1 **High resilience of the mycorrhizal community to prescribed seasonal burnings in** 2 **a Mediterranean woodland**

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26 **Abstract**

27 Fire effects on ecosystems range from destruction of aboveground vegetation
 28 to direct and indirect effects on belowground microorganisms. Although variation in
 29 such effects is expected to be related to fire severity, another potentially important and
 30 poorly understood factor is the effects of fire seasonality on soil microorganisms. We
 31 carried out a large-scale field experiment examining the effects of spring versus
 32 autumn burns on the community composition of soil fungi in a typical Mediterranean
 33 woodland. Although the intensity and severity of our prescribed burns were largely
 34 consistent between the two burning seasons, we detected differential fire season
 35 effects on the composition of the soil fungal community, driven by changes in the
 36 saprotrophic fungal guild. The community composition of ectomycorrhizal fungi,
 37 assayed both in pine seedling bioassays and from soil sequencing, appeared to be
 38 resilient to the variation inflicted by seasonal fires. Since changes in the soil
 39 saprotrophic fungal community can directly influence carbon emission and
 40 decomposition rates, we suggest that regardless of their intensity and severity,
 41 seasonal fires may cause changes in ecosystem functioning.

42 **Keywords:** *Cistus*, ecosystem resilience, ectomycorrhizal fungi, fungal richness,
 43 Illumina MiSeq, Mediterranean, *Pinus halepensis*, prescribed burns, seasonality.

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49 **Conflicts of interest/Competing interests**

50 We declare no conflicts of interest and that this material has not been
51 submitted for publication elsewhere.

52 **Ethics approval** Not applicable

53 **Consent to participate** Not applicable

54 **Consent for publication** Not applicable

55 **Availability of data and material**

56 Sequences were submitted to the National Center for Biotechnology
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58 **Code availability** Not applicable

59 **Authors' contributions**

60 OO HS TB YO YC conceived and designed the experiment. SSL YA HM AT
61 performed the experiment. SIG provided the pipeline scripts, and guidance in
62 bioinformatics work and analyses. SLL OO HS wrote the paper and analyzed the
63 data, and all authors contributed substantially to revisions.

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73 sequencing library, and Uri Yogeve for his help with the soil properties analyses.

74

75 **Introduction**

76 Fire is one of the most common natural and anthropogenic disturbances
77 leading to secondary succession of both plant and fungal communities (Marlon et al.
78 2009). Exploring the effects of fire on ecosystem functioning is of high priority,
79 especially due to the increase in fire risk associated with climate change (Moriondo et
80 al. 2006; Pechony and Shindell 2010; Westerling et al. 2006). The extent of damage
81 fires inflict on plant communities is manifested not only through the destruction of
82 plant tissues, but also in destruction of symbiotic soil microbes, which may be
83 necessary to buffer against fire effects, thus increasing plant community resilience
84 (Johnstone et al. 2010; Kipfer et al. 2011). Most of the temperate and boreal trees
85 around the globe are obligately symbiotic with ectomycorrhizal fungi (EMF),
86 meaning that their establishment is dependent upon the occurrence of an appropriate
87 symbiont community (Miller et al. 1998). Therefore, fire effects on the belowground
88 biota may be far-reaching with regard to vegetation regeneration and growth during
89 the first few post-fire years (Neary et al. 1999). For example, a fire study based on
90 chrono-sequence found that fire temporarily shifted the fungal community structure
91 and function by increasing the abundance of saprotrophic fungi (Sun et al. 2015).
92 Eventually the community returned to its pre-fire state, but at a very slow rate (Sun et
93 al. 2015). Such a community shift towards saprotrophic fungi may have a detrimental
94 effect on ecosystem functioning because it may shift the balance between obligate
95 symbiotic EMF, associated with tree roots, and saprotrophic fungi. Besides the clear
96 negative outcome of reduced symbionts available for plants (Collier and Bidartondo
97 2009), competition between these two fungal guilds can suppress decomposition rates
98 (i.e., the Gadgil effect, Fernandez and Kennedy 2016; Gadgil and Gadgil 1975;
99 Gadgil and Gadgil 1971).

100 Various studies have demonstrated both direct and indirect effects of fire on
101 the EMF community while consequently influencing the post-fire regeneration of the
102 plant community (Buscardo et al. 2010; Glassman et al. 2016b; Johnson 1995;
103 Marlon et al. 2009; Miller and Urban 1999; Taudière et al. 2017; Veen et al. 2008).
104 Although such effects are expected to be related to fire severity, which often varies
105 during the year, less is known about the specific effect of fire season on the EMF
106 community. The aboveground importance of fire season is well established.
107 Specifically, compared with spring fires, autumn fires consume greater portions of the
108 landscape area, standing plant biomass and other organic material (Knapp et al. 2005),
109 while having more profound negative effects on the understory vegetation richness
110 (see Knapp et al. 2009 for a thorough review). However, less attention has been given
111 to the effect of fire season on the subterranean part of the ecosystem (but see, de
112 Roman and de Miguel 2005; Smith et al. 2004).

113 Examining the effects of prescribed burns on the EMF community in a natural
114 setting of ponderosa pine stands in eastern Oregon, Smith *et al.* (2004) found that
115 autumn fires had long lasting, devastating effects on the mycorrhizal community, with
116 a reduction of 80% in molecular species richness. Spring fires, however, did not differ
117 from the unburned control. Smith et al. (2004) suggested that observed differences in
118 the EMF community composition were the result of inter-season variation in fire
119 severity. Specifically, the low moisture content in the fuel and in the soil during late
120 season, resulted in higher soil temperatures and increased microbial mortality. Such
121 extreme soil temperatures may damage the mycorrhizal community directly by
122 destroying the mycelia, or indirectly by host death, both can lead to a long lasting
123 negative effect on the EMF community (Klopatek et al. 1994). On the contrary,
124 spring fires usually occur after the wet season when soil moisture is high and heat

125 transfer is highly efficient, compensating for the increase in soil temperature caused
126 by these fires, and thus having a weaker detrimental effect on the soil biota. Seasonal
127 fire effects can be also related the phenological stages of both plants and fungi,
128 resulting in a differential effect on their community composition. For example, in
129 many fungal species characterizing Mediterranean habitats, the amount of mycelium
130 decreases in summer, probably due to hot and dry conditions, whereas in autumn it
131 increases again (De la Varga et al. 2013). Furthermore, during the hot dry
132 Mediterranean summer selection may favor fungal species which can better cope with
133 these extreme conditions, resulting in a seasonal shift in the composition of the fungal
134 community. Clearly, these shifts should be more pronounced in open canopy gaps
135 created by spring fires, where the soil is more exposed to direct sun radiation. All of
136 the above imply that fire timing can play a major role in shaping the soil fungal
137 community in general and the EMF community in particular, which in turn can
138 determine species-specific plant establishment and growth (Klironomos et al. 2010;
139 Livne-Luzon et al. 2017b), and plant species' richness (Klironomos 2002). We thus
140 hypothesized that fire season should have a differential effect on the composition of
141 the soil fungal community, shifting the balance between obligate symbiotic EMF,
142 associated with tree roots, and saprotrophic fungi.

