3 mm in summer, respectively.
156 The soil is a Lithic leptosol ~~(FAO, 1988)~~, associated with Chromic Luvisol, a very
157 shallow soil over calcareous hard rock (FAO, 1988). This area was a natural uneven-
158 aged stand, composed mainly of a dominant canopy tree layer of *Pinus pinaster* Ait.
159 subsp. *mesogeensis* (Mediterranean Maritime pine) with a subdominant tree layer of
160 *Quercus ilex* L. subsp. *ballota* (Holm oak). However, in July 2001, a wildfire affected
161 the area with a moderate-high severity (López-Serrano et al., 2016). The fire led to
162 natural post-fire plant regeneration with a very high density of sprouted Holm oak.

163 Six years later, in October 2007, the burnt stand was composed principally of Holm oak,
164 with an average density of 20000 standards ha^{-1}, and some Maritime pine (an average of
165 350 trees ha^{-1}). Some shrubs - such as *Sideritis incana* L., *Thymus vulgaris* L.,
166 *Rosmarinus officinalis* L., *Santolina chamaecyparissus* L., *Bupleurum fruticescens* L.,

Lavandula latifolia L., and *Genista scorpius* L. - were also observed (Lopez-Serrano et al., 2016).

In January 2008, thinning was performed in a 1.25-ha experimental area within the burnt stand. This reduced the Holm oak density to 3500 standards ha^{-1}. Dead, diseased, and suppressed standards were felled with a clearing saw and left on the ground (an average of 2.7 t ha^{-1} of plant dry biomass was provided to the soil by this thinning activity). ~~The tree density in the unthinned area was about 20000 standards ha^{-1}.~~

2.2.Experimental design for thinning and water restriction

This study had a full factorial design with three factors: i) induced drought (that started in 2012), with two levels (natural rainfall vs rainfall reduction or induced drought), ii) forest management (thinning), with two levels (thinning or no thinning), and iii) season, with two sampling seasons (summer and winter). The abbreviations utilized in this study are as follows: CU (unthinned control soil); DU (unthinned soil subjected to drought); CT (thinned control soil); and DT (thinned soil subjected to drought). Thus, in October 2012, a total of 12 plots (20 m x 20 m) (i.e. 2 levels (rainfall) x 2 levels (thinning) x 3 replicates) were set up, six of them in thinned areas and six in unthinned areas. Each group of six plots was split into two subgroups: three plots received natural rainfall and three plots, partially covered by PVC gutters, received reduced precipitation to simulate drought-induced conditions. The PVC gutters were suspended at about 50 cm above the soil surface and occupied about 25% of the plot surface, intercepting the corresponding rainfall. This rainfall reduction was within the range predicted by climate models (15-30% for 2080, IPCC 2013).

Soil sampling was performed in January 2017 (winter) and July 2017 (summer). Six soil samples per plot were taken at 0-15 cm depth, after removing the plant litter within each plot, and mixed to obtain one composite sample per plot. The samples were immediately sieved (2 mm) after sampling and kept at 4ºC during 3 weeks~~,~~ for chemical and enzyme analyses, ~~or~~and during 3 months at -20ºC~~,~~ for fatty acids and genomic analyses.

~~The abbreviations utilized in this study are as follows: CU (unthinned control soil); DU (unthinned soil subjected to drought); CT (thinned control soil); and DT (thinned soil subjected to drought).~~ In 2014, the total biomass of Holm oak was slightly greater in thinned plots (31.25 ± 9.4 t ha^{-1}) than in unthinned ones (25.29 ± 2.31 t ha^{-1}), although there was no significant difference. The root-shoot ratio was 5-times higher in thinned than in unthinned plots. The ~~aerial~~ aboveground biomass was greater in unthinned plots (12.87 ± 1.17 t ha^{-1}) than in thinned plots (5.55 ± 2.01 t ha^{-1}), while the underground biomass was greater in thinned plots (25.71 ± 7.9 t ha^{-1}) than in unthinned ones (12.42 ± 1.14 t ha^{-1}). The total shrub biomass was greater in thinned plots (5.2 ± 0.2 t ha^{-1}) than in unthinned plots (4.3 ± 0.5 t ha^{-1}). Further, the increase in Holm oak biomass was 3-times greater in DU than in DT.

2.3.Chemical analyses, enzyme activities, and phospholipid fatty acids (PLFAs) analysis

The total nitrogen (N) and total organic C contents were determined using an Elemental Analyzer (C/N Flash EA 112 Series-Leco Truspec). The pH of an aqueous extract (soil:water, 1:5 (w:v)) was measured using a pH meter (Crison mod. 2001, Barcelona, Spain). The water-soluble C (WSC) and N (WSN) of the soil were extracted with distilled water (1:5, w:v) by shaking for 2 h, followed by centrifugation at 13000 rpm

for 15 min and filtration. The analysis of the C and N contents in the extracts was performed in an analyzer for liquid samples (Multi N/C 3100, Analytik Jena).

The urease activity in the soil was determined by the buffered method of Kandeler and Gerber (1988). The alkaline phosphatase and β-glucosidase activities were determined by following the methods described by Tabatabai and Bremner (1969) and a modification of Tabatabai's method (1982), respectively. Polyphenol oxidase was determined by the method of Allison (2006), with 50 mM pyrogallol as the substrate.

Phospholipids were extracted from 6 g of soil using chloroform-methanol extraction, as described by Bligh and Dyer (1959), and were fractionated and quantified using the procedure described by Frostegard et al. (1993). They were transformed into fatty acid methyl esters (FAMEs) by alkaline methanolysis and designated as described by Frostegard et al. (1993). The complete dried FAME fraction was dissolved in isooctane containing 0.23 mg ml^{-1} of 21:0 FAME as internal standard. The analysis was performed using a Trace Ultra Thermo Scientific gas chromatograph fitted with a 60-m capillary column (Thermo TR-FAME 60 m x 0.25 mm ID x 0.25 µm film), using helium as carrier gas. The following fatty acids are characteristic bacterial fatty acids and were chosen as bacterial biomarkers: i15:0, a15:0, 15:0, i16:0, i17:0, cy17:0, cy19:0, 16:1ω7c, 16:1ω7t, 18:1ω9c, and 18:1ω9t. The fatty acid 18:2ω6 was used as the indicator of fungal biomass. The Gram-positive representative fatty acids used were i15:0, a15:0, i16:0, and i17:0. The Gram-negative fatty acids used were cy17:0, cy19:0, 16:1ω7c, 16:1ω7t, 18:1ω9c, and 18:1ω9t. The 10Me-branched FAMEs (10Me16:0 and 10Me18:0) were taken as specific actinobacterial biomarkers within the Gram-positive bacteria.

2.4.DNA extraction and amplification

The DNA was extracted from 0.5 g of freeze-dried soil of each sample with the Fast DNA Spin Kit for soil (MP Biomedicals, France). The V4 region of bacterial 16S ribosomal RNA (rRNA) was amplified using the barcoded primers 515F and 806R, as described previously (Caporaso et al., 2012). The PCR amplification of the fungal ITS2 region from DNA was performed using barcoded gITS7 and ITS4 (Ihrmark et al., 2012) in three PCR reactions per sample, as described previously (Zifcakova et al., 2016). The PCR products were cleaned using a MiniElute Kit (Qiagen) and their concentrations measured by Qubit. After libraries were prepared, sequencing of fungal and bacterial amplicons was performed on Illumina MiSeq. The amplicon sequencing data were processed using the pipeline SEED 2 (Vetrovsky et al., 2018). Briefly, pair-end reads were merged using FASTQ-join (Aronesty, 2013). Whole amplicons were processed for bacterial 16S, whereas the ITS2 region was extracted using ITS EXTRACTOR 1.0.8 before processing. Chimeric sequences were detected using USEARCH 7.0.1090 and deleted, and sequences were clustered, using UPARSE implemented within USEARCH (Edgar, 2013), at a 97% similarity level. Consensus sequences were constructed for each cluster, and the closest hits at the genus or species level were identified using BLASTn against the Ribosomal Database Project (Cole et al., 2014) and Genbank databases (for bacteria) or UNITE (Koljalg et al., 2013) and GenBank (for fungi). Sequences identified as nonbacterial or nonfungal were discarded. The Shannon–Wiener index was calculated for 4700 sequences per sample. The pipeline SEED 2 was used for data pre-processing and diversity calculations (Vetrovsky et al., 2018). The DNA sequences have been deposited in the MG-RAST public database (mgm4829212.3 for bacterial communities and mgm4829213.3 for fungal communities).

2.5.Data analysis and statistics

The normality and variance homogeneity of variables were tested by the Kolmogorov-Smirnov and Levene tests, respectively. The variables were subjected to three-way ANOVA. The three factors included in this experimental design were: i) *Drought*, which had two levels: natural rainfall conditions and induced drought; ii) *Forest management*, which also had two levels: thinning and control; and iii) *Season:* summer and winter. For each season, chemical parameters, PLFAs, and enzyme activities were subjected to one-way ANOVA followed by post hoc analysis using Tukey's significant difference test. Differences at $P < 0.05$ were regarded as statistically significant. Non-metric multidimensional scaling (NMDS) on Bray-Curtis distances was used to visualize the differences in the microbial community structure. PERMANOVA with 9999 permutations was applied to test the influence of the factors analyzed on the structure of the microbial communities. Spearman correlations between variables were determined. The statistical analyses were performed with IBM-SPSS Statistics (version 24.0) and R software v.3.1.3. ~~R studio package 1.1.383.~~

3.Results

3.1.Moisture, soil chemistry, and soil enzyme activities

Soil moisture was significantly affected by all factors (drought, management, and season) (Table S1, Supporting Information). Soil moisture was greater in winter than in summer, and in soils that were not subjected to induced drought ~~(Table 1)~~. Soil moisture was generally higher in unthinned plots than in the corresponding thinned plots (Table 1). Drought and season, but not management, influenced the soil organic C (SOC) content. The interaction management-drought influenced the ~~soil organic C~~SOC content: it was higher in control thinned plots (CT) than in drought thinned plots (DT); and lower in unthinned control plots (CU) than in unthinned plots subjected to induced

drought (DU) (Table 1; Table S1). Moreover, soil moisture and SOC content were significantly higher in DU than DT in both seasons. Soil moisture, but not SOC content, was higher in CU than in CT (Table 1). The triple interaction (season-management-drought) did not significantly influence the soil moisture and the SOC content (Table S1).

All factors (drought, management, and season) influenced the contents of water-soluble C and N. WSC and WSN were higher in unthinned drought plots (DU) than in the control treatments (CU) (Table 1). A similar pattern was observed in winter and summer. However, the WSC and WSN contents were significantly higher in summer than in winter.

Drought and season significantly impacted all the enzyme activities measured. Forest management influenced significantly all enzyme activities except ~~that of p-~~phenol oxidase (Table S1). The interaction between management and drought influenced the activity of the enzymes involved in C cycling (β-glucosidase and ~~p-~~phenol oxidase), but not the others. In winter, drought reduced the activity of all enzymes (except ~~, with the exception of that of p-~~phenol oxidase~~,~~ which increased in drought plots) (Fig. 1). In summer, drought had different effects depending on the enzyme: the urease activity of ~~thinned drought plots (~~DT~~)~~ was higher than that in ~~the thinned plots under control conditions (~~CT~~)~~; the β-glucosidase and ~~p-~~phenol oxidase activities were lower in ~~thinned drought plots (~~DT~~)~~ in comparison to their values in ~~the thinned plots under control conditions (~~CT~~)~~; and the phosphatase activity remained quite stable under drought conditions in summer.

3.2.The PLFA content

313 Neither management nor drought impacted the total contents of bacterial and fungal
314 fatty acids. Drought did impact the abundance of Gram-positive bacterial fatty acids
315 (Table S1). The season influenced the abundance of bacterial PLFAs, including both
316 Gram-positive and Gram-negative biomarkers. The interaction management-drought did
317 not influence any fatty acid group. The interaction season-management influenced the
318 content of fungal fatty acids and the interaction season-drought influenced the content
319 of all PLFA groups.

320 In winter, drought had a negative impact on the bacterial and fungal PLFA contents (in
321 both thinned and unthinned plots) (Fig. 2). Conversely, the bacterial and fungal
322 biomasses were higher in drought plots in summer. The same results were observed for
323 actinobacterial biomarkers.

