

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
57 Information Sequence Read Archive under accession numbers SRRXXX□SRRXXX.

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 Yogev 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 *pines*. 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

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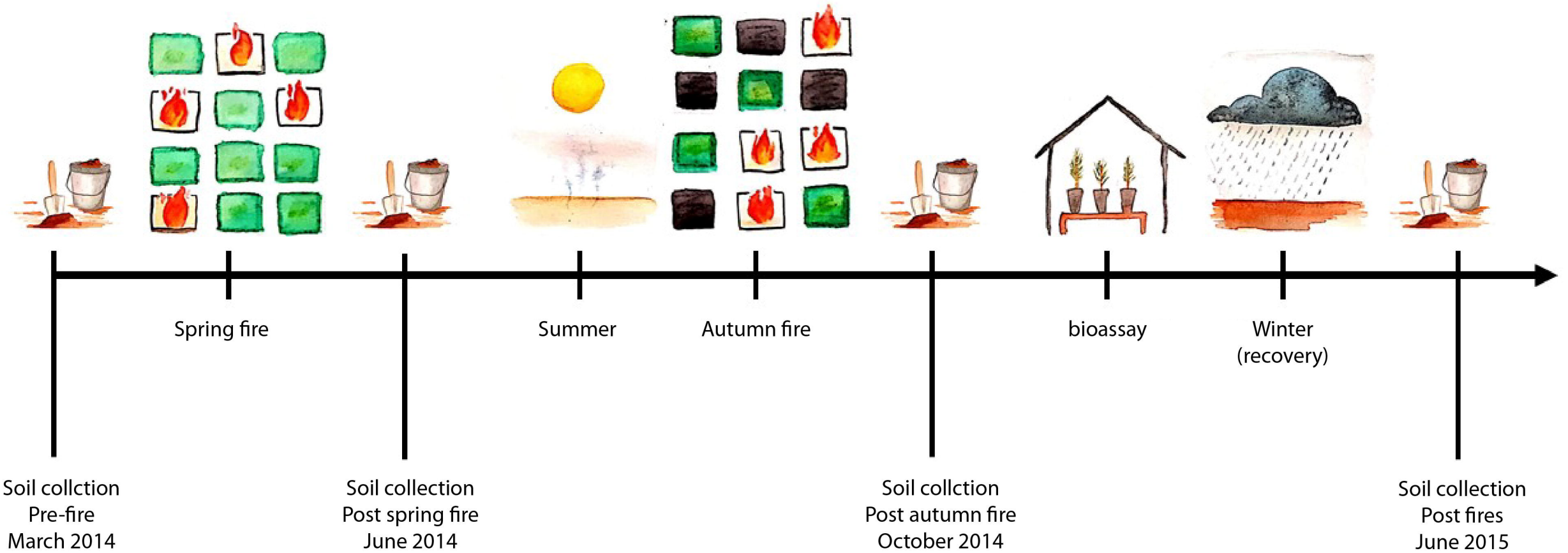
Figure legends:

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

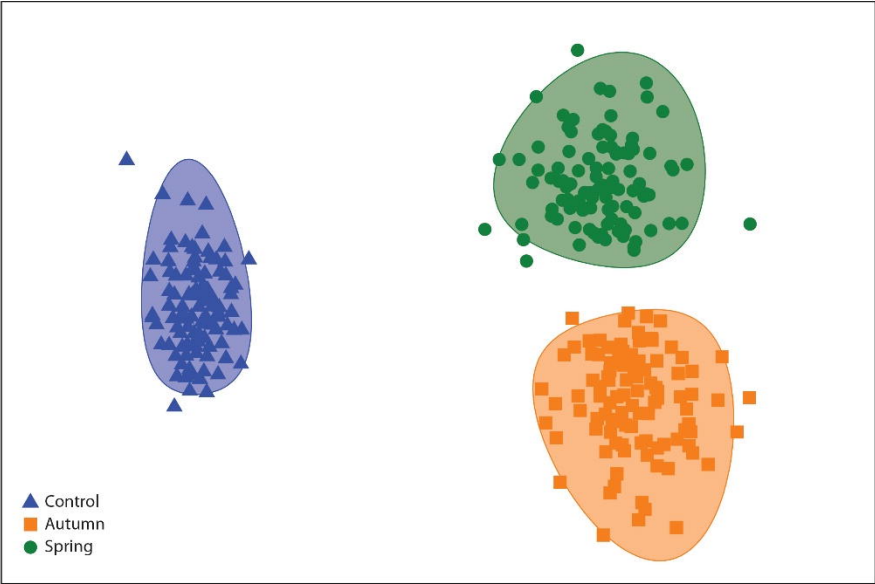
Figure 2: a) Non-metric multi-dimensional scaling (nMDS) ordination with bootstrap of fire season averages, illustrating that soil fungi detected in the post-fire sampling period (i.e., June 2015) vary significantly among fire treatments. Circles represent 95% CI. b) Results of SIMPER analysis illustrating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

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

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



a)



b)