143 Most studies on the post-fire dynamics of the EMF community have been
144 performed in conifer forests (Dove and Hart 2017), located in temperate and boreal
145 areas. In comparison, much less is known about the effect of fire on the EMF
146 community in fire-prone Mediterranean ecosystems. Notably, a few recent studies
147 have brought new attention to fire effects on EMF communities in Mediterranean
148 habitats dominated by *Quercus* or *Cistus* sp. (Buscardo et al. 2015; Buscardo et al.
149 2010; de Roman and de Miguel 2005; Hernández-Rodríguez et al. 2013), emphasizing

150 the need to explore these more neglected habitats. Our research aimed to study the
151 effects of fire season on the soil fungal community and specifically on the EMF
152 community in a *Cistus* dominated eastern Mediterranean ecosystem. We manipulated
153 fire seasonality using early and late season prescribed burns, and examined the
154 various effects of fire season on the soil- and ectomycorrhizal fungal communities
155 through both sequencing and pine seedling bioassays.

156 Succession in Mediterranean woodlands often begins with a pioneer stage of
157 *Cistus salviifolius* followed by *Pinus halepensis* colonization (Ne'eman 1997; Sheffer
158 2012). *Cistus* is considered an early 'pioneer' species that increases in density,
159 especially after fire disturbance (Ne'eman and Izhaki 1999). *Pinus halepensis* is a
160 dominant tree species in natural (Liphschitz and Biger 2001) and planted (Osem et al.
161 2008) forests in Israel, known for its adaptive post-fire regeneration (Ne'eman 1997;
162 Ne'eman et al. 2004). Since at early successional stages *Cistus* shrubs are the main
163 EMF hosts, we hypothesized that *P. halepensis* colonization should be facilitated by
164 the EMF community characterizing *Cistus*. We therefore compared the pine-
165 associated EMF community under *Cistus* shrubs with that of adjacent open canopy
166 gaps using pine bioassays. Doing so allowed us to examine the soil fungal spore bank,
167 essential for the post-fire regeneration of pines in this ecosystem (Glassman et al.
168 2016b).

169 **Materials and Methods**

170 *The study area*

171 The study site was located in Har Yaaran in the Judean lowlands of Israel (600
172 m ASL, Fig. S1). The climate is Mediterranean, with an average annual precipitation
173 of 500-600 mm; between May and October it hardly rains, while solar radiation is

174 very high (Goldreich 2003). The soil is clayish and shallow due to large limestone
175 plates. The vegetation cover is of a Mediterranean shrubland (garrigue), with patches
176 of small multi-stem trees (e.g., *Quercus calliprinos* and *Rhamnus lycioides*), shrubs
177 (1-1.5 m high; e.g., *Pistacia lentiscus*, *Rhamnus lycioide* and *Calicotome villosa*),
178 dwarf-shrubs (≤ 1 m; e.g., *Cistus salviifolius*, *Cistus creticus* and *Teucrium*
179 *divaricatum*), and patches of herbaceous vegetation. The main ectomycorrhizal hosts
180 in the study area are (by order of dominance) *C. salviifolius*, *Q. calliprinos* and *C.*
181 *creticus*. There is an adjacent planted pine (*P. halepensis* and *P. brutia*) forest uphill
182 of the study area, but there were no mature pine trees and only a few pine seedlings
183 were found in the research plots.

184 *Experimental design*

185 The experimental system consisted of twelve 50×30 m plots, each divided into
186 eight 5×5 m sampling subplots (Fig. S1). Plots were randomly assigned to one of the
187 three following fire treatments (four plots per treatment): 1) spring burns (due to
188 exceptionally late rains, spring burnings were conducted on the 1st of June 2014), 2)
189 autumn burns (11th September 2014), and 3) unburned control plots.

190 *Soil sampling*

191 Soil samples were collected at four different sampling periods: 1) Pre-fire soil
192 samples (i.e., March 2014), 2) Post-Spring fire (two weeks after the spring burns, i.e.,
193 June 2014), 3) Post-Autumn fire (two weeks after the autumn burns, i.e., Oct- 2014),
194 and 4) Post-fires (~1 year after the collection of pre-fire samples, i.e., June 2015).
195 This experimental design and sampling scheme (Fig.1), enabled us to quantify the net
196 effects of spring and autumn burns on the soil fungal community composition,
197 conveying the actual effects of seasonal fires in typical Mediterranean woodlands.

198 All samples were collected using the following protocol: three soil cores (10
199 cm depth, ~ 0.5L) were collected from each of the eight subplots located within each
200 experimental plot. Since the field site is characterized by several rocky patches, each
201 soil core was collected from wherever possible within the 5×5 m subplot, staying
202 within 0.5 m from a *Cistus* shrub (the dominant EMF host in the study area). Each soil
203 core was bagged separately, and all tools were sterilized with 70% ethanol when
204 moving among different subplots to avoid cross-sample contamination. Upon
205 returning to the lab, the three soil cores of each subplot were sieved (2 mm) and
206 homogenized. Then 0.25 g of soil from each sample was directly added to Powersoil
207 DNA tubes (MoBio, Carlsbad, CA USA), and stored in 4°C up to one week before
208 DNA extraction. The remaining soil from the Pre-fire (March 2014) and Post-fires
209 (June 2015) was kept (4°C) in a zip-lock bag for later soil property analysis.

210 *Greenhouse bioassays*

211 Fungal DNA extracted from the soil may originate from active hyphae or from
212 the soil EMF spore bank (Lindahl et al. 2013; Taylor and Bruns 1999). To assess the
213 inoculation potential of the post-fire EMF community, we bioassayed the soils
214 collected from the study area with *P. halepensis* using standard protocols (Glassman
215 et al. 2016a).