324 Drought impacted significantly the Gram-positive to Gram-negative PLFA ratio,
325 whereas management and season (but not drought) influenced the fungal-to-bacterial
326 PLFA ratio; in winter, the fungal-to-bacterial PLFA~~this~~ ratio was higher in unthinned
327 plots than in thinned ones (Fig. S1; Supporting Information; Table S1). No differences
328 between the drought and control treatments were observed. In summer, the fungal-to-
329 bacterial PLFA ratio did not differ between management treatments, but tended to be
330 higher in drought plots.

331

332 *3.3. Diversity indices*

333 Drought influenced the Shannon-Wiener index of the soil bacterial community, but not
334 ~~those~~ that of the fungal community (Table ~~S~~2). Forest management influenced the
335 Shannon-Wiener index of the fungal community. The management-drought interaction
336 influenced the Shannon-Wiener index of the bacteria (this was the only significant

effect of the interactions on microbial diversity). The Shannon-Wiener index of the bacterial community tended to be greater in DU than in CU plots (Table S2). In winter, the Shannon-Wiener index of the fungal community was higher in thinned than in unthinned plots. In summer, this pattern was only observed in the control, non-drought plots.

3.4. The composition of the bacterial community

The PERMANOVA, performed with axes 1 and 2 from the NMDS, indicated that the composition (β-diversity) of the bacterial community were significantly influenced by drought (R^2 = 0.087; *P* = 0.011), forest management (R^2 = 0.105; *P* = 0.0044), and season (R^2 = 0.204; *P* = 0.0001) (Table S2). The structure of the bacterial community was influenced by the interaction between forest management and drought (R^2 = 0.223; *P* = 0.0002). In this sense, the differences between thinned and unthinned plots were greater when they were subjected to drought than in the non-drought conditions (Fig. 3).

Drought influenced the relative abundance of *Actinobacteria* (i.e. *Rubrobacter*) and *Bacteroidetes* (i.e. *Flavisolibacter*), that tended to decrease with drought. Forest management impacted the abundance of *Acidobacteria* (including *Pyrinomonas*) and *Betaproteobacteria* (including *Herbaspirillum*, *Massilia*, *Methyloversatilis*, and *Ramlibacter*) (Fig. 4; Table S3). The abundance of both groups was increased in unthinned plots, particularly in the case of *Betaproteobacteria*. The season influenced the relative abundance of *Acidobacteria* (increased in summer); *Actinobacteria* (including *Blastococcus*), that decreased in summer, particularly in thinned plots; *Bacteroidetes* (including *Flavisolibacter*), that increased in summer; *Lysinibacillus* (increased in summer); and some *Betaproteobacteria*, such as *Massilia* and *Methyloversatilis*, that were more abundant in winter. The interaction forest

362 management-drought influenced the abundance of *Actinobacteria* (including
363 *Blastococcus*), that decreased in summer, particularly in thinned plots; *Firmicutes*
364 (including *Bacillus* and *Lysinibacillus*), which had higher values in unthinned plots
365 when they were not subjected to drought; and some *Alphaproteobacteria*, such as
366 *Bradyrhizobium* and *Microvirga*.

367

368 *3.5. The composition of the fungal community*

369 The PERMANOVA indicated that the composition (β-diversity) of the fungal
370 community were solely and significantly influenced by forest management (R^2 = 0.232;
371 P = 0.0062), but not by drought (P = 0.827) or season (P = 0.274). Further, no
372 interaction influenced the structure of the fungal community. In summer, there was a
373 clear impact of forest management on the structure of the fungal community (Fig. 3):
374 CT and DT were grouped separately from CU and DU. In winter, the structure of the
375 fungal community under drought conditions differed between the managed and non-
376 managed plots.

377 Neither drought nor forest management, nor their interaction, influenced the abundance
378 of fungal ~~populations at the phylum level~~phyla. The season did influence it in the case
379 of *Ascomycota* and *Basidiomycota* (Fig 5; Table S4). Drought influenced the relative
380 abundance of the following fungal genera: *Amphinema*, *Cortinarius*, *Exophiala*,
381 *Hydnocystis*, *Kabatiella*, *Lyophyllum*, *Sebacina*, *Tricholoma*, and *Tuber*. Forest
382 management altered the abundance of *Alternaria*, *Amphinema*, *Astraeus*, *Cortinarius*,
383 *Exophiala*, *Hygrocybe*, *Hydnocystis*, *Lyophyllum*, *Penicillium*, *Sebacina*, *Tricholoma*,
384 and *Tuber*. The season influenced the abundance of *Ascomycota* and *Basidiomycota*,
385 and the abundance of the following genera: *Alternaria*, *Amphinema*, *Cortinarius*,
386 *Hydnobolites*, *Hydnocystis*, *Inocybe*, *Lyophyllum*, *Sebacina*, and *Tuber*.

3.6. Relationship between the diversity of the microbial community and enzyme activities

The Shannon-Wiener index of diversity of bacteria and fungi did not significantly correlate with any soil enzyme activity ($P > 0.05$; Table S5). The composition of soil bacterial community correlated with soil enzyme activities. In detail, the NMDS1 coordinates of bacterial community correlated significantly with phenol-oxidase activity, and the NMDS2 correlated with all the enzyme activities. In contrast, the composition of the fungal community (neither NMDS1 nor NMDS2) did not correlate with soil enzyme activities ($P > 0.05$; Table S5).

4.Discussion

4.1.The effects of forest management (CT vs CU)

Soil microorganisms are involved in organic matter (OM) cycling and thus it is expected that any external influence that impacts the soil content of organic C and N may affect the soil microbial community (Fuchslueger et al., 2014). Forest management practices that affect soil C stocks can also influence the soil microbial community (Hodel and Treseder, 2013; Bastida et al., 2017a). Here, the thinning in CT plots increased the bacterial biomass, in comparison to unthinned plots (CU), particularly in winter. The increases in bacterial biomass associated with thinning may be related to the higher contents of total organic C and N in CT plots, relative to CU plots. These results are in consonance with the greater Holm oak biomass in thinned (31.2 ± 5.4 t ha^{-1}) than in unthinned (25.3 ± 2.3 t ha^{-1}) plots. Moreover, after thinning, *Q. ilex* roots can survive and regrow, as indicated by the higher root to shoot ratio in CT (457 ± 104 %), in

411 comparison to CU (96 ± 0.6 %), which points to the roots of *Q. ilex* as an important
412 source of OM. Other studies found a greater SOM content in thinned plots of *Pinus*
413 *tabuliformis* plantations (Dang et al., 2018) and promotion of the microbial biomass -
414 without significant effects on soil enzyme activities - after thinning in *Pinus densiflora*
415 plantations (Kim et al., 2018). Further, it could be hypothesized that the incidence of
416 thinning in soil moisture and temperature could also influence litter decomposition and
417 be, partially, responsible on the greater SOC content in thinned plots. For instance,
418 thinning reduced soil moisture (particularly in winter) and could also increase the
419 averaged soil temperature (not measured) due to the increased light penetration as a
420 consequence of the lower aboveground biomass. Furthermore, the thinning of Holm oak
421 consisted of the removal of ~~aerial~~ aboveground plant ~~parts~~tissues, which were thereafter
422 abandoned on the soil surface. Indeed, thinning initially added 1.48 t ha^{-1} of plant
423 biomass to the soil surface in 2008. Such plant debris is decomposed and can be
424 incorporated into the SOM through the stimulation of the microbial biomass and
425 enzyme activities. Further, the establishment of shrubs and herbs after thinning could
426 result in higher decomposition rates and an increase in the SOM content (He and
427 Barclay, 2000). Indeed, our results show that the shrub biomass in thinned plots was
428 greater (5.2 ± 0.2 t ha^{-1}) than in unthinned plots (4.3 ± 0.7 t ha^{-1}). These results contrast
429 with the impacts of thinning in *Pinus halepensis*–dominated ecosystems under a
430 semiarid climate: in this case the plant remains produced by thinning were removed and
431 so did not contribute substantially to the accumulation of OM (Bastida et al., 2017).

432 The fungal-to-bacterial PLFA ratio was higher in unthinned plots (CU), but only in
433 winter. This may be indicative of the dominance of fungi in unthinned plots when the
434 moisture content was higher. Moreover, it has been proposed that the soil microbial
435 community becomes increasingly dominated by fungi as primary succession proceeds,

436 suggesting that decomposition and nutrient cycling are increasingly fungal-driven as an
437 ecosystem develops (Bardgett and Walker, 2004). Since no management practices were
438 carried out in the CU forest plots, the microbial communities in this soil developed
439 naturally, becoming more dominated by fungi.

440 As discussed above, thinning impacted the microbial biomass, but it is unclear if these
441 responses were accompanied or not by changes in the composition of the microbial
442 communities. The composition of the fungal community was affected uniquely by forest
443 management. Fungi depend on the exogenous inputs of carbohydrates and metabolites
444 from plants that can be modified through forest management activities, finally
445 impacting the composition of the soil fungal community (Broeckling et al., 2008;
446 Kohout et al., 2018). For instance, the abundance of *Basidiomycota* was lower in
447 thinned plots (CT) than in unthinned plots (CU) in winter, and *vice versa* in summer.
448 Dang et al. (2018) also observed a negative impact of thinning on the abundance of
449 *Basidiomycota*. Among the genera that were more abundant in thinned plots, we noticed
450 saprotrophs such as *Cladophialophora*, *Chalara*, and *Penicillium*, and ectomycorrhizal
451 fungi such as *Cenococcum*. These saprotrophs have been identified recently as
452 degraders of carbohydrates and plant-derived compounds in the oak forest soil (López-
453 Mondéjar et al., 2018), so it is possible that these ~~fungal populations~~fungi took
454 advantage of the SOM derived from the plant remains produced by thinning.

455 The composition of the bacterial community was affected by management but was also
456 subjected to seasonal effects. Among *Alphaproteobacteria*, the abundance of
457 *Bradyrhizobium*, a genus of N-fixing bacteria, was greater in CT than CU. Other
458 authors have also found that thinning can modify the forest microenvironment and
459 increase the abundance of N-fixing bacteria (Dang et al., 2018). Further, it has been
460 suggested that some groups of *Proteobacteria* are copiotrophic organisms with higher

abundance in soils richer in OM (Fierer et al., 2007; Bastida et al., 2016). In contrast, the abundance of *Betaproteobacteria* was higher in CU than in CT soil in winter, which is to say that they were more abundant in soils with lower amounts of total organic C and N. However, we have found previously that the WSC content has a greater influence on the abundance of copiotrophs than the total organic C (Bastida et al., 2016). Indeed, the ~~superior~~ greater abundance of *Betaproteobacteria* in CU ~~soil~~ in winter matched the pattern of WSC which was also higher in CU than in CT. In Mediterranean ecosystems, it has been observed that the amount of labile SOM is higher in the dry season (as observed here) and that this fraction can act as a substrate for microorganisms in more humid seasons (i.e. winter) through the improved diffusion of C (Schaeffer et al., 2017). In this respect, the abundance of *Massilia*, *Herbaspirillum*, and *Ramlibacter* (*Betaproteobacteria*), *Devosia* (*Alphaproteobacteria*), *Cohnella* (*Firmicutes*), and *Pseudonocardia* (*Actinobacteria*) were greater in CU than in CT soil in winter. Some of these taxa (*Massilia*, *Herbaspirillum*, *Cohnella*, *etc.*) are associated with plant roots whose exudates have a higher WSC content (Ofek et al., 2012). Moreover, Verastegui et al. (2014) found that *Devosia* had the capacity to assimilate multiple carbohydrates. We suggest that these ~~populations~~ groups (*Betaproteobacteria*, such as *Massilia*, *Devosia*, etc.), which are abundant in the rhizosphere, feed on the larger amount of root exudates present in CU soil (higher WSC content) relative to CT, in winter, while the amount of root exudates is probably limited by the dry conditions in summer.

