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ORIGINAL ARTICLE

High resilience of the mycorrhizal community to prescribed seasonal burnings in eastern Mediterranean woodlands

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Abstract

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

Keywords *Cistus* · Ecosystem resilience · Ectomycorrhizal fungi · Fungal richness · Illumina MiSeq · Mediterranean · *Pinus halepensis* · Prescribed burns · Seasonality

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Introduction

Fire is one of the most common natural and anthropogenic disturbances leading to secondary succession of both plant and fungal communities (Marlon et al. 2009). Exploring the effects of fire on ecosystem functioning is of high priority, especially due to the increase in fire risk associated with climate change (Moriondo et al. 2006; Pechony and Shindell 2010; Westerling et al. 2006). The extent of damage fires inflict on plant communities is manifested not only through the destruction of plant tissue but also in destruction of symbiotic soil microbes, which may be necessary to buffer against fire effects, thus increasing plant community resilience (Johnstone et al. 2010; Kipfer et al. 2011). Most of the temperate and boreal trees around the globe are obligately symbiotic with ectomycorrhizal fungi (EMF), meaning that their establishment is dependent upon the occurrence of an appropriate symbiont community (Miller et al. 1998). Therefore, fire effects on the belowground biota may be far-reaching with regard to vegetation regeneration and growth during the first few post-fire years (Neary et al. 1999). For example, a fire study based on chrono-sequence found that

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fire temporarily shifted the fungal community structure and function by increasing the abundance of saprotrophic fungi (Sun et al. 2015). Eventually, the community returned to its pre-fire state, but at a very slow rate (Sun et al. 2015). Such a community shift towards saprotrophic fungi may have a detrimental effect on ecosystem functioning because it may shift the balance between obligate symbiotic EMF, associated with tree roots, and saprotrophic fungi. Besides the clear negative outcome of reduced symbionts available for plants (Collier and Bidartondo 2009), competition between these two fungal guilds can suppress decomposition rates (i.e., the Gadgil effect; Fernandez and Kennedy 2016; Gadgil and Gadgil 1971, 1975).

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

Examining the effects of prescribed burns on the EMF community in a natural setting of ponderosa pine stands in eastern Oregon, Smith et al. (2004) found that late-dry season fires had long lasting, devastating effects on the mycorrhizal community, with a reduction of 80% in molecular species richness. Early-dry season fires, however, did not differ from the unburned control. Smith et al. (2004) suggested that observed differences in the EMF community composition were the result of inter-season variation in fire severity. Specifically, the low moisture content in the fuel and in the soil during the late-dry season resulted in higher soil temperatures and increased microbial mortality. Such extreme soil temperatures may damage the mycorrhizal community directly by destroying the mycelia or indirectly by host death; both can lead to a long lasting, negative effect on the EMF community (Klopatek et al. 1994). On the contrary, in the Mediterranean region spring fires occur after the wet season when soil moisture is high and heat transfer is highly efficient (early-dry season fires), compensating for the increase in soil temperature caused by these fires, and thus having a weaker detrimental effect on the soil biota. Differential fire season effects can be also related to the phenological stage of both the plants and fungi at the time of fire. For example, in many fungal species occupying Mediterranean habitats, the amount of mycelium decreases in summer, probably due to hot and dry conditions, whereas in autumn it increases again (De la Varga et al. 2013). Furthermore, the hot dry Mediterranean summer can act as an environmental stressor driving the filtering (the successful or failed persistence of species in a given environment) of fungal species that vary in their ability to cope with these extreme conditions, resulting in a seasonal shift in the composition of the fungal community. Clearly, these shifts should be more pronounced in open canopy gaps created by early-dry season fires, where the soil is more exposed to direct sun radiation. All of the above imply that fire timing can play a major role in shaping the soil fungal community in general and the EMF community in particular, which in turn can determine species-specific plant establishment and growth (Klironomos et al. 2010; Livne-Luzon et al. 2017a, b), and plant species' richness (Klironomos 2002). We thus hypothesized that the fire season should have a differential effect on the composition of the soil fungal community, shifting the balance between obligate symbiotic EMF, associated with tree roots, and saprotrophic fungi.

Most studies on the post-fire dynamics of the EMF community have been performed in conifer forests (Dove and Hart 2017) located in temperate and boreal areas. In comparison, much less is known about the effect of fire on the EMF community in fire-prone Mediterranean ecosystems. Notably, a few recent studies have brought new attention to fire effects on EMF communities in Mediterranean habitats dominated by *Quercus* or *Cistus* sp. (Buscardo et al. 2015, 2010; de Román and de Miguel 2005; Hernández-Rodríguez et al. 2013), emphasizing the need to explore these poorly studied habitats. Our research aimed to study the effects of fire season (early- vs. late-dry season burns) on the soil fungal community and specifically on the EMF community in a *Cistus* dominated eastern Mediterranean ecosystem. We manipulated fire seasonality using early-dry season (spring) and late-dry season (autumn) prescribed burns and examined the various effects of fire season on the soil and ectomycorrhizal fungal communities through both sequencing and pine seedling bioassays.

Succession in Mediterranean woodlands often begins with a pioneer stage of *Cistus salviifolius* followed by *Pinus halepensis* colonization (Ne'eman 1997; Schiller et al. 1997; Sheffer 2012). *Cistus* is considered an early "pioneer" species that increases in density, especially after fire disturbance (Ne'eman and Izhaki 1999). *Pinus halepensis* is a dominant tree species in natural (Liphschitz and Biger 2001) and planted (Osem et al. 2008) forests in Israel, known for its adaptive post-fire regeneration (Ne'eman 1997; Ne'eman et al. 2004). Since at early successional stages

Cistus shrubs are the main EMF hosts, we hypothesized that *P. halepensis* colonization should be facilitated by the EMF community characterizing *Cistus*. We therefore compared the pine-associated EMF community under *Cistus* shrubs with that of adjacent open canopy gaps using pine bioassays. Doing so allowed us to examine the soil fungal spore bank, essential for the post-fire regeneration of pines in this ecosystem (Glassman et al. 2016).

Materials and methods

The study area

The study site was located in Har Yaaran in the Judean lowlands of Israel (600 m ASL; Fig. S1). The climate is Mediterranean, with an average annual precipitation of 500–600 mm; between May and October it hardly