216 We used the same protocol described above to collect soil samples for the
217 greenhouse bioassay from each of the twelve experimental plots, while distinguishing
218 between two different microhabitats: 1) under a *Cistus* shrub, and 2) an open area
219 without any perennial shrub cover (12 plots × 8 subplots × 2 microhabitats = 192 soil
220 samples). Soil sampling occurred in October 2014, two weeks after the autumn burns
221 and ~4 months after the spring burns, i.e., before the rainy season when most fungi

retains activity. Soil samples were air-dried to kill active vegetative fungal hyphae before assaying for resistant propagules (Glassman et al. 2015). *Pinus halepensis* seeds were soaked in water for 48 h, after which they germinated in inert growing media – vermiculite, under controlled conditions in a growth chamber (22 °C, 80% rh, 17 days), and were then planted in the dried soil from each of the 192 bioassay soil samples. Pine seedlings were planted in 200 mL containers using a 1:1 ratio of dried soil and autoclaved sand (121 °C for 20 min ×2), to improve drainage. We controlled for the presence of airborne fungal spores in the greenhouse by adding fifteen replicates of pots containing pine seedlings planted in autoclaved sand. Plants were watered daily and grown in the greenhouse under semi-controlled conditions without fertilizer for approximately six months before harvesting. Treatments were randomized among trays upon initial planting. In total, 207 seedlings were planted (12 plots × 8 subplots × 2 microhabitats = 192 soil samples + 15 controls). After six months, due to harsh summer conditions, only 119 seedlings had survived (8-14 seedlings per plot). Upon harvesting, plants were removed intact from the pots and washed under tap water. Then, roots were thoroughly scanned under a dissecting microscope for colonized root tips. All colonized root tips were removed using sterilized forceps (70% ethanol), inserted into a 1.5 ml Eppendorf tube, and immediately stored in a -20°C freezer. The tubes were immersed in liquid nitrogen at the end of the day, and stored in a -80°C freezer until DNA extraction.

Molecular identification of species and bioinformatics

Molecular identification of species followed the methods of Glassman et al. (2016b) with minor modifications during the DNA extraction stage. Generally, the ITS1 region was PCR targeted, barcoded and sequenced using Illumina MiSeq technology. For full description of the molecular identification of species and the

247 respective bioinformatic analyses see Supplement S1 and Table S1 in online resource
248 1. Illumina data were processed using a combination of the UPARSE (Edgar 2013)
249 and QIIME (Caporaso et al. 2010) pipelines following the methods of Smith and Peay
250 (Smith and Peay 2014), and Glassman et al. (Glassman et al. 2016b) with minor
251 modifications related to software updates. Taxonomic assignments were made in
252 QIIME based on the UNITE database (Koljalg et al. 2005). FUNguild was then used
253 to parse OTUs into ecological guilds (Nguyen et al. 2016).

254 In the greenhouse bioassay, we had ten control pots containing only potting
255 material and plants (no added experimental soil). These root tip samples had low
256 colonization resulting in a total of 48 fungal OTU's (for all of the controls) with low
257 read abundance (55.08 ± 1.23 ; mean ± 1 SE), we thus subtracted these read abundances
258 from the respective data of the bioassay samples. Negative controls from the DNA
259 extraction and PCR stages had all zero reads in them.

260 *Statistical analyses*

261 Multivariate analyses were performed in PRIMER v.6 of the Plymouth Marine
262 Laboratory (Clarke and Warwick 1994). Relative abundances were fourth-root
263 transformed (Clarke and Warwick 1994; Clarke et al. 2008). A permutational
264 MANOVA (PERMANOVA) based on Bray–Curtis similarity matrix (Anderson et al.
265 2001) followed by non-metric multi-dimensional scaling (nMDS) ordination was
266 performed to test for the combined effect of fire season (whole plot) and sampling
267 treatments (within plot) on the entire fungal community composition (and on the EMF
268 community) using a split-plot experimental design. A similar analysis was used to
269 examine the combined effects of fire season (whole plot treatment) and microhabitat
270 (*Cistus* vs. Open; within plot treatment) on the fungal community composition

271 associated with pine roots (greenhouse bioassay experiment). We examined the same
 272 effects on the relative abundance of each fungal OTU in order to search for specific
 273 fungal species that were differentially expressed among these bioassay treatments, the
 274 p-values obtained from these tests were then corrected for multiple testing using the
 275 false discovery rate correction (Benjamini and Hochberg 1995) implemented in the
 276 p.adjust function of the R Stats Package (R Development Core Team 2010). To
 277 identify the percentage contribution of different fungal OTU's to observed differences
 278 in community composition, we used a similarity percentages routine (SIMPER)
 279 (Anderson et al. 2001). In all cases, qualitative similar results were obtained when a
 280 square-root or no transformation were applied, as well as when using a Jaccard
 281 similarity matrix (Clarke and Warwick 1994) based on presence/absence data (Clarke
 282 and Warwick 1994), so unless otherwise mentioned all results refer to the fourth-root
 283 Bray-Curtis similarity matrix. To test for the combined effect of fire season and
 284 sampling period on the ratio of saprotrophic to EM fungi ((saprotrophic/EM)/total
 285 OUT's) and on the bioassay OTU richness (and several other diversity indexes), we
 286 used split-plot ANOVAs with fire season as the whole-plot factor and sampling
 287 period as the within-plot factor. These analyses were performed using STATISTICA
 288 v.12 (StatSoft, Inc., Tulsa, OK, USA).

289 **Results**

290 Fire intensity and severity were largely consistent between spring and autumn
 291 burns (Table S2). Notably, the amount of variation in fire intensity and severity
 292 among experimental plots was higher during spring than during autumn burns (Fig.
 293 S2). For full description of the environmental conditions monitored pre- and during
 294 the burns, proxies of fire intensity and severity measured during and post the burns,
 295 and analyses of these variables see Supplement S2 in online resource 1. Monthly

precipitation, daily precipitation, number of rainy days and ambient temperature during the sampling periods are presented in Fig. S3.

Soil properties including phosphate, nitrite, nitrate, total ammonia-nitrogen, soil organic matter content, and pH were all consistent among fire seasons (Table S3). For full description of these variables and their respective analyses, see Supplement S3 in online resource 1.

Soil fungal community

Time and seasonal fire effects on soil fungal richness and diversity

OTU richness and diversity of soil fungi varied significantly among sampling periods (Tables S4-S6 for the entire soil fungal, EMF and saprotrophic fungal communities, respectively; for the most abundant EMF and saprotrophic fungal taxa see Table S7). The effect of fire season was inconsistent among sampling periods (i.e., Fire season $\times$ Sampling period interaction). However, this pattern was significant only when examining the entire fungal community, irrespective of the diversity index used (Tables S4-S6). There were no significant differences in OTU richness and diversity among fire treatments in all sampling periods (March 2014, October 2014 and June 2015), except for the post-spring fire period (June 2014; Fig. S4). Immediately after the spring burns, there was a significant reduction in OTU richness and diversity in burned compared to unburned control plots. Approximately one year after the spring burns these differences diminished (Fig. S4).

Time and seasonal fire effects on soil fungal Community composition

Community composition of soil fungi varied significantly by season (PERMANOVA: $F_{3,25,81} = 12.75$, $P = 0.0001$; Table S8; Fig. S5a), but both fire season

319 ($F_{2,10.53} = 0.85$, $P = 0.621$) and the interaction between fire season and sampling period
 320 ($F_{5,23.62} = 0.67$, $P = 0.921$) were not significant. However, there was higher variation
 321 in fungal community composition among plots subjected to spring burns ($F_{10,249} =$
 322 3.42 , $P = 0.0001$). Similar patterns were observed when examining the EMF and
 323 saprotrophic fungal community composition (Tables S9 & S10; Fig. S5).