4.2.The effects of drought (CU vs DU)

Drought could be expected to negatively affect plant understory development and the allocation of C compounds to soil, hence lowering the content of SOM (Fuchslueger et

al., 2014). However, here, when comparing CU vs DU, drought increased the amounts of total organic C and total N in both seasons. Given the fact that drought did not affect the *Q. ilex* biomass in unthinned plots, the accumulation of SOM could have resulted from the reduction of some enzyme activities as a consequence of drought. Drought ~~lowered~~ reduced soil moisture in winter and this probably lowered ~~this, probably, reduced~~ pore connectivity ~~– that would have limited the diffusion of substrates in drought-affected plots~~ and the diffusion of substrates from plants to soil~~in winter. In these conditions, the transfer of plant organic compounds to soil is limited~~ (Canarini et al., 2016). ~~,~~This phenomenon could ~~-~~negatively affect~~ing~~ soil hydrolase activities related to the C (β-glucosidase) and P (phosphatase) cycles in winter. Moreover, the drought resistance of some extracellular enzyme activities in summer may be related to the resistance of the microbial biomass that is producing these enzymes; but, most likely, it is related to their association with humic compounds that are abundant in forest soils and can preserve the activity of enzymes even in the absence of the producer cells (Burns et al., 2013). Further, induced drought in summer probably has less impact because rainfall is, *per se*, scarce in this season. In comparison to hydrolases, ~~The p-~~ phenol oxidase activity ~~differed from that of hydrolase since it~~ increased with drought in both seasons. Soils with higher oxygen availability, such as those with no moisture, may have superior oxidative enzyme activity (Sinsabaugh, 2010). Nevertheless, under tropical climate, it has been found that the activity of soil phenol oxidase increased in the rainy season due to the greater SOC content in such season (Singh et al., 2018).

In winter, the decrease in the activity of some hydrolases coincided with the decrease in soil microbial biomass in DU in comparison to CU. Negative effects of drought on soil microbial biomass have been shown previously (Sheik et al., 2011; Baldrian et al., 2013). However, we observed that the bacterial and fungal PLFA contents, as indicators

511 of microbial biomass, were greater in DU than in CU soil in summer. There are several
512 mechanisms through which the soil microbial community of a forest ecosystem can
513 present resistance (and even resilience) to drought under a semiarid climate. Soil OM
514 improves water retention and hence the greater OM content in the drought-affected plots
515 may have increased the drought resistance of the soil microbial biomass in summer
516 (Hueso et al., 2012). This increased water-sorption capacity can reduce the episodes of
517 water shortage in the soil (Bastida et al., 2017b). Further, the accumulation of fatty
518 acids does not necessarily match microbial growth. It is possible that the higher PLFA
519 content in drought plots in summer was a mechanism of osmolyte accumulation for
520 protection against dehydration rather than a signal of microbial growth *per se* (Schimel
521 et al., 2007; Bastida et al., 2017b). Moreover, via aerobic degradation of lipids by β-
522 oxidation, the citric acid cycle, and oxidation of the reducing equivalents produced,
523 microorganisms can gain more water per unit of lipid than per unit of carbohydrate or
524 protein oxidized (Hauschild et al., 2017). Thus, lipids accumulation can represent a
525 cellular mechanism for the maintenance of the basic energetic demands when
526 environmental conditions are harsher.

527 The composition of the fungal community was affected less by drought (CU vs DU)
528 than were those of the bacterial community, in agreement with the generalized greater
529 resistance of fungi to drought (Evans and Wallenstein, 2012). This is illustrated by the
530 relative stability of the fungal community composition in the face of drought and by the
531 slightly greater soil fungal-to-bacterial PLFA ratio in DU than in CU in summer. This
532 ratio has been proposed as an indicator of drought because hyphae can explore
533 micropores whereas bacteria rely on water films for substrate diffusion (de Boer et al.,
534 2005; de Vries et al., 2012).

We observed that the composition of the bacterial community in this semiarid ecosystem dominated by Holm oak may be more strongly modulated by the indirect and long-term effects of drought - the accumulation of SOM, which is a limiting factor in semiarid ecosystems (Bastida et al., 2016) - rather than by the direct effect of the lower water content imposed by water restriction. Moreover, these changes in the bacterial community composition were statistically related to changes in soil enzyme activities (Table S5). Some groups of *Proteobacteria* are copiotrophic ~~populations~~ organisms that utilize such carbon stocks (Fierer et al., 2007; López-Mondéjar et al., 2018). Here, we show that the SOM content can be a more important driver of the abundance of these ~~populations~~ groups than the water supply. These proteobacterial ~~populations~~ groups were more abundant in drought plots (DU), which also had greater OM contents. Some ~~populations~~ groups (i.e. *Bradyrhizobium* (*Alphaprotoebacteria*) and *Herbaspirillum* (*Betaproteobacteria*)) could have outcompeted some classical drought-resistant ~~populations~~ groups such as *Actinobacteria* (including *Rubrobacter* and *Solirubrobacter*) (Marasco et al., 2012; Bastida et al., 2017a; Ochoa-Hueso et al., 2018).

4.3.Does thinning influence the impacts of drought? (DT vs DU)

The contents of total organic C and N were higher in unthinned drought plots (DU) than in thinned drought plots (DT), in accordance with the greater increment in biomass per tree in DU. This pattern was the opposite of that observed in CT and CU plots. It is conceivable that the plant remains left after thinning did not contribute to the SOM under drought conditions. This can be probably ~~–~~due to the ~~limitation of the~~limited diffusion of carbon and hence of microbial activity in DT soil, ~~by~~ as a consequence of the lower moisture content. However, our results suggest that thinning had a protective influence on the soil hydrolase activities of drought plots, particularly in winter (DT >

DU). It is possible that microbes can produce more enzymes when there is a demand for substrates (Burns et al., 2013), as was the case of DT. There were no differences in the bacterial and fungal PLFA content between DT and DU (with the sole exception of the fungal PLFA content in DU, which was higher in winter). The absence of parallel patterns of the PLFA content and enzyme activities can be explained by the fact that the PLFA content is not directly related to growth (Schimel et al., 2007; Bastida et al., 2017b) and/or that soil enzymes can be immobilized in humic substances even when the producer cell is no longer alive (Burns et al., 2013).

Both drought and forest management altered the composition of the bacterial community, but management was the only factor that impacted the composition of the fungal community. ~~Forest management can have a direct influence on C cycling and hence it is logical that the community of fungi, which are often considered the principal decomposers of dead plant biomass (Voriskova et al., 2014), was altered.~~ In this sense, thinning in drought plots (DT) increased the abundance of a few ectomychorrizal fungi (*Astraeus*, *Tomentella*, *Inocybe*) but decreased the abundance of other ectomycorrhiza such as *Tricholoma*. In the same way, DT plots also showed higher abundance of a few saprotrophs, such as *Peziza*, but in general the abundance of other saprotrophic genera - such as *Penicillium*, *Hydnobolites*, and *Exophiala* - was decreased. A recent study highlighted a role for *Tomentella* in glucose accumulation, and in plant biomass decomposition for *Exophiala* and *Penicillium* (López-Mondéjar et al., 2018). Here, DU soil had a higher content of organic C than DT, which likely derived from plant compounds that could have been utilized by these fungi.

The composition of the bacterial community differed between DT and DU in both seasons. The abundance of some *Actinobacteria* (i.e. *Blastococcus* and *Solirubrobacter*) in winter, and of *Firmicutes* (*Bacillus* and *Lysinibacillus*) in both seasons, was higher in

thinned drought plots (DT) than in unthinned drought plots (DU). Contrarily, a higher abundance of *Acidobacteria* (*Pyrinomonas*), *Alphaproteobacteria* (*Bradyrhizobium*), *Betaproteobacteria* (*Herbaspirillum* and *Ramlibacter*), and *Domibacillus* was observed in DU, in comparison to DT. The dominance of *Actinobacteria* in semiarid ecosystems has been pointed out in several studies (Bastida et al., 2017b), highlighting the capacity of this phylum to survive under harsh conditions (Battistuzzi and Hedges, 2009). Probably, under drought conditions, the plant debris remaining after thinning did not accumulate in soil sufficiently to increase the soil C content. Under such conditions, *Actinobacteria* become more competitive. Conversely, the accumulation of SOM was higher in DU than in DT, and favored typical copitrophic ~~populations~~ groups such as *Betaproteobacteria* (Fierer et al., 2007; Bastida et al., 2016). Furthermore, we found different intra-phylum patterns. For instance, the abundance of *Bacillus* and *Lysinibacillus* was promoted by thinning in drought plots, while the abundance of *Domibacillus* was promoted in unthinned ones. These results illustrate the need to delve into lower taxonomic levels for a complete understanding of the impacts of drought and forest management on the soil microbial community and the possible role of specific populations.

5. Conclusions

The interplay of forest management and drought affected the soil microbial community of a Holm oak Mediterranean ecosystem, hence defining the response of soil microbiomes to drought. As hypothesized, drought altered the bacterial community but not the fungal community, which was influenced more by thinning. Further, the impact of thinning was dependent on the drought conditions. Our results indicate that thinning

did not seem a suitable strategy for soil carbon sequestration in ecosystems under drought conditions.

We conclude that thinning can promote drought resistance in the soil microbial community, particularly at the functional level, as indicated by the enzyme activities related to C, N and P cycles. These responses could be related to variations in the composition of the bacterial and fungal communities in thinned drought plots, in comparison to unthinned drought ones.

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849

850 **Figure Captions**

851 **Figure 1**. β-glucosidase (A), alkaline phosphatase (B), urease (C), and ~~p~~ phenol oxidase
852 (D) activities in the studied soils. CT (thinned soil without induced drought); DT
853 (thinned soil with induced drought); CU (unthinned soil without induced drought); DU
854 (unthinned soil with induced drought). For each season, data followed by the same letter
855 are not significantly different ($P < 0.05$).

856 **Figure 2**. The content of bacterial (A), fungal (B), Gram-positive (C), Gram-negative
857 (D), and actinobacterial (E) phospholipid fatty acids (PLFAs) in the studied soils. CT
858 (thinned soil without induced drought); DT (thinned soil with induced drought); CU
859 (unthinned soil without induced drought); DU (unthinned soil with induced drought).

860 For each season, data followed by the same letter are not significantly different ($P <$
861 0.05).

862 **Figure 3**. The non-metrical multimensional scaling (NMDS) of bacterial (A) and fungal
863 (B) communities in the studied soils

864 **Figure 4**. Bacterial community composition in the studied soils at the phylum (A) and
865 genus (B) taxonomic levels.

866 **Figure 5**. Fungal community composition in the studied soils at the phylum (A) and
867 genus (B) taxonomic levels.

868

*Graphical Abstract

Hoalm oak dominated Mediterranean ecosystem

Thinning
Drought
Seasonality

Thinning

Thinning + Drought

Bacterial community

Fungal community

Greater resistance microbial community

SOC reduction

Soil enzyme activities

HIGHLIGHTS

- The impacts of thinning and drought were evaluated in the soil microbial community.
- Thinning increased organic C and microbial biomass in a Holm oak dominated-soil.
- The composition of fungal community was solely affected by thinning.
- Drought reduced the activity of extracellular soil enzymes.
- The resistance of the soil microbiome to drought was fostered by thinning.

TITLE PAGE

When drought meets forest management: effects on the soil microbial community of a Holm oak forest ecosystem

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Abstract

The growth and survival of plants in semiarid Mediterranean forests can be improved through the benefits conferred by thinning, a forest management practice that removes trees and reduces the competition between the remaining ones. Here, we evaluate the impacts of induced drought (the exclusion of 25% of the natural rainfall for 5 years) and thinning, and their interaction, with the objective of determining whether the thinning of Holm oak (*Quercus ilex* L.) modulates the resistance of the soil microbial community to drought. Sequencing of 16S rRNA and ITS amplicons revealed that drought, thinning, and their interaction influenced the composition of the bacterial community, while the fungal community was exclusively affected by thinning. Thinning consisted of the removal of the aboveground parts of the Holm oak trees, which were thereafter left in forest stand. Thinning contributed to the C and N contents, with parallel increases in microbial biomass, particularly in summer. Drought increased the amounts of total organic C and total N, likely due to the reduced enzyme activities. Indeed, the composition of the bacterial community was modulated primarily by the indirect and long-term effects of drought - the accumulation of soil organic matter - rather than by the direct effect of the lower water content imposed by the drought treatments. Thinning under drought conditions did not increase soil organic C (SOC) content. However, the

resistance of the soil microbial community to drought was fostered by thinning, particularly at the functional level, as indicated by the enzyme activities related to C, N and P cycles. These responses were associated to variations in the composition of the microbial communities in thinned, drought-exposed plots, in comparison to unthinned, drought-exposed plots. In conclusion, the interaction between forest management and drought influenced the soil microbial community of a Holm oak-dominated Mediterranean ecosystem.

Keywords: soil microbial community; semiarid; thinning; drought; enzyme activity; Holm oak

1. Introduction

Forest soils store large amounts of organic matter from aboveground(Crowther et al., 2014; Bastida et al., 2015; Lladó et al. 2017). Consequently, practices that affect plant development in forests and soil properties may have crucial impacts on ecosystem functions and C feedbacks between the soil and atmosphere (Crowther et al., 2014). Several forest management practices are designed to improve ecosystem functioning. In this sense, thinning is a common management practice that involves the removal of trees to improve the growth rate and health of the remaining trees, by reducing competition for resources such as water, nutrients, or light (Yang et al., 2017). Thinning has been widely practiced in areas that have suffered wildfires, as a strategy to provide sufficient resources to the regenerated plants (Lopez-Serrano et al., 2016). Hence, given the strong recurrence of wildfires in Mediterranean areas in the last few decades (Certini et al., 2011), thinning practices were demonstrated to have a strong influence on the ecosystem function of these forests (López-Serrano et al., 2015, 2016). Moreover, the limited precipitation and the higher temperatures forecasted by climate change models will increase the chance of wildfires in Mediterranean ecosystems (Lindner et al., 2010; Hedo de Santiago et al., 2015).

Although its effects depend on the climate, timing and forest stand characteristics, thinning has an impact on the understory plant community due to the changes in the canopy density, plant community and microclimate (Dang et al., 2018). These changes in plant communities impact the quality and quantity of soil organic matter (SOM) and nutrients (López-Serrano et al., 2016; Bastida et al., 2017a; Dang et al., 2018) as well as the interactions between aboveground and belowground communities (Wardle et al., 2004; Prescott and Grayston, 2013). Although the impacts of thinning on the understory plant community have been widely studied (Long and Vacchiano, 2014), its

consequences for the belowground soil bacterial and fungal communities are less known (Bastida et al., 2017a; Dang et al., 2018). Importantly, the impacts of thinning on the soil microbial community of forest dominated by Holm oak (*Quercus ilex* L.), which is a central and emblematic plant species in the Iberian Peninsula, have not been evaluated so far. Besides the availability of organic C, there is another factor that largely drives the plant and microbial productivity in semiarid areas: the availability of water (Bastida et al., 2017a). This is particularly important when considering the predictions of climate change models that point to a reduction in precipitation in Southern Europe (IPCC 2013). However, there is a lack of knowledge about the interactions between forest management and climate change, and there is no doubt that both factors will contribute to the ecosystem sustainability in Mediterranean forests.

In soil, microbial communities perform key ecosystem functions and biogeochemical processes (i.e., litter decomposition) (van der Heijden et al., 2008; Baldrian et al., 2011; Bastida et al., 2016; Ochoa-Hueso et al., 2018). Drought may affect the biomass, activity and composition of soil microbial communities (Bastida et al., 2017a; Ochoa-Hueso et al., 2018), fungi being commonly considered as more resistant to drought than bacteria (de Vries et al., 2012; Evans and Wallenstein, 2012). In principle, the impacts of forest management in the soil microbial community can alter soil functioning and the provision ecosystem services, although the functional redundancy of soil microbial communities may minimize these impacts (Allison and Martiny, 2008; Curiel-Yuste et al., 2014). Further, it is known that land use and forest management can affect the resistance and resilience of soil microbial communities to drought (de Vries et al., 2012; Karlowsky et al., 2017). However, until now, only one study has evaluated the impacts of the interaction between climatic factors and thinning on the soil microbial community (Bastida et al., 2017a). This previous study was carried out in a *Pinus*

halepensis L. forest, where all the plant remains were completely removed after the thinning activities.

Here, we studied the long-term effects of Holm oak thinning on the soil microbial community under a drought scenario. Particularly, we determined the impacts of five years of drought on the soil microbial community and evaluate whether thinning influences the responses of the soil fungal and bacterial communities to drought. For these purposes, a multi-parametric study was performed that included the analysis of microbial biomass, enzyme activities, and composition and diversity of microbial communities, as well as chemical soil properties. Given the fact that in Holm oak thinning (this technique is known as 'resalveo' -coppicing with standards- in Spanish, López-Serrano et al. (2010)) the plant remains are left on the ground, we hypothesized that this forest management practice will largely impact the biomass and composition of soil fungal communities rather than the bacterial community, because fungi are strongly connected to the decomposition of cellulose and lignin from plant biomass (López-Mondéjar et al., 2018). Further, we expected that drought affects soil bacterial community more than fungal community, and that this impact will be shaped by thinning. Moreover, given the strong seasonality of Mediterranean forest ecosystems (dry summer vs humid winter), we expect that the effects of rainfall exclusion will be more severe in the humid season. Overall, this study aims to provide new evidence on how plant-microbe interactions are modulated by climate change and forest management, opening the door to the development of applied strategies for improving the sustainability of ecosystems in the predicted climate-change scenario.

2.Material and Methods

2.1.Study site

144 The study area is an original Mediterranean Maritime pine-Holm oak mixed forest
145 stand, located in the Castilla-La Mancha region (central-eastern Spain) in a forest called
146 "Dehesa de Abajo" (1,000 m.a.s.l., 39° 40′ N, 1° 55′W). This forest is considered a
147 mountain plain, where slopes do not exceed a 3% gradient. The mean annual
148 temperature and precipitation are 15.5 ºC and 510 mm, respectively (López-Serrano et
149 al., 2016). For the period 2008-2010, the mean seasonal temperature and precipitation
150 was 7.5 ºC and 170.3 mm in winter, and 24.0 ºC and 24.3 mm in summer, respectively.
151 The soil is a Lithic leptosol , associated with Chromic Luvisol, a very shallow soil over
152 calcareous hard rock (FAO, 1988). This area was a natural uneven-aged stand,
153 composed mainly of a dominant canopy tree layer of *Pinus pinaster* Ait. subsp.
154 *mesogeensis* (Mediterranean Maritime pine) with a subdominant tree layer of *Quercus*
155 *ilex* L. subsp. *ballota* (Holm oak). However, in July 2001, a wildfire affected the area
156 with a moderate-high severity (López-Serrano et al., 2016). The fire led to natural post-
157 fire plant regeneration with a very high density of sprouted Holm oak.

158 Six years later, in October 2007, the burnt stand was composed principally of Holm oak,
159 with an average density of 20000 standards ha^{-1}, and some Maritime pine (an average of
160 350 trees ha^{-1}). Some shrubs - such as *Sideritis incana* L., *Thymus vulgaris* L.,
161 *Rosmarinus officinalis* L., *Santolina chamaecyparissus* L., *Bupleurum fruticescens* L.,
162 *Lavandula latifolia* L., and *Genista scorpius* L. - were also observed (Lopez-Serrano et
163 al., 2016).

164 In January 2008, thinning was performed in a 1.25-ha experimental area within the
165 burnt stand. This reduced the Holm oak density to 3500 standards ha^{-1}. Dead, diseased,
166 and suppressed standards were felled with a clearing saw and left on the ground (an
167 average of 2.7 t ha^{-1} of plant dry biomass was provided to the soil by this thinning
168 activity).

169

170 *2.2.Experimental design for thinning and water restriction*

171 This study had a full factorial design with three factors: i) induced drought (that started
172 in 2012), with two levels (natural rainfall vs rainfall reduction or induced drought), ii)
173 forest management (thinning), with two levels (thinning or no thinning), and iii) season,
174 with two sampling seasons (summer and winter). The abbreviations utilized in this
175 study are as follows: CU (unthinned control soil); DU (unthinned soil subjected to
176 drought); CT (thinned control soil); and DT (thinned soil subjected to drought). Thus, in
177 October 2012, a total of 12 plots (20 m x 20 m) (i.e. 2 levels (rainfall) x 2 levels
178 (thinning) x 3 replicates) were set up, six of them in thinned areas and six in unthinned
179 areas. Each group of six plots was split into two subgroups: three plots received natural
180 rainfall and three plots, partially covered by PVC gutters, received reduced precipitation
181 to simulate drought-induced conditions. The PVC gutters were suspended at about 50
182 cm above the soil surface and occupied about 25% of the plot surface, intercepting the
183 corresponding rainfall. This rainfall reduction was within the range predicted by climate
184 models (15-30% for 2080, IPCC 2013).

185 Soil sampling was performed in January 2017 (winter) and July 2017 (summer). Six soil
186 samples per plot were taken at 0-15 cm depth, after removing the plant litter within each
187 plot, and mixed to obtain one composite sample per plot. The samples were
188 immediately sieved (2 mm) after sampling and kept at 4ºC during 3 weeks for chemical
189 and enzyme analyses, and during 3 months at -20ºC for fatty acids and genomic
190 analyses.

191 In 2014, the total biomass of Holm oak was slightly greater in thinned plots (31.25 ± 9.4
192 t ha^{-1}) than in unthinned ones (25.29 ± 2.31 t ha^{-1}), although there was no significant
193 difference. The root-shoot ratio was 5-times higher in thinned than in unthinned plots.

194 The aboveground biomass was greater in unthinned plots (12.87 ± 1.17 t ha^{-1}) than in
195 thinned plots (5.55 ± 2.01 t ha^{-1}), while the underground biomass was greater in thinned
196 plots (25.71 ± 7.9 t ha^{-1}) than in unthinned ones (12.42 ± 1.14 t ha^{-1}). The total shrub
197 biomass was greater in thinned plots (5.2 ± 0.2 t ha^{-1}) than in unthinned plots (4.3 ± 0.5 t
198 ha^{-1}). Further, the increase in Holm oak biomass was 3-times greater in DU than in DT.

199

200 *2.3.Chemical analyses, enzyme activities, and phospholipid fatty acids (PLFAs) analysis*

201 The total nitrogen (N) and total organic C contents were determined using an Elemental
202 Analyzer (C/N Flash EA 112 Series-Leco Truspec). The pH of an aqueous extract
203 (soil:water, 1:5 (w:v)) was measured using a pH meter (Crison mod. 2001, Barcelona,
204 Spain). The water-soluble C (WSC) and N (WSN) of the soil were extracted with
205 distilled water (1:5, w:v) by shaking for 2 h, followed by centrifugation at 13000 rpm
206 for 15 min and filtration. The analysis of the C and N contents in the extracts was
207 performed in an analyzer for liquid samples (Multi N/C 3100, Analytik Jena).

208 The urease activity in the soil was determined by the buffered method of Kandeler and
209 Gerber (1988). The alkaline phosphatase and β-glucosidase activities were determined
210 by following the methods described by Tabatabai and Bremner (1969) and a
211 modification of Tabatabai's method (1982), respectively. Polyphenol oxidase was
212 determined by the method of Allison (2006), with 50 mM pyrogallol as the substrate.

213 Phospholipids were extracted from 6 g of soil using chloroform-methanol extraction, as
214 described by Bligh and Dyer (1959), and were fractionated and quantified using the
215 procedure described by Frostegard et al. (1993). They were transformed into fatty acid
216 methyl esters (FAMEs) by alkaline methanolysis and designated as described by
217 Frostegard et al. (1993). The complete dried FAME fraction was dissolved in isooctane

containing 0.23 mg ml^{-1} of 21:0 FAME as internal standard. The analysis was performed using a Trace Ultra Thermo Scientific gas chromatograph fitted with a 60-m capillary column (Thermo TR-FAME 60 m x 0.25 mm ID x 0.25 µm film), using helium as carrier gas. The following fatty acids are characteristic bacterial fatty acids and were chosen as bacterial biomarkers: i15:0, a15:0, 15:0, i16:0, i17:0, cy17:0, cy19:0, 16:1ω7c, 16:1ω7t, 18:1ω9c, and 18:1ω9t. The fatty acid 18:2ω6 was used as the indicator of fungal biomass. The Gram-positive representative fatty acids used were i15:0, a15:0, i16:0, and i17:0. The Gram-negative fatty acids used were cy17:0, cy19:0, 16:1ω7c, 16:1ω7t, 18:1ω9c, and 18:1ω9t. The 10Me-branched FAMEs (10Me16:0 and 10Me18:0) were taken as specific actinobacterial biomarkers within the Gram-positive bacteria.