324 PERMANOVA pair-wise comparisons (Table 1) indicated that there were no
 325 significant differences in community composition of the soil fungi among fire seasons
 326 during the pre-fire sampling period (*i.e.*, March 2014), and the same holds true when
 327 examining the pre-fire EMF and saprotrophic fungal communities. After spring burns
 328 (*i.e.*, June 2014), community composition of the entire and saprotrophic fungal
 329 communities varied significantly between the control and spring burned plots
 330 ($t_{11,1}=1.52$, $p=0.024$ and $t_{11,1}=1.59$, $p=0.020$, for the entire and saprotrophic fungal
 331 communities, respectively). However, after autumn burns (*i.e.*, October 2014), these
 332 differences disappeared ($t_{6,14}=1.04$, $p=0.409$), probably due to the large difference in
 333 community composition between the control and recently burned autumn plots
 334 ($t_{6,14}=1.67$, $p=0.006$, $t_{6,14}=1.53$, $p=0.007$ and $t_{6,14}=1.40$, $p=0.025$, for the entire, EM
 335 and saprotrophic fungal communities, respectively). This strong effect of autumn
 336 burns on the entire soil fungal community translated into nearly-significant
 337 differences between the autumn and spring burned plots ($t_{6,05}=1.24$, $p=0.058$). In the
 338 post-fires sampling period (*i.e.*, June 2015), we observed a significant difference
 339 between the control and autumn burned plots ($t_{6,06}=1.39$, $p=0.031$), but only when
 340 examining the entire fungal community. In addition, there was a minor nearly
 341 significant difference between the control and spring burned plots ($t_{6,08}=1.25$,
 342 $p=0.058$), but not between autumn and spring burned plots ($t_{6,1}=1.02$, $p=0.406$). When
 343 examining a subset of the data including only the post-fire sampling period (*i.e.*, June

2015), there were significant differences in species composition of soil fungi among the three fire treatments (PERMANOVA: $F_{2,9,93} = 1.49$, $P = 0.008$; Table S11; Fig. 2a; this effect was weaker when examining subsets of the EM and saprotrophic fungal communities: Tables S12 & S13; Figs. S6). In particular, the soil fungal communities varied between the autumn burned and unburned control plots (PERMANOVA pairwise contrast: $t_{6,05} = 1.38$, $P = 0.030$; Table S14). SIMPER analysis (Fig. 2b) illustrated that numerous OUT's generated 90% of the dissimilarity among fire treatments and the contribution of each one of them was ~1%. Putative EMF taxa such as *Tuber* and *Inocybe* contributed ~14% to this dissimilarity, while putative saprotrophic fungi generated 22-25% of the dissimilarity among fire treatments. These saprotrophic fungi were attributed to various functional guilds such as wood and dung saprotrophs, but most of these were unidentified to a level allowing for the assessment of their exact function (for the most abundant EMF and SAP taxa in the various soil samples see Table S7). Nevertheless, the ratio of saprotrophic to EM fungi ((saprotrophic/EMF)/total OUT's) varied among both sampling periods (split plot ANOVA: $F_{3,22} = 41.58$ $p < 0.001$; Table S15; Fig.3) and fire season treatments ($F_{2,10} = 8.26$ $p = 0.008$). Prior to the burns there were no significant differences between experimental plots ($t = 0.39$, $p = 0.415$). Spring burns led to a reduction in the ratio of saprotrophic fungi, but this pattern was not significant ($t = -0.64$, $p = 0.520$). Autumn burns led to a significant reduction in the ratio of saprotrophic fungi compared to that of unburned control plots ($t = 3.12$, $p = 0.002$), and this reduction resulted in a significant difference between the autumn and spring burned plots ($t = 2.66$, $p = 0.008$). Approximately one year after the spring burns (i.e., June 2015), the differences among the control and burned plots disappeared ($t = 1.12$, $p = 0.259$). However, there were still significant differences between the autumn and spring burned plots ($t = 2.14$, $p = 0.032$).

369 Greenhouse bioassay EMF community

370 Consistent with the results of the soil EMF community, OTU richness and
371 diversity did not vary among fire treatments, nor between microhabitats (Table S16).

372 Community composition of the bioassay samples did not vary among fire
373 treatments (PERMANOVA: $F_{2,9} = 0.84$, $P = 0.605$; Table S16; Fig. S6c). Also, there
374 was no significant effect of microhabitat (PERMANOVA: $F_{1,9} = 1.76$, $P = 0.138$), nor
375 was there a significant fire treatment by microhabitat interaction (PERMANOVA:
376 $F_{2,9} = 0.45$, $P = 0.916$).

377 Description of the bioassay EMF community

378 Regardless of fire season and microhabitat, pine roots in the bioassay were
379 dominated by three major fungal species: *Tuber oligospermum* (21-58%), *Tomentella*
380 *sp.1* (27-51%) and *Suillus collintus* sp.1 (10-27%). Other fungal species belonging to
381 EMF genera (e.g. other *Tuber spp.*, *Inocybe* etc.) accounted for 1-24% of the EMF
382 community (Fig. 4a). These three dominant species were differentially abundant
383 between the *Cistus* and the open microhabitat: *Tuber oligospermum* had higher
384 abundance under *Cistus* shrubs than in the open gaps, while *S. collintus* and *Terfezia*
385 had higher abundance in the open microhabitat (Fig. 4b). Differential expression
386 analysis demonstrated that this pattern was only significant ($p < 0.01$) for *Tuber*
387 *oligospermum*.

388 **Discussion**

389 We report here the results of the first comprehensive field experiment
390 quantifying the effects of seasonal fires on the soil fungal community in the eastern
391 Mediterranean basin. Fire season caused differential effects on the community

392 composition of soil fungi (Fig.2), driven by alterations within the saprotrophic fungal
393 community (Fig. 3), with the EMF community demonstrating high resilience.

394 *Differential fire season effects on soil fungi*

395 Our spring and autumn burns did not differ in their intensity and severity,
396 probably due to the specific environmental conditions required for prescribed burns
397 (Allen et al. 1968). Yet, they led to differential modifications in the soil fungal
398 community. Specifically, spring burns caused reductions in soil OTU richness and
399 diversity. But, ~one year after the burns these differences disappeared. Moreover, fire
400 season induced changes in the community composition of soil fungi, which were
401 mostly driven by alterations within the saprotrophic fungal guild (Fig. 2, Fig. 3).

402 The most parsimonious explanation for the variation in fungal community
403 composition between burning seasons is the time passed since the fires (i.e., areas
404 subjected to spring burns had a longer time to recover before the soil sampling).
405 Another possible explanation is related to fire timing effects. Specifically, seasonal
406 fires occur at different phenological stages of the fungi, potentially resulting in a
407 differential effect on their community composition. For example, fire occurring
408 during the fruiting season might inflict a greater damage than fires occurring during
409 dormancy. Nevertheless, variation in fire intensity and severity among our
410 experimental plots was higher during spring than during autumn burns.
411 Correspondingly, also the variation in community composition of soil fungi was
412 higher during spring, suggesting that fire intensity or severity, regardless of fire
413 season, can lead to changes in the soil fungal community.