2.4.DNA extraction and amplification

The DNA was extracted from 0.5 g of freeze-dried soil of each sample with the Fast DNA Spin Kit for soil (MP Biomedicals, France). The V4 region of bacterial 16S ribosomal RNA (rRNA) was amplified using the barcoded primers 515F and 806R, as described previously (Caporaso et al., 2012). The PCR amplification of the fungal ITS2 region from DNA was performed using barcoded gITS7 and ITS4 (Ihrmark et al., 2012) in three PCR reactions per sample, as described previously (Zifcakova et al., 2016). The PCR products were cleaned using a MiniElute Kit (Qiagen) and their concentrations measured by Qubit. After libraries were prepared, sequencing of fungal and bacterial amplicons was performed on Illumina MiSeq. The amplicon sequencing data were processed using the pipeline SEED 2 (Vetrovsky et al., 2018). Briefly, pair-end reads were merged using FASTQ-join (Aronesty, 2013). Whole amplicons were processed for bacterial 16S, whereas the ITS2 region was extracted using ITS EXTRACTOR 1.0.8 before

processing. Chimeric sequences were detected using USEARCH 7.0.1090 and deleted, and sequences were clustered, using UPARSE implemented within USEARCH (Edgar, 2013), at a 97% similarity level. Consensus sequences were constructed for each cluster, and the closest hits at the genus or species level were identified using BLASTn against the Ribosomal Database Project (Cole et al., 2014) and Genbank databases (for bacteria) or UNITE (Koljalg et al., 2013) and GenBank (for fungi). Sequences identified as nonbacterial or nonfungal were discarded. The Shannon–Wiener index was calculated for 4700 sequences per sample. The pipeline SEED 2 was used for data pre-processing and diversity calculations (Vetrovsky et al., 2018). The DNA sequences have been deposited in the MG-RAST public database (mgm4829212.3 for bacterial communities and mgm4829213.3 for fungal communities).

2.5.Data analysis and statistics

The normality and variance homogeneity of variables were tested by the Kolmogorov-Smirnov and Levene tests, respectively. The variables were subjected to three-way ANOVA. The three factors included in this experimental design were: i) *Drought*, which had two levels: natural rainfall conditions and induced drought; ii) *Forest management*, which also had two levels: thinning and control; and iii) *Season:* summer and winter. For each season, chemical parameters, PLFAs, and enzyme activities were subjected to one-way ANOVA followed by post hoc analysis using Tukey's significant difference test. Differences at $P < 0.05$ were regarded as statistically significant. Non-metric multidimensional scaling (NMDS) on Bray-Curtis distances was used to visualize the differences in the microbial community structure. PERMANOVA with 9999 permutations was applied to test the influence of the factors analyzed on the structure of the microbial communities. Spearman correlations between variables were

268 determined. The statistical analyses were performed with IBM-SPSS Statistics (version
269 24.0) and R software v.3.1.3.

270

271 **3.Results**

272 *3.1.Moisture, soil chemistry, and soil enzyme activities*

273 Soil moisture was significantly affected by all factors (drought, management, and
274 season) (Table S1, Supporting Information). Soil moisture was greater in winter than in
275 summer, and in soils that were not subjected to induced drought. Soil moisture was
276 generally higher in unthinned plots than in the corresponding thinned plots (Table 1).
277 Drought and season, but not management, influenced the soil organic C (SOC) content.
278 The interaction management-drought influenced the SOC content: it was higher in
279 control thinned plots (CT) than in drought thinned plots (DT); and lower in unthinned
280 control plots (CU) than in unthinned plots subjected to induced drought (DU) (Table 1;
281 Table S1). Moreover, soil moisture and SOC content were significantly higher in DU
282 than DT in both seasons. Soil moisture, but not SOC content, was higher in CU than in
283 CT (Table 1). The triple interaction (season-management-drought) did not significantly
284 influence the soil moisture and the SOC content (Table S1).

285 All factors (drought, management, and season) influenced the contents of water-soluble
286 C and N. WSC and WSN were higher in unthinned drought plots (DU) than in the
287 control treatments (CU) (Table 1). A similar pattern was observed in winter and
288 summer. However, the WSC and WSN contents were significantly higher in summer
289 than in winter.

290 Drought and season significantly impacted all the enzyme activities measured. Forest
291 management influenced significantly all enzyme activities except phenol oxidase (Table

S1). The interaction between management and drought influenced the activity of the enzymes involved in C cycling (β-glucosidase and phenol oxidase), but not the others. In winter, drought reduced the activity of all enzymes (except phenol oxidase which increased in drought plots) (Fig. 1). In summer, drought had different effects depending on the enzyme: the urease activity of DT was higher than that in CT; the β-glucosidase and phenol oxidase activities were lower in DT in comparison to their values in CT; and the phosphatase activity remained quite stable under drought conditions in summer.

3.2.The PLFA content

Neither management nor drought impacted the total contents of bacterial and fungal fatty acids. Drought did impact the abundance of Gram-positive bacterial fatty acids (Table S1). The season influenced the abundance of bacterial PLFAs, including both Gram-positive and Gram-negative biomarkers. The interaction management-drought did not influence any fatty acid group. The interaction season-management influenced the content of fungal fatty acids and the interaction season-drought influenced the content of all PLFA groups.

In winter, drought had a negative impact on the bacterial and fungal PLFA contents (in both thinned and unthinned plots) (Fig. 2). Conversely, the bacterial and fungal biomasses were higher in drought plots in summer. The same results were observed for actinobacterial biomarkers.

Drought impacted significantly the Gram-positive to Gram-negative PLFA ratio, whereas management and season (but not drought) influenced the fungal-to-bacterial PLFA ratio; in winter, the fungal-to-bacterial PLFA ratio was higher in unthinned plots than in thinned ones (Fig. S1; Supporting Information; Table S1). No differences

316 between the drought and control treatments were observed. In summer, the fungal-to-
317 bacterial PLFA ratio did not differ between management treatments, but tended to be
318 higher in drought plots.

319

320 *3.3. Diversity indices*

321 Drought influenced the Shannon-Wiener index of the soil bacterial community, but not
322 that of the fungal community (Table 2). Forest management influenced the Shannon-
323 Wiener index of the fungal community. The management-drought interaction influenced
324 the Shannon-Wiener index of the bacteria (this was the only significant effect of the
325 interactions on microbial diversity). The Shannon-Wiener index of the bacterial
326 community tended to be greater in DU than in CU plots (Table 2). In winter, the
327 Shannon-Wiener index of the fungal community was higher in thinned than in
328 unthinned plots. In summer, this pattern was only observed in the control, non-drought
329 plots.

330

331 *3.4. The composition of the bacterial community*

332 The PERMANOVA, performed with axes 1 and 2 from the NMDS, indicated that the
333 composition (β-diversity) of the bacterial community were significantly influenced by
334 drought (R^2 = 0.087; P = 0.011), forest management (R^2 = 0.105; P = 0.0044), and
335 season (R^2 = 0.204; P = 0.0001) (Table S2). The structure of the bacterial community
336 was influenced by the interaction between forest management and drought (R^2 = 0.223;
337 P = 0.0002). In this sense, the differences between thinned and unthinned plots were
338 greater when they were subjected to drought than in the non-drought conditions (Fig. 3).

Drought influenced the relative abundance of *Actinobacteria* (i.e. *Rubrobacter*) and *Bacteroidetes* (i.e. *Flavisolibacter*), that tended to decrease with drought. Forest management impacted the abundance of *Acidobacteria* (including *Pyrinomonas*) and *Betaproteobacteria* (including *Herbaspirillum*, *Massilia*, *Methyloversatilis*, and *Ramlibacter*) (Fig. 4; Table S3). The abundance of both groups was increased in unthinned plots, particularly in the case of *Betaproteobacteria*. The season influenced the relative abundance of *Acidobacteria* (increased in summer); *Actinobacteria* (including *Blastococcus*), that decreased in summer, particularly in thinned plots; *Bacteroidetes* (including *Flavisolibacter*), that increased in summer; *Lysinibacillus* (increased in summer); and some *Betaproteobacteria*, such as *Massilia* and *Methyloversatilis*, that were more abundant in winter. The interaction forest management-drought influenced the abundance of *Actinobacteria* (including *Blastococcus*), that decreased in summer, particularly in thinned plots; *Firmicutes* (including *Bacillus* and *Lysinibacillus*), which had higher values in unthinned plots when they were not subjected to drought; and some *Alphaproteobacteria*, such as *Bradyrhizobium* and *Microvirga*.

3.5. The composition of the fungal community

The PERMANOVA indicated that the composition (β-diversity) of the fungal community were solely and significantly influenced by forest management (R^2 = 0.232; P = 0.0062), but not by drought (P = 0.827) or season (P = 0.274). Further, no interaction influenced the structure of the fungal community. In summer, there was a clear impact of forest management on the structure of the fungal community (Fig. 3): CT and DT were grouped separately from CU and DU. In winter, the structure of the

363 fungal community under drought conditions differed between the managed and non-
364 managed plots.

365 Neither drought nor forest management, nor their interaction, influenced the abundance
366 of fungal phyla. The season did influence it in the case of *Ascomycota* and
367 *Basidiomycota* (Fig 5; Table S4). Drought influenced the relative abundance of the
368 following fungal genera: *Amphinema*, *Cortinarius*, *Exophiala*, *Hydnocystis*, *Kabatiella*,
369 *Lyophyllum*, *Sebacina*, *Tricholoma*, and *Tuber*. Forest management altered the
370 abundance of *Alternaria*, *Amphinema*, *Astraeus*, *Cortinarius*, *Exophiala*, *Hygrocybe*,
371 *Hydnocystis*, *Lyophyllum*, *Penicillium*, *Sebacina*, *Tricholoma*, and *Tuber*. The season
372 influenced the abundance of *Ascomycota* and *Basidiomycota*, and the abundance of the
373 following genera: *Alternaria*, *Amphinema*, *Cortinarius*, *Hydnobolites*, *Hydnocystis*,
374 *Inocybe*, *Lyophyllum*, *Sebacina*, and *Tuber*.

375

376 *3.6. Relationship between the diversity of the microbial community and enzyme*
377 *activities*

378 The Shannon-Wiener index of diversity of bacteria and fungi did not significantly
379 correlate with any soil enzyme activity ($P > 0.05$; Table S5). The composition of soil
380 bacterial community correlated with soil enzyme activities. In detail, the NMDS1
381 coordinates of bacterial community correlated significantly with phenol-oxidase
382 activity, and the NMDS2 correlated with all the enzyme activities. In contrast, the
383 composition of the fungal community (neither NMDS1 nor NMDS2) did not correlate
384 with soil enzyme activities ($P > 0.05$; Table S5).

385

386 **4.Discussion**

387 *4.1.The effects of forest management (CT vs CU)*

388 Soil microorganisms are involved in organic matter (OM) cycling and thus it is
389 expected that any external influence that impacts the soil content of organic C and N
390 may affect the soil microbial community (Fuchslueger et al., 2014). Forest management
391 practices that affect soil C stocks can also influence the soil microbial community
392 (Hodel and Treseder, 2013; Bastida et al., 2017a). Here, the thinning in CT plots
393 increased the bacterial biomass, in comparison to unthinned plots (CU), particularly in
394 winter. The increases in bacterial biomass associated with thinning may be related to the
395 higher contents of total organic C and N in CT plots, relative to CU plots. These results
396 are in consonance with the greater Holm oak biomass in thinned (31.2 ± 5.4 t ha^{-1}) than
397 in unthinned (25.3 ± 2.3 t ha^{-1}) plots. Moreover, after thinning, *Q. ilex* roots can survive
398 and regrow, as indicated by the higher root to shoot ratio in CT (457 ± 104 %), in
399 comparison to CU (96 ± 0.6 %), which points to the roots of *Q. ilex* as an important
400 source of OM. Other studies found a greater SOM content in thinned plots of *Pinus*
401 *tabuliformis* plantations (Dang et al., 2018) and promotion of the microbial biomass -
402 without significant effects on soil enzyme activities - after thinning in *Pinus densiflora*
403 plantations (Kim et al., 2018). Further, it could be hypothesized that the incidence of
404 thinning in soil moisture and temperature could also influence litter decomposition and
405 be, partially, responsible on the greater SOC content in thinned plots. For instance,
406 thinning reduced soil moisture (particularly in winter) and could also increase the
407 averaged soil temperature (not measured) due to the increased light penetration as a
408 consequence of the lower aboveground biomass. Furthermore, the thinning of Holm oak
409 consisted of the removal of aboveground plant tissues, which were thereafter abandoned
410 on the soil surface. Indeed, thinning initially added 1.48 t ha^{-1} of plant biomass to the
411 soil surface in 2008. Such plant debris is decomposed and can be incorporated into the

412 SOM through the stimulation of the microbial biomass and enzyme activities. Further,
413 the establishment of shrubs and herbs after thinning could result in higher
414 decomposition rates and an increase in the SOM content (He and Barclay, 2000).
415 Indeed, our results show that the shrub biomass in thinned plots was greater (5.2 ± 0.2 t
416 ha^{-1}) than in unthinned plots (4.3 ± 0.7 t ha^{-1}). These results contrast with the impacts of
417 thinning in *Pinus halepensis*–dominated ecosystems under a semiarid climate: in this
418 case the plant remains produced by thinning were removed and so did not contribute
419 substantially to the accumulation of OM (Bastida et al., 2017).

420 The fungal-to-bacterial PLFA ratio was higher in unthinned plots (CU), but only in
421 winter. This may be indicative of the dominance of fungi in unthinned plots when the
422 moisture content was higher. Moreover, it has been proposed that the soil microbial
423 community becomes increasingly dominated by fungi as primary succession proceeds,
424 suggesting that decomposition and nutrient cycling are increasingly fungal-driven as an
425 ecosystem develops (Bardgett and Walker, 2004). Since no management practices were
426 carried out in the CU forest plots, the microbial communities in this soil developed
427 naturally, becoming more dominated by fungi.

428 As discussed above, thinning impacted the microbial biomass, but it is unclear if these
429 responses were accompanied or not by changes in the composition of the microbial
430 communities. The composition of the fungal community was affected uniquely by forest
431 management. Fungi depend on the exogenous inputs of carbohydrates and metabolites
432 from plants that can be modified through forest management activities, finally
433 impacting the composition of the soil fungal community (Broeckling et al., 2008;
434 Kohout et al., 2018). For instance, the abundance of *Basidiomycota* was lower in
435 thinned plots (CT) than in unthinned plots (CU) in winter, and *vice versa* in summer.
436 Dang et al. (2018) also observed a negative impact of thinning on the abundance of

Basidiomycota. Among the genera that were more abundant in thinned plots, we noticed saprotrophs such as *Cladophialophora*, *Chalara*, and *Penicillium*, and ectomycorrhizal fungi such as *Cenococcum*. These saprotrophs have been identified recently as degraders of carbohydrates and plant-derived compounds in the oak forest soil (López-Mondéjar et al., 2018), so it is possible that these fungi took advantage of the SOM derived from the plant remains produced by thinning.

The composition of the bacterial community was affected by management but was also subjected to seasonal effects. Among *Alphaproteobacteria*, the abundance of *Bradyrhizobium*, a genus of N-fixing bacteria, was greater in CT than CU. Other authors have also found that thinning can modify the forest microenvironment and increase the abundance of N-fixing bacteria (Dang et al., 2018). Further, it has been suggested that some groups of *Proteobacteria* are copiotrophic organisms with higher abundance in soils richer in OM (Fierer et al., 2007; Bastida et al., 2016). In contrast, the abundance of *Betaproteobacteria* was higher in CU than in CT soil in winter, which is to say that they were more abundant in soils with lower amounts of total organic C and N. However, we have found previously that the WSC content has a greater influence on the abundance of copiotrophs than the total organic C (Bastida et al., 2016). Indeed, the greater abundance of *Betaproteobacteria* in CU in winter matched the pattern of WSC which was also higher in CU than in CT. In Mediterranean ecosystems, it has been observed that the amount of labile SOM is higher in the dry season (as observed here) and that this fraction can act as a substrate for microorganisms in more humid seasons (i.e. winter) through the improved diffusion of C (Schaeffer et al., 2017). In this respect, the abundance of *Massilia*, *Herbaspirillum*, and *Ramlibacter* (*Betaproteobacteria*), *Devosia* (*Alphaproteobacteria*), *Cohnella* (*Firmicutes*), and *Pseudonocardia* (*Actinobacteria*) were greater in CU than in CT soil

in winter. Some of these taxa (*Massilia, Herbaspirillum, Cohnella, etc.*) are associated with plant roots whose exudates have a higher WSC content (Ofek et al., 2012). Moreover, Verastegui et al. (2014) found that *Devosia* had the capacity to assimilate multiple carbohydrates. We suggest that these groups (*Betaproteobacteria*, such as *Massilia*, *Devosia*, etc.), which are abundant in the rhizosphere, feed on the larger amount of root exudates present in CU soil (higher WSC content) relative to CT, in winter, while the amount of root exudates is probably limited by the dry conditions in summer.

4.2.The effects of drought (CU vs DU)

Drought could be expected to negatively affect plant understory development and the allocation of C compounds to soil, hence lowering the content of SOM (Fuchslueger et al., 2014). However, here, when comparing CU vs DU, drought increased the amounts of total organic C and total N in both seasons. Given the fact that drought did not affect the *Q. ilex* biomass in unthinned plots, the accumulation of SOM could have resulted from the reduction of some enzyme activities as a consequence of drought. Drought reduced soil moisture in winter and this probably lowered pore connectivity and the diffusion of substrates from plants to soil(Canarini et al., 2016). This phenomenon could negatively affect soil hydrolase activities related to the C (β-glucosidase) and P (phosphatase) cycles in winter. Moreover, the drought resistance of some extracellular enzyme activities in summer may be related to the resistance of the microbial biomass that is producing these enzymes; but, most likely, it is related to their association with humic compounds that are abundant in forest soils and can preserve the activity of enzymes even in the absence of the producer cells (Burns et al., 2013). Further, induced drought in summer probably has less impact because rainfall is, *per se*, scarce in this

487 season. In comparison to hydrolases, phenol oxidase activity increased with drought in
488 both seasons. Soils with higher oxygen availability, such as those with no moisture, may
489 have superior oxidative enzyme activity (Sinsabaugh, 2010). Nevertheless, under
490 tropical climate, it has been found that the activity of soil phenol oxidase increased in
491 the rainy season due to the greater SOC content in such season (Singh et al., 2018).

492 In winter, the decrease in the activity of some hydrolases coincided with the decrease in
493 soil microbial biomass in DU in comparison to CU. Negative effects of drought on soil
494 microbial biomass have been shown previously (Sheik et al., 2011; Baldrian et al.,
495 2013). However, we observed that the bacterial and fungal PLFA contents, as indicators
496 of microbial biomass, were greater in DU than in CU soil in summer. There are several
497 mechanisms through which the soil microbial community of a forest ecosystem can
498 present resistance (and even resilience) to drought under a semiarid climate. Soil OM
499 improves water retention and hence the greater OM content in the drought-affected plots
500 may have increased the drought resistance of the soil microbial biomass in summer
501 (Hueso et al., 2012). This increased water-sorption capacity can reduce the episodes of
502 water shortage in the soil (Bastida et al., 2017b). Further, the accumulation of fatty
503 acids does not necessarily match microbial growth. It is possible that the higher PLFA
504 content in drought plots in summer was a mechanism of osmolyte accumulation for
505 protection against dehydration rather than a signal of microbial growth *per se* (Schimel
506 et al., 2007; Bastida et al., 2017b). Moreover, via aerobic degradation of lipids by β-
507 oxidation, the citric acid cycle, and oxidation of the reducing equivalents produced,
508 microorganisms can gain more water per unit of lipid than per unit of carbohydrate or
509 protein oxidized (Hauschild et al., 2017). Thus, lipids accumulation can represent a
510 cellular mechanism for the maintenance of the basic energetic demands when
511 environmental conditions are harsher.

512 The composition of the fungal community was affected less by drought (CU vs DU)
513 than were those of the bacterial community, in agreement with the generalized greater
514 resistance of fungi to drought (Evans and Wallenstein, 2012). This is illustrated by the
515 relative stability of the fungal community composition in the face of drought and by the
516 slightly greater soil fungal-to-bacterial PLFA ratio in DU than in CU in summer. This
517 ratio has been proposed as an indicator of drought because hyphae can explore
518 micropores whereas bacteria rely on water films for substrate diffusion (de Boer et al.,
519 2005; de Vries et al., 2012).

520 We observed that the composition of the bacterial community in this semiarid
521 ecosystem dominated by Holm oak may be more strongly modulated by the indirect and
522 long-term effects of drought - the accumulation of SOM, which is a limiting factor in
523 semiarid ecosystems (Bastida et al., 2016) - rather than by the direct effect of the lower
524 water content imposed by water restriction. Moreover, these changes in the bacterial
525 community composition were statistically related to changes in soil enzyme activities
526 (Table S5). Some groups of *Proteobacteria* are copiotrophic organisms that utilize such
527 carbon stocks (Fierer et al., 2007; López-Mondéjar et al., 2018). Here, we show that the
528 SOM content can be a more important driver of the abundance of these groups than the
529 water supply. These proteobacterial groups were more abundant in drought plots (DU),
530 which also had greater OM contents. Some groups (i.e. *Bradyrhizobium*
531 (*Alphaprotoebacteria*) and *Herbaspirillum* (*Betaproteobacteria*)) could have
532 outcompeted some classical drought-resistant groups such as *Actinobacteria* (including
533 *Rubrobacter* and *Solirubrobacter*) (Marasco et al., 2012; Bastida et al., 2017a; Ochoa-
534 Hueso et al., 2018).

535

536 *4.3.Does thinning influence the impacts of drought? (DT vs DU)*

The contents of total organic C and N were higher in unthinned drought plots (DU) than in thinned drought plots (DT), in accordance with the greater increment in biomass per tree in DU. This pattern was the opposite of that observed in CT and CU plots. It is conceivable that the plant remains left after thinning did not contribute to the SOM under drought conditions. This can be probably due to the limited diffusion of carbon and hence of microbial activity in DT soil,as a consequence of the lower moisture content. However, our results suggest that thinning had a protective influence on the soil hydrolase activities of drought plots, particularly in winter (DT > DU). It is possible that microbes can produce more enzymes when there is a demand for substrates (Burns et al., 2013), as was the case of DT. There were no differences in the bacterial and fungal PLFA content between DT and DU (with the sole exception of the fungal PLFA content in DU, which was higher in winter). The absence of parallel patterns of the PLFA content and enzyme activities can be explained by the fact that the PLFA content is not directly related to growth (Schimel et al., 2007; Bastida et al., 2017b) and/or that soil enzymes can be immobilized in humic substances even when the producer cell is no longer alive (Burns et al., 2013).

Both drought and forest management altered the composition of the bacterial community, but management was the only factor that impacted the composition of the fungal community. In this sense, thinning in drought plots (DT) increased the abundance of a few ectomychorrizal fungi (*Astraeus*, *Tomentella*, *Inocybe*) but decreased the abundance of other ectomycorrhiza such as *Tricholoma*. In the same way, DT plots also showed higher abundance of a few saprotrophs, such as *Peziza*, but in general the abundance of other saprotrophic genera - such as *Penicillium*, *Hydnobolites*, and *Exophiala* - was decreased. A recent study highlighted a role for *Tomentella* in glucose accumulation, and in plant biomass decomposition for *Exophiala* and

562 *Penicillium* (López-Mondéjar et al., 2018). Here, DU soil had a higher content of
563 organic C than DT, which likely derived from plant compounds that could have been
564 utilized by these fungi.

565 The composition of the bacterial community differed between DT and DU in both
566 seasons. The abundance of some *Actinobacteria* (i.e. *Blastococcus* and *Solirubrobacter*)
567 in winter, and of *Firmicutes* (*Bacillus* and *Lysinibacillus*) in both seasons, was higher in
568 thinned drought plots (DT) than in unthinned drought plots (DU). Contrarily, a higher
569 abundance of *Acidobacteria* (*Pyrinomonas*), *Alphaproteobacteria* (*Bradyrhizobium*),
570 *Betaproteobacteria* (*Herbaspirillum* and *Ramlibacter*), and *Domibacillus* was observed
571 in DU, in comparison to DT. The dominance of *Actinobacteria* in semiarid ecosystems
572 has been pointed out in several studies (Bastida et al., 2017b), highlighting the capacity
573 of this phylum to survive under harsh conditions (Battistuzzi and Hedges, 2009).
574 Probably, under drought conditions, the plant debris remaining after thinning did not
575 accumulate in soil sufficiently to increase the soil C content. Under such conditions,
576 *Actinobacteria* become more competitive. Conversely, the accumulation of SOM was
577 higher in DU than in DT, and favored typical copitrophic groups such as
578 *Betaproteobacteria* (Fierer et al., 2007; Bastida et al., 2016). Furthermore, we found
579 different intra-phylum patterns. For instance, the abundance of *Bacillus* and
580 *Lysinibacillus* was promoted by thinning in drought plots, while the abundance of
581 *Domibacillus* was promoted in unthinned ones. These results illustrate the need to delve
582 into lower taxonomic levels for a complete understanding of the impacts of drought and
583 forest management on the soil microbial community and the possible role of specific
584 populations.

585

586 **5. Conclusions**

The interplay of forest management and drought affected the soil microbial community of a Holm oak Mediterranean ecosystem, hence defining the response of soil microbiomes to drought. As hypothesized, drought altered the bacterial community but not the fungal community, which was influenced more by thinning. Further, the impact of thinning was dependent on the drought conditions. Our results indicate that thinning did not seem a suitable strategy for soil carbon sequestration in ecosystems under drought conditions.