414 Experiencing no significant change due to disturbance (i.e., resistance) and
415 being capable of returning to their pre-disturbance composition (i.e., resilience) are

two important features of healthy ecosystems (Shade et al. 2012). Similar to findings from high severity wildfires in CA conifer forests (Glassman et al. 2016b), and in various conifer wildfires in the west-Mediterranean basin (Buscardo et al. 2012; Buscardo et al. 2015; Buscardo et al. 2010), the EMF community represented by both soil samples and pine seedling bioassays appeared to be both resistant and resilient to seasonal fire effects. First, we could not detect a significant effect of fire on EMF richness or diversity. This result is in contrast to most studies of prescribed burns, illustrating a negative effect of fire on EMF richness (Taudière et al. 2017). Second, autumn burns affected EMF community composition, but these differences between the control and autumn burned plots faded quickly and disappeared by the next sampling period (Table 1). Numerous studies have demonstrated that prescribed burns induce changes in the soil fungal community composition (Anderson et al. 2007; Bastias et al. 2006; Hernández-Rodríguez et al. 2015). However, we could not detect a significant effect of fire season on the EMF community composition, neither in soil samples, nor in pine seedling root tips examined in the bioassay experiment (Fig. 2), suggesting high resilience of the EMF spore bank community to fires. This was somewhat surprising, since we are aware of only one other study that did not detect a significant effect of prescribed burns on the soil EMF community composition or richness (Southworth et al. 2011). However, examining the EMF spore bank community after a high-intensity wildfire, Glassman *et al.* (2016b) also demonstrated high resilience of the EMF community in greenhouse bioassays. The ability of the EMF community to survive fire perturbations should contribute to ecosystem stability, since changes in the EMF community can result in structural and functional changes in the respective plant community (Bever et al. 2010).

Temporal shift in soil fungi

Fungal seasonality has recently been identified as a key feature of natural fungal communities (Averill et al. 2019). Notably, our field experiment lends support to this idea. While examining the soil fungal community, we observed high temporal variation in the soil fungi among sampling periods regardless of fire treatment (i.e., differences appeared also in the unburned control plots). Such variation among sampling periods suggest that fires occurring at different seasons are impacting different pre-fire fungal communities.

Furthermore, EM and saprotrophic fungi compete for limiting resources held within the soil organic matter (Gadgil and Gadgil 1975). These groups have complementary roles in the cycling of nutrients through soil organic matter (Talbot et al. 2014). The observed temporal differences in community composition were mostly related to changes in the relative abundance of saprotrophic fungi among sampling periods (Fig. 2b, Fig.3), which can be related to temporal variation in precipitation attributes. Even though there were no differences in the amount of rain, the number of rainy days marginally varied among sampling periods and was lowest prior to autumn burns (0.8 ± 0.89 , mean $\pm$ 1SE; Fig. S3). Conversely, Bell *et al.* (Bell et al. 2009) showed that the saprophytic community of a desert grassland was unaffected by precipitation frequency, however, they suggested that soil temperature, rather than soil moisture strongly influenced fungal carbon use and community structure, and function dynamics. This makes sense, since temperature (i.e., evapotranspiration) affects how much of the soil water will remain available for both plants and fungi. As expected, ambient temperature was higher before autumn burns (25.00 ± 0.91 before autumn vs. 17.35 ± 1.64 before spring burns, mean $\pm$ 1SE). Even though soil moisture was only slightly lower during autumn (3.3 ± 0.22 , mean $\pm$ 1SE) than during spring burns (7.69 ± 0.39 , mean $\pm$ 1SE), plant water content during autumn burns (0.18 ± 0.01)

466 was half of that measured during spring burns (0.28 ± 0.02 , mean $\pm$ 1SE). This suggests
467 that both temperature and precipitation can influence water availability, thus playing
468 an important role in shaping fungal communities in the semi-arid Mediterranean
469 ecosystem.

470 *The EMF community of Cistus-dominated East-Mediterranean ecosystem*

471 In the eastern Mediterranean ecosystem *Cistus* shrubs are often the main EMF
472 hosts at early successional stages, followed by later successional species such as
473 *Pinus halepensis*. Our data represent the first comprehensive description of the EMF
474 community associated with the understudied *Cistus*-dominated eastern Mediterranean
475 ecosystem. We hypothesized that *P. halepensis* colonization should be facilitated by
476 the EMF community characterizing *Cistus*. We observed high abundance of *Tuber*
477 *oligospermum* associating with pines grown on soils collected underneath *Cistus*
478 shrubs (Fig. 4), suggesting a newly described link between *T. oligospermum*, *P.*
479 *halepensis* and the local *Cistus* shrubs (*C. salviifolius*, *C. creticus*). This finding is in
480 concurrence with other studies of *Cistus* dominated ecosystems in the western
481 Mediterranean basin (Comandini et al. 2006), describing the association between
482 *Cistus* and various *Tuber* species. Similarly, but in a conifer-dominated ecosystem,
483 Buscardo et al. (2012) and Glassman et al. (2016b) found *Rhizopogon* (a Pinaceae
484 specific truffle) increasing in abundance after fire, possibly indicating a pre-
485 adaptation of these hypogeous fungi to fire survival. Since mycorrhizal interactions
486 are often not species-specific (e.g., a mature tree facilitating the establishment of
487 seedlings of a different tree species; Bai et al. 2009; Dickie et al. 2002; Grau et al.
488 2010; Henry et al. 2015; Kennedy et al. 2003; Kennedy et al. 2012; Richard et al.
489 2009), these newly described association suggests that interspecific mycorrhizal
490 facilitation is one possible mechanism of facilitation between *Cistus* and *pinus*. Such

491 facilitation processes can play an important role in shaping plant community
492 dynamics, vegetation structure and ecosystem functioning (Hayward et al. 2015;
493 Horton et al. 1999). However, the mechanisms governing such facilitation processes
494 are yet to be unravel.

495 Open canopy gaps were mostly dominated by *Suillus* spores (Fig. 4b). *Suillus*
496 is known for its long-lived (Nguyen et al. 2012), long dispersal distance spores
497 (Glassman et al. 2017; Glassman et al. 2015; Peay et al. 2012) and are known for
498 dominating open microhabitats spore banks. Another dominant fungal genus was
499 *Tomentella*, colonizing pine seedlings grown on soil collected from all fire treatment
500 plots. Similarly, Buscardo *et al.* (Buscardo et al. 2012) reported that *Tomentella ellisii*
501 colonized both pine and oak seedling grown in soil obtained from a short fire return
502 interval site dominated by *Cistus ladanifer*. Interestingly, in a previous study from
503 northern Israel, the genus *Tomentella* also dominated pine seedlings grown in soils
504 collected from a mixed forest site (Livne-Luzon et al. 2017a).