We conclude that thinning can promote drought resistance in the soil microbial community, particularly at the functional level, as indicated by the enzyme activities related to C, N and P cycles. These responses could be related to variations in the composition of the bacterial and fungal communities in thinned drought plots, in comparison to unthinned drought ones.

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832

833 **Figure Captions**

834 **Figure 1**. β-glucosidase (A), alkaline phosphatase (B), urease (C), and phenol oxidase
835 (D) activities in the studied soils. CT (thinned soil without induced drought); DT
836 (thinned soil with induced drought); CU (unthinned soil without induced drought); DU
837 (unthinned soil with induced drought). For each season, data followed by the same letter
838 are not significantly different ($P < 0.05$).

839 **Figure 2**. The content of bacterial (A), fungal (B), Gram-positive (C), Gram-negative
840 (D), and actinobacterial (E) phospholipid fatty acids (PLFAs) in the studied soils. CT
841 (thinned soil without induced drought); DT (thinned soil with induced drought); CU
842 (unthinned soil without induced drought); DU (unthinned soil with induced drought).

For each season, data followed by the same letter are not significantly different ($P < 0.05$).

Figure 3. The non-metrical multimensional scaling (NMDS) of bacterial (A) and fungal (B) communities in the studied soils

Figure 4. Bacterial community composition in the studied soils at the phylum (A) and genus (B) taxonomic levels.

Figure 5. Fungal community composition in the studied soils at the phylum (A) and genus (B) taxonomic levels.

Table 1. Soil moisture, soil C and N contents, and water-soluble fractions in the studied soils

	Soil moisture	TOC	Total N	WSC	WSN
Winter					
CT	8.66 (1.14)b	3.67 (0.51)b	0.27 (0.05)a	62.66 (1.05)a	5.69 (1.20)c
DT	5.94 (0.92)a	2.58 (0.12)a	0.18 (0.04)a	56.79 (3.37)a	3.17 (0.82)b
CU	11.55 (0.84)c	2.33 (0.13)a	0.19 (0.03)a	87.75 (7.11)b	0.47 (0.14)a
DU	9.28 (0.50)b	3.82 (0.25)b	0.24 (0.04)a	131.10 (7.90)c	3.58 (0.52)b
Summer					
CT	1.23 (0.25)b	3.57 (0.38)b	0.27 (0.05)b	213.14 (20.04)bc	20.10 (1.37)b
DT	0.70 (0.18)a	2.54 (0.25)a	0.17 (0.02)a	177.44 (20.09)ab	12.02 (2.82)a
CU	1.62 (0.27)c	2.07 (0.16)a	0.18 (0.03)a	147.89 (16.44)a	13.01 (2.37)a
DU	1.09 (0.41)a	4.33 (0.63)b	0.21 (0.02)ab	225.25 (18.48)c	12.84 (1.69)a

Soil moisture (%); TOC (total organic C, g 100 g^{-1} soil); total N (g 100 g^{-1} soil); WSC (water-soluble C, mg kg^{-1} soil); WSN (water-soluble N, mg kg^{-1} soil). The standard deviation is included in parentheses. CT (thinned soil without induced drought); DT (thinned soil with induced drought); CU (unthinned soil without induced drought); DU (unthinned soil with induced drought). For each season, data followed by the same letter are not significantly different ($P < 0.05$).

Table 2. The Shannon-Wiener diversity index (H) for the bacterial and fungal communities in the studied soils

	$H_{bacteria}$	H_{fungi}
Winter		
CT	6.36 (0.09)a	4.33 (0.65)b
DT	6.25 (0.17)a	4.60 (0.63)b
CU	6.19 (0.25)a	3.28 (0.80)a
DU	6.35 (0.08)a	3.58 (0.42)a
Summer		
CT	6.23 (0.02)b	4.41 (0.20)b
DT	6.27 (0.04)b	4.52 (0.06)b
CU	5.88 (0.09)a	3.62 (0.17)a
DU	6.34 (0.05)b	4.42 (0.15)b

CT (thinned soil without induced drought); DT (thinned soil with induced drought); CU (unthinned soil without induced drought); DU (unthinned soil with induced drought). The standard deviation is included in parentheses. For each season, data followed by the same letter are not significantly different ($P < 0.05$).

Figures revised

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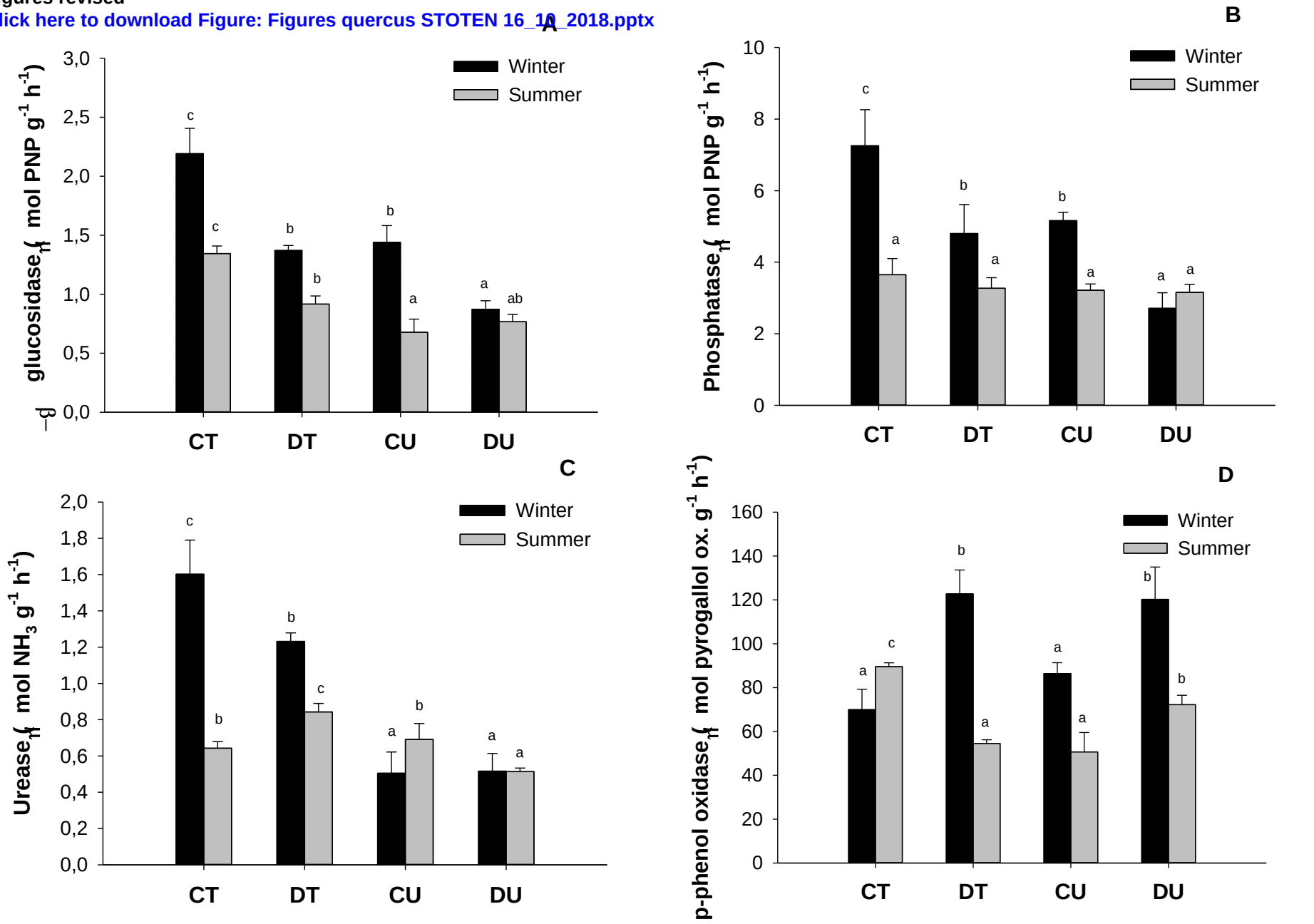

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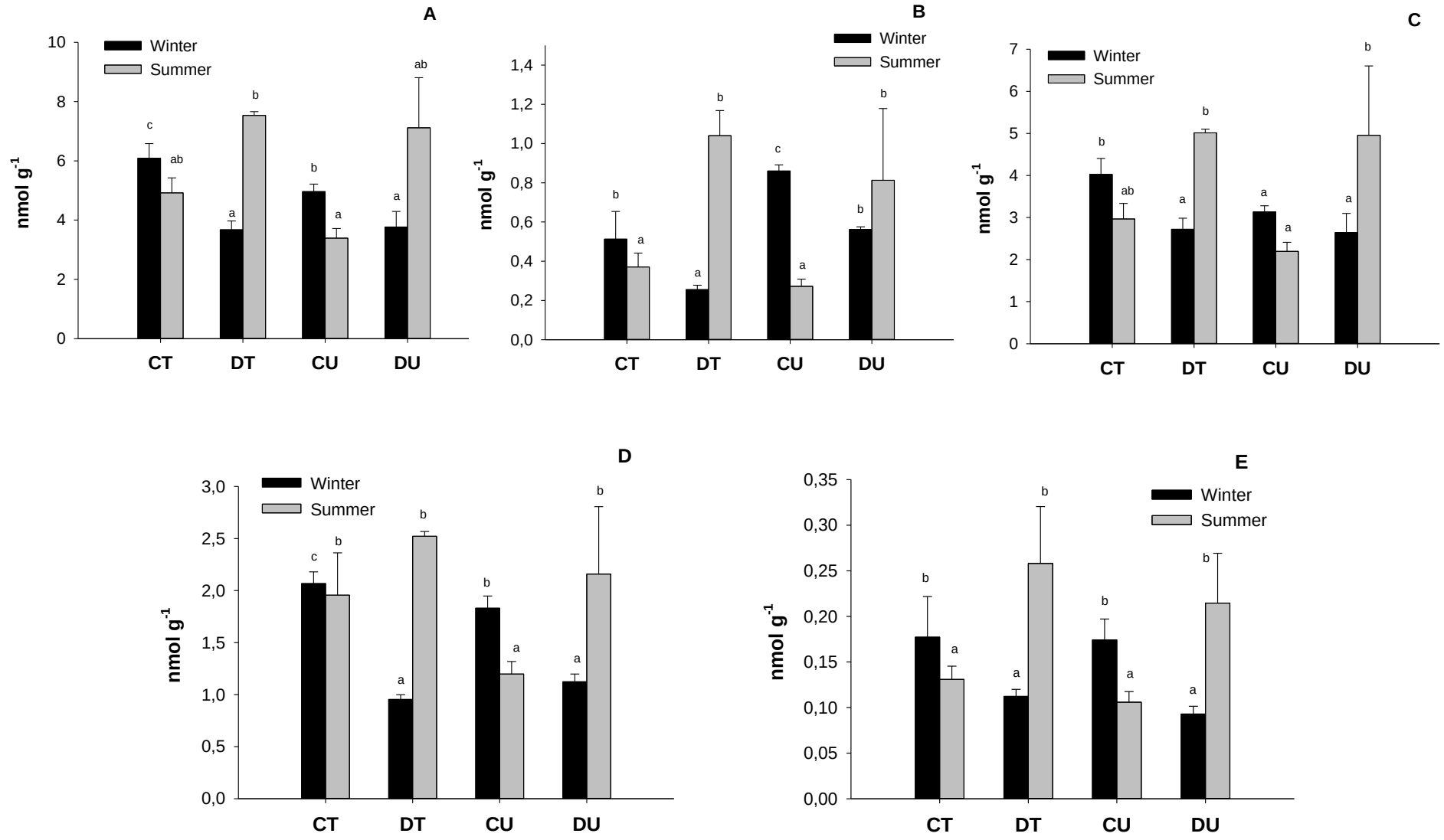

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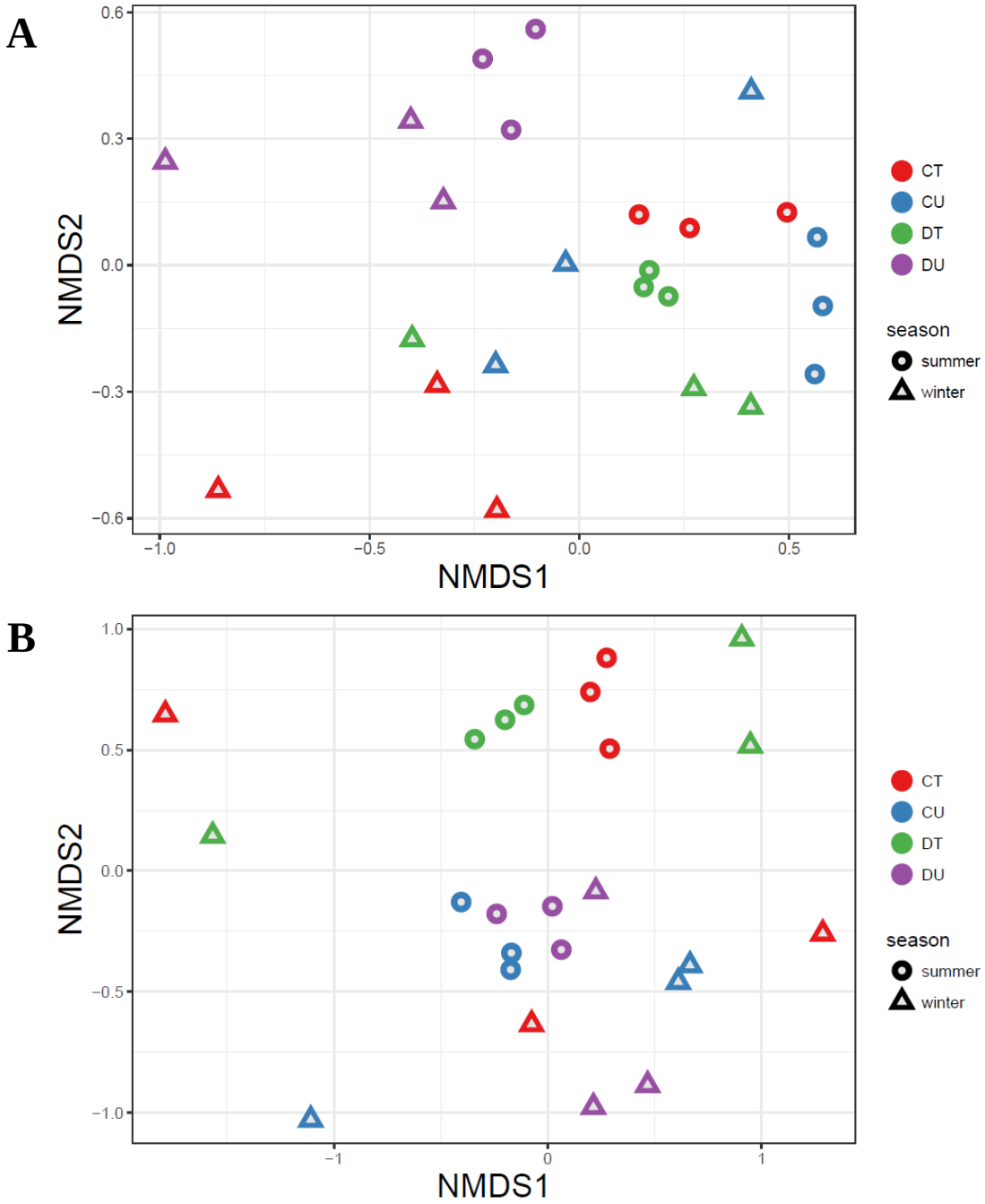
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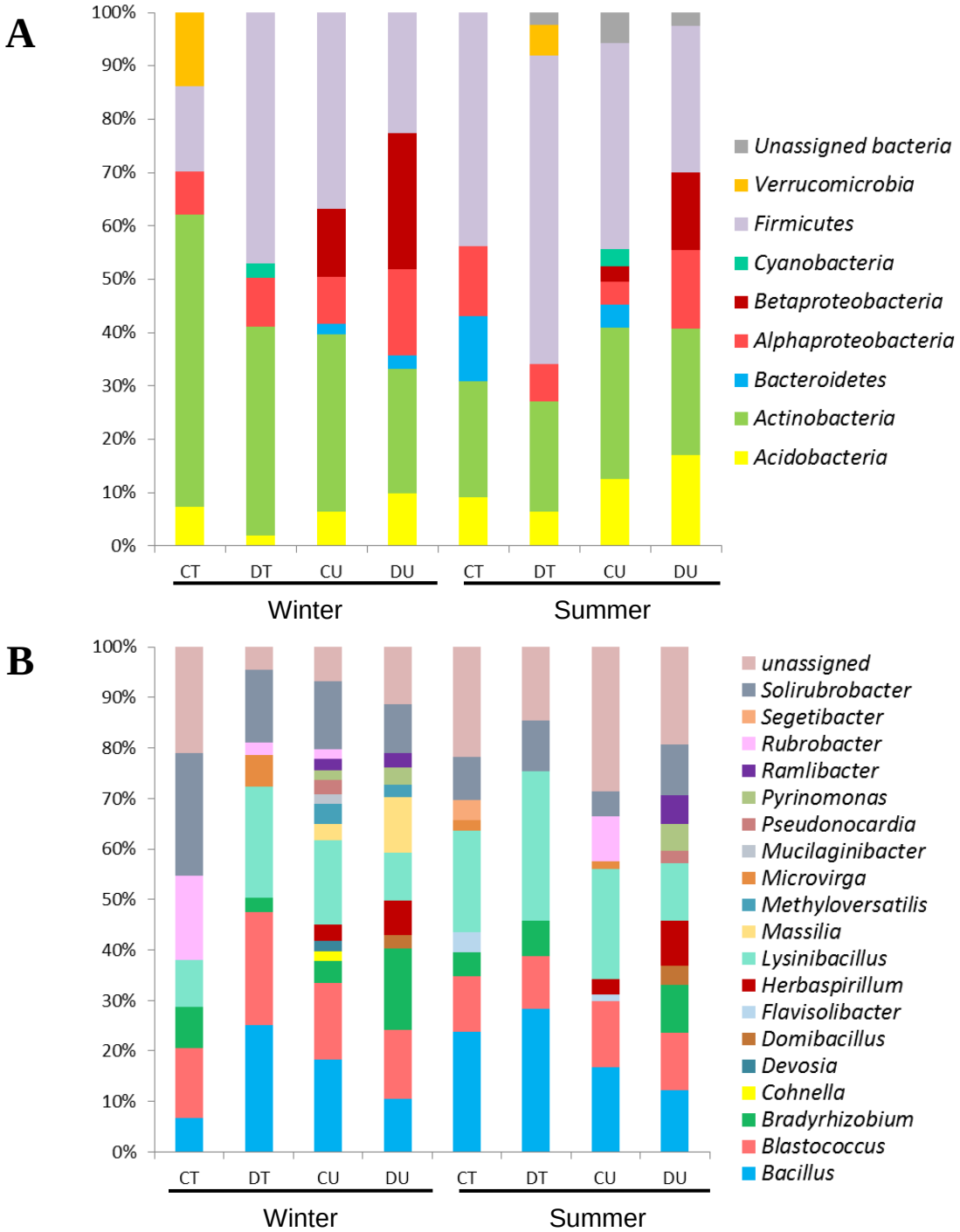
Figure 3.

Figure 4.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Betaproteobacteria
Alphaproteobacteria
Bacteroidetes
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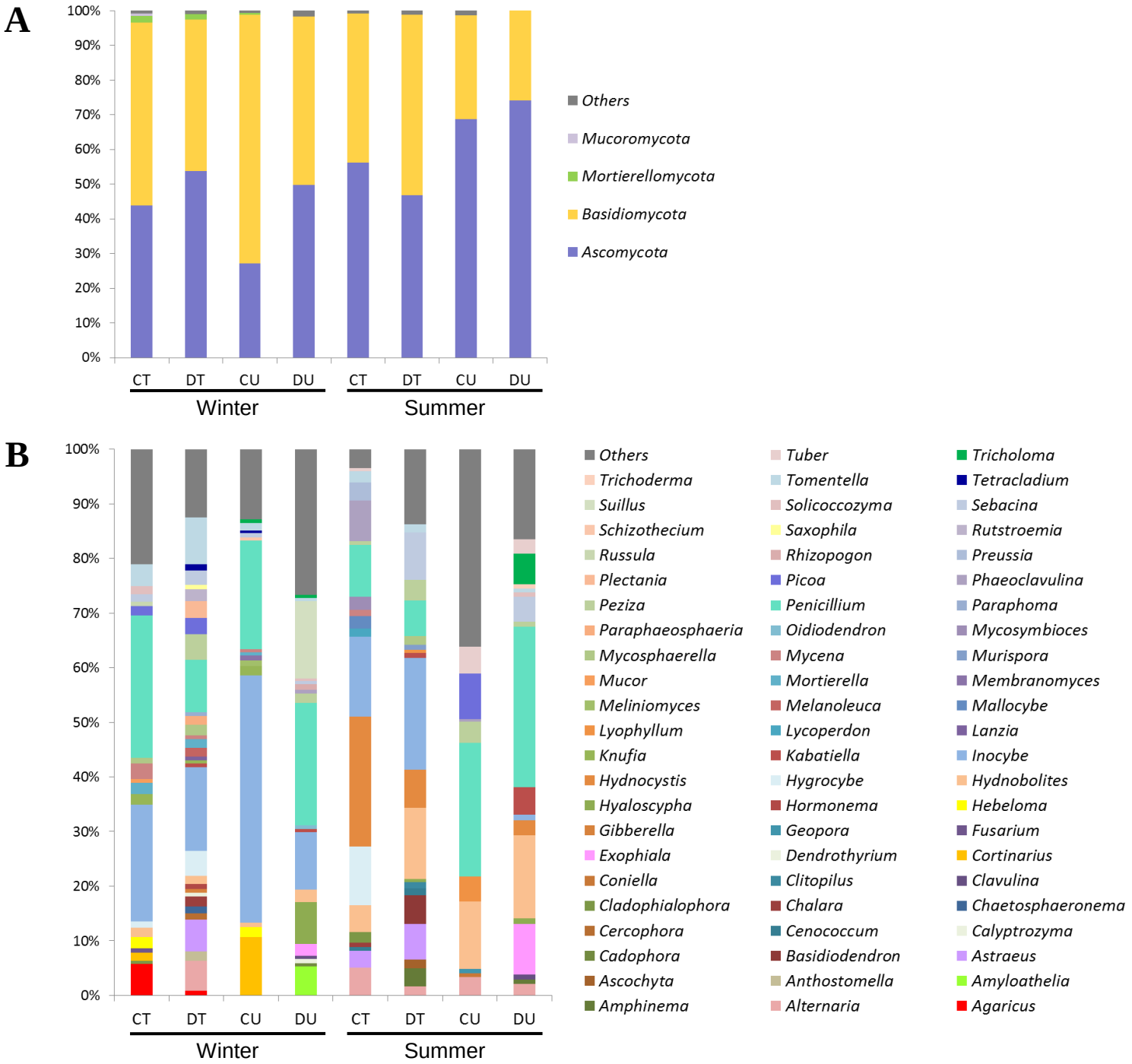
Rubrobacter
Ramlibacter
Pyrinomonas
Pseudonocardia
Mucilaginibacter
Microvirga
Methyloversatilis
Massilia
Lysinibacillus
Herbaspirillum
Flavisolibacter
Domibacillus
Devosia
Cohnella
Bradyrhizobium
Blastococcus
Bacillus

Figure 5.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Winter
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Others
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Mortierellomycota
Basidiomycota
Ascomycota
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Winter
Summer
Others
Trichoderma
Suillus
Schizothecium
Russula
Plectania
Peziza
Paraphaeosphaeria
Mycosphaerella
Mucor
Meliniomyces
Lyophyllum
Knufia
Hydnocystis
Hyaloscypha
Gibberella
Exophiala
Coniella
Cladophialophora
Cercophora
Cadophora
Ascochyta
Amphinema
Tuber
Tomentella
Solicoccozyma
Saxophila
Rhizopogon
Picoa
Penicillium
Oidiodendron
Mycena
Mortierella
Melanoleuca
Lycoperdon
Kabatiella
Hygrocybe
Hormonema
Geopora
Dendrothyrium
Clitopilus
Chalara
Cenococcum
Basidiodendron
Anthostomella
Alternaria
Tricholoma
Tetracladium
Sebacina
Rutstroemia
Preussia
Phaeoclavulina
Paraphoma
Mycosymbioces
Murispora
Membranomyces
Mallocybe
Lanzia
Inocybe
Hydnobolites
Hebeloma
Fusarium
Cortinarius
Clavulina
Chaetosphaeronema
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