505 *Conclusions*

506 We observed largest differences due to fire season in the total soil fungal,
507 rather than in the EMF community, and this effect was largely driven by alternations
508 within the saprotrophic fungal guild. Most data on fungal response to fire comes from
509 northwestern USA (Taudière et al. 2017), where fires are typically of higher severity
510 than in the eastern Mediterranean basin. Such fires, as those attained in our study, are
511 less likely to lead to host death, or to inflict direct damage to soil microorganisms.
512 Therefore, Mediterranean fires might induce different selection pressure on the soil
513 biota. Even though the EMF community appeared to be resilient to fire, saprotrophic
514 and EM fungi were documented to compete over similar niche requirements in many

515 ecosystems (Leake et al. 2002). Therefore, these changes in the saprotrophic
516 community composition might have an additional indirect effect on the EMF
517 community composition. Since small-scale changes in carbon inputs can cascade to
518 affect decomposition rates and carbon emissions (Hawlena et al. 2012; Schmitz et al.
519 2014), these small yet distinct differences in the soil fungal community composition
520 can further affect ecosystem functioning.

521

522 **References**

- 523 Allen M, Berry R, Gill D, Hayes G, Truesdell P, Zwolinski M, Perovich J (1968)
524 Guide to prescribed fire in the Southwest Tucson, AZ: Southwest Interagency
525 Fire Council
- 526 Anderson IC, Bastias BA, Genney DR, Parkin PI, Cairney JW (2007) Basidiomycete
527 fungal communities in Australian sclerophyll forest soil are altered by
528 repeated prescribed burning MYCOLOGICAL RESEARCH 111:482-486
- 529 Anderson JB, Wickens C, Khan M, Cowen LE, Federspiel N, Jones T, Kohn LM
530 (2001) Infrequent genetic exchange and recombination in the mitochondrial
531 genome of *Candida albicans* Journal of Bacteriology 183:865-872
- 532 Averill C, Cates LL, Dietze MC, Bhatnagar JM (2019) Spatial vs. temporal controls
533 over soil fungal community similarity at continental and global scales The
534 ISME journal:1
- 535 Bai S-L, Li G-L, Liu Y, Dumroese RK, Lv R-H (2009) *Ostryopsis davidiana*
536 seedlings inoculated with ectomycorrhizal fungi facilitate formation of
537 mycorrhizae on *Pinus tabulaeformis* seedlings Mycorrhiza 19:425-434
- 538 Bastias BA, Huang ZQ, Blumfield T, Xu Z, Cairney JW (2006) Influence of repeated
539 prescribed burning on the soil fungal community in an eastern Australian wet
540 sclerophyll forest Soil Biology and Biochemistry 38:3492-3501
- 541 Bell CW, Acosta-Martinez V, McIntyre NE, Cox S, Tissue DT, Zak JC (2009)
542 Linking microbial community structure and function to seasonal differences in
543 soil moisture and temperature in a Chihuahuan desert grassland Microb Ecol
544 58:827-842
- 545 Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and
546 powerful approach to multiple testing Journal of the royal statistical society
547 Series B (Methodological):289-300
- 548 Bever JD et al. (2010) Rooting theories of plant community ecology in microbial
549 interactions Trends in Ecology & Evolution 25:468-478
550 doi:10.1016/j.tree.2010.05.004
- 551 Buscardo E et al. (2012) Is the potential for the formation of common mycorrhizal
552 networks influenced by fire frequency? Soil Biology and Biochemistry
553 46:136-144

- 554 Buscardo E, Rodríguez-Echeverría S, Freitas H, De Angelis P, Pereira JS, Muller LA
- 555 (2015) Contrasting soil fungal communities in Mediterranean pine forests
- 556 subjected to different wildfire frequencies *Fungal Diversity* 70:85-99
- 557 Buscardo E, Rodríguez-Echeverría S, Martín MP, De Angelis P, Pereira JS, Freitas H
- 558 (2010) Impact of wildfire return interval on the ectomycorrhizal resistant
- 559 propagules communities of a Mediterranean open forest *Fungal Biology*
- 560 114:628-636
- 561 Caporaso JG et al. (2010) QIIME allows analysis of high-throughput community
- 562 sequencing data *Nature Methods* 7:335-336
- 563 Clarke K, Warwick R (1994) Change in marine communities : an approach to
- 564 statistical analysis and interpretation. 2nd edn. Plymouth, U.K. : PRIMER-E
- 565 Ltd., 2001,
- 566 Clarke KR, Somerfield PJ, Gorley RN (2008) Testing of null hypotheses in
- 567 exploratory community analyses: similarity profiles and biota-environment
- 568 linkage *Journal of Experimental Marine Biology and Ecology* 366:56-69
- 569 Collier FA, Bidartondo MI (2009) Waiting for fungi: the ectomycorrhizal invasion of
- 570 lowland heathlands *J Ecol* 97:950-963
- 571 Comandini O, Contu M, Rinaldi A (2006) An overview of *Cistus* ectomycorrhizal
- 572 fungi *Mycorrhiza* 16:381-395
- 573 De la Varga H, Águeda B, Ágreda T, Martínez-Peña F, Parladé J, Pera J (2013)
- 574 Seasonal dynamics of *Boletus edulis* and *Lactarius deliciosus* extraradical
- 575 mycelium in pine forests of central Spain *Mycorrhiza* 23:391-402
- 576 de Roman M, de Miguel AM (2005) Post-fire, seasonal and annual dynamics of the
- 577 ectomycorrhizal community in a *Quercus ilex* L. forest over a 3-year period
- 578 *Mycorrhiza* 15:471-482
- 579 Dickie IA, Koide RT, Steiner KC (2002) Influences of established trees on
- 580 mycorrhizas, nutrition, and growth of *Quercus rubra* seedlings *Ecological*
- 581 *Monographs* 72:505-521
- 582 Dove NC, Hart SC (2017) Fire reduces fungal species richness and in situ mycorrhizal
- 583 colonization: a meta-analysis *Fire Ecology* 13:37-65
- 584 Edgar RC (2013) UPARSE: highly accurate OTU sequences from microbial amplicon
- 585 reads *Nat Methods* 10:996-+ doi:10.1038/nmeth.2604
- 586 Fernandez CW, Kennedy PG (2016) Revisiting the ‘Gadgil effect’: do interguild
- 587 fungal interactions control carbon cycling in forest soils? *New Phytol*
- 588 209:1382-1394
- 589 Gadgil PD, Gadgil RL (1975) Suppression of litter decomposition by mycorrhizal
- 590 roots of *Pinus radiata*. New Zealand Forest Service,
- 591 Gadgil RL, Gadgil PD (1971) Mycorrhiza and litter decomposition *Nature* 233:133
- 592 Glassman SI, Levine CR, DiRocco AM, Battles JJ, Bruns TD (2016a)
- 593 Ectomycorrhizal fungal spore bank recovery after a severe forest fire: some
- 594 like it hot *The ISME journal* 10:1228
- 595 Glassman SI, Levine CR, DiRocco AM, Battles JJ, Bruns TD (2016b)
- 596 Ectomycorrhizal fungal spore bank recovery after a severe forest fire: some
- 597 like it hot *ISME J* 10:1228-1239 doi:10.1038/ismej.2015.182
- 598 Glassman SI, Lubetkin KC, Chung JA, Bruns TD (2017) The theory of island
- 599 biogeography applies to ectomycorrhizal fungi in subalpine tree “islands” at a
- 600 fine scale *Ecosphere* 8:e01677
- 601 Glassman SI et al. (2015) A continental view of pine-associated ectomycorrhizal
- 602 fungal spore banks: a quiescent functional guild with a strong biogeographic
- 603 pattern *New Phytol* 205:1619-1631

- 604 Goldreich Y (2003) The climate of Israel: observation, research and application.
605 Kluwer Academic/Plenum Publishers,
- 606 Grau O, Rautio P, Heikkinen J, Saravesi K, Kozlov MV, Markkola A (2010) An
607 ericoid shrub plays a dual role in recruiting both pines and their fungal
608 symbionts along primary succession gradients Oikos 119:1727-1734
- 609 Hawlena D, Strickland MS, Bradford MA, Schmitz OJ (2012) Fear of Predation
610 Slows Plant-Litter Decomposition Science 336:1434-1438
- 611 Hayward J, Horton TR, Pauchard A, Nunez MA (2015) A single ectomycorrhizal
612 fungal species can enable a Pinus invasion Ecology 96:1438-1444
- 613 Henry C, Raivoarisoa J-F, Razafimamonjy A, Ramanankierana H,
614 Andrianaivomahefa P, Selosse M-A, Ducousso M (2015) *Asteropeia*
615 *mcphersonii*, a potential mycorrhizal facilitator for ecological restoration in
616 Madagascar wet tropical rainforests For Ecol Manage 358:202-211
- 617 Hernández-Rodríguez M, Oria-de-Rueda JA, Martín-Pinto P (2013) Post-fire fungal
618 succession in a Mediterranean ecosystem dominated by *Cistus ladanifer* L
619 Forest Ecology And Management 289:48-57
- 620 Hernández-Rodríguez M, Oria-de-Rueda JA, Pando V, Martín-Pinto P (2015) Impact
621 of fuel reduction treatments on fungal sporocarp production and diversity
622 associated with *Cistus ladanifer* L. ecosystems Forest Ecology And
623 Management 353:10-20
- 624 Horton TR, Bruns TD, Parker T (1999) Mycorrhizal fungi associated with
625 *Arctostaphylos* facilitate the establishment of *Pseudotsuga menziesii* during
626 succession. Can J Bot 77:93-102
- 627 Johnson CN (1995) Interactions between fire, mycophagous mammals, and dispersal
628 of ectomycorrhizal fungi in Eucalyptus forests Oecologia (Berlin) 104:467-
629 475
- 630 Johnstone JF, Chapin FS, Hollingsworth TN, Mack MC, Romanovsky V, Turetsky M
631 (2010) Fire, climate change, and forest resilience in interior Alaska Canadian
632 Journal Of Forest Research 40:1302-1312
- 633 Kennedy P, Izzo A, Bruns T (2003) There is high potential for the formation of
634 common mycorrhizal networks between understorey and canopy trees in a
635 mixed evergreen forest J Ecol 91:1071-1080
- 636 Kennedy PG, Smith DP, Horton TR, Molina RJ (2012) *Arbutus menziesii* (Ericaceae)
637 facilitates regeneration dynamics in mixed evergreen forests by promoting
638 mycorrhizal fungal diversity and host connectivity American Journal Of
639 Botany 99:1691-1701
- 640 Kipfer T, Moser B, Egli S, Wohlgemuth T, Ghazoul J (2011) Ectomycorrhiza
641 succession patterns in *Pinus sylvestris* forests after stand-replacing fire in the
642 Central Alps Oecologia 167:219 doi:10.1007/s00442-011-1981-5
- 643 Klironomos J et al. (2010) Forces that structure plant communities: quantifying the
644 importance of the mycorrhizal symbiosis New Phytol 189:366-370
- 645 Klironomos JN (2002) Feedback with soil biota contributes to plant rarity and
646 invasiveness in communities Nature 417:67-70
- 647 Klopatek CC, Freise CF, Allen MF, Klopatek JM (1994) Comparisons of laboratory
648 and field burning experiments on mycorrhizae distribution, density and
649 diversity Journal of the American forestry Society 94:762-776
- 650 Knapp EE, Estes BL, Skinner CN (2009) Ecological Effects of Prescribed Fire
651 Season: A Literature Review and Synthesis for Managers. United States
652 Department of Agriculture - Forest Service,

- 653 Knapp EE, Keeley JE, Ballenger EA, Brennan TJ (2005) Fuel reduction and coarse
654 woody debris dynamics with early season and late season prescribed fire in a
655 Sierra Nevada mixed conifer forest *Forest Ecology and Management* 208:383-
656 397
- 657 Koljalg U et al. (2005) UNITE: a database providing web-based methods for the
658 molecular identification of ectomycorrhizal fungi *New Phytol* 166:1063-1068
- 659 Leake J, Donnelly D, Boddy L (2002) Interactions between ecto-mycorrhizal and
660 saprotrophic fungi. In: *Mycorrhizal Ecology*. Springer, pp 345-372
- 661 Lindahl BD et al. (2013) Fungal community analysis by high-throughput sequencing
662 of amplified markers—a user's guide *New Phytol* 199:288-299
- 663 Liphshitz N, Biger G (2001) Past distribution of Aleppo pine (*Pinus halepensis*) in
664 the mountains of Israel (Palestine) *The Holocene* 11:427-436
- 665 Livne-Luzon S, Avidan Y, Weber G, Migael H, Bruns T, Ovadia O, Shemesh H
666 (2017a) Wild boars as spore dispersal agents of ectomycorrhizal fungi:
667 consequences for community composition at different habitat types
668 *Mycorrhiza* 27:165-174
- 669 Livne-Luzon S et al. (2017b) Small-scale spatial variability in the distribution of
670 ectomycorrhizal fungi affects plant performance and fungal diversity *Ecol Lett*
671 20:1192-1202 doi:10.1111/ele.12816
- 672 Marlon JR et al. (2009) Wildfire responses to abrupt climate change in North America
673 *Proceedings of the National Academy of Sciences* 106:2519-2524
- 674 Miller C, Urban DL (1999) Interactions between forest heterogeneity and surface fire
675 regimes in the southern Sierra Nevada *Canadian Journal of Forest Research*
676 29:202-212
- 677 Miller SL, McClean TM, Stanton NL, Williams SE (1998) Mycorrhization,
678 physiognomy, and first-year survivability of conifer seedlings following
679 natural fire in Grand Teton National Park *Can J For Res-Rev Can Rech For*
680 28:115-122
- 681 Moriondo M, Good P, Durao R, Bindi M, Giannakopoulos C, Corte-Real J (2006)
682 Potential impact of climate change on fire risk in the Mediterranean area
683 *Climate Research* 31:85-95
- 684 Ne'eman G (1997) Regeneration of natural pine forest—review of work done after the
685 1989 fire in Mount Carmel, Israel *International Journal of Wildland Fire*
686 7:295-306
- 687 Ne'eman G, Goubitz S, Nathan R (2004) Reproductive traits of *Pinus halepensis* in the
688 light of fire - a critical review *Plant Ecology* 171:69-79
- 689 Ne'eman G, Izhaki I (1999) The effect of stand age and microhabitat on soil seed
690 banks in Mediterranean Aleppo pine forests after fire *Plant Ecology* 144:115-
691 125
- 692 Nguyen NH, Hynson NA, Bruns TD (2012) Stayin' alive: survival of mycorrhizal
693 fungal propagules from 6-yr-old forest soil *Fungal Ecology* 5:741-746
694 doi:10.1016/j.funeco.2012.05.006
- 695 Nguyen NH et al. (2016) FUNGuild: an open annotation tool for parsing fungal
696 community datasets by ecological guild *Fungal Ecology* 20:241-248
- 697 Osem Y, Ginsberg P, Tauber I, Atzmon N, Perevolotsky A (2008) Sustainable
698 management of Mediterranean planted coniferous forests: an Israeli definition
699 *Journal of Forestry* 106:38-46
- 700 Peay KG, Schubert MG, Nguyen NH, Bruns TD (2012) Measuring ectomycorrhizal
701 fungal dispersal: macroecological patterns driven by microscopic propagules
702 *Molecular Ecology* 21:4122-4136 doi:10.1111/j.1365-294X.2012.05666.x

- 703 Pechony O, Shindell DT (2010) Driving forces of global wildfires over the past
704 millennium and the forthcoming century Proceedings of the National
705 Academy of Sciences 107:19167-19170
- 706 R Development Core Team (2010) R: A Language and Environment for Statistical
707 Computing. R Foundation for Statistical Computing. [http://www.R-](http://www.R-project.org)
708 [project.org](http://www.R-project.org).
- 709 Richard F, Selosse M-A, Gardes M (2009) Facilitated establishment of *Quercus ilex*
710 in shrub-dominated communities within a Mediterranean ecosystem: do
711 mycorrhizal partners matter? FEMS Microbiology Ecology 68:14-24
- 712 Schmitz OJ et al. (2014) Animating the carbon cycle Ecosystems 17:344-359
- 713 Shade A et al. (2012) Fundamentals of microbial community resistance and resilience
714 Frontiers in microbiology 3:417
- 715 Sheffer E (2012) A review of the development of Mediterranean pine–oak ecosystems
716 after land abandonment and afforestation: are they novel ecosystems? Annals
717 of Forest Science 69:429-443
- 718 Smith DP, Peay KG (2014) Sequence depth, not PCR replication, improves ecological
719 inference from next generation DNA sequencing Plos One 9:e90234
- 720 Smith JE, McKay D, Niwa CG, Thies WG, Brenner G, Spatafora JW (2004) Short-
721 term effects of seasonal prescribed burning on the ectomycorrhizal fungal
722 community and fine root biomass in ponderosa pine stands in the Blue
723 Mountains of Oregon Can J For Res-Rev Can Rech For 34:2477-2491
724 doi:10.1139/x04-124
- 725 Southworth D, Donohue J, Frank JL, Gibson J (2011) Mechanical mastication and
726 prescribed fire in conifer–hardwood chaparral: differing responses of
727 ectomycorrhizae and truffles International Journal of Wildland Fire 20:888-
728 896
- 729 Sun H et al. (2015) Fungal community shifts in structure and function across a boreal
730 forest fire chronosequence Applied and Environmental Microbiology 81:7869-
731 7880
- 732 Talbot JM et al. (2014) Endemism and functional convergence across the North
733 American soil mycobiome Proceedings of the National Academy of Sciences
734 111:6341-6346
- 735 Taudière A, Richard F, Carcaillet C (2017) Review on fire effects on ectomycorrhizal
736 symbiosis, an unachieved work for a scalding topic Forest Ecology And
737 Management 391:446-457
- 738 Taylor DL, Bruns TD (1999) Community structure of ectomycorrhizal fungi in a
739 *Pinus muricata* forest: minimal overlap between the mature forest and
740 resistant propagule communities. Mol Ecol 8:1837-1850
- 741 Veen GF, Blair JM, Smith MD, Collins SL (2008) Influence of grazing and fire
742 frequency on small-scale plant community structure and resource variability in
743 native tallgrass prairie Oikos 117:859-866
- 744 Westerling AL, Hidalgo HG, Cayan DR, Swetnam TW (2006) Warming and earlier
745 spring increase western US forest wildfire activity Science 313:940-943

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749 **Figure legends:**

750 **Figure 1:** Experimental timescale and sampling scheme. Soil samples were
751 collected from burned and unburned sites before and after spring and autumn
752 burns. Bioassay samples were collected just before the rainy season, reflecting the
753 soil spore bank that germinating plants encounter in the field. This sampling
754 scheme was designed to allow us to quantify the net effects of spring and autumn
755 burns on the soil fungal community composition, conveying the actual effects of
756 seasonal fires in typical Mediterranean woodlands

757 **Figure 2:** a) Non-metric multi-dimensional scaling (nMDS) ordination with
758 bootstrap of fire season averages, illustrating that soil fungi detected in the post-
759 fire sampling period (i.e., June 2015) vary significantly among fire treatments.
760 Circles represent 95% CI. b) Results of SIMPER analysis illustrating the relative
761 contribution of each of the major fungal functional groups (saprotrophic, EM and
762 AM fungi) to the dissimilarity (Bray-Curtis) among fire treatments. Data includes
763 unassigned fungal sequences and other fungal sequence assignments (e.g., plant
764 pathogen, animal pathogen endophytes etc., each accounted for less than 3% of
765 the dissimilarity).

766 **Figure 3:** The ratio between OUT's identified as putative saprotrophic to putative
767 EM fungi out of all OUT's identified ((saprotrophic/EMF)/total OUT's) in each
768 sample. * denote significant differences ($p < 0.05$) among the autumn and spring
769 burns within sampling periods.

770 **Figure 4: (a)** Relative sequence abundance of the root-associated EMF species in
771 each of the six treatment combinations of fire treatment and microhabitat. **(b)**
772 Differences in the relative read abundance of the various root-associated EMF
773 species between the two microhabitats (underneath *Cistus* shrubs and open gaps).
774 Positive values are indicative for higher sequence abundance in the *Cistus*
775 microhabitat and negative values indicate higher abundance in open gaps.

a)

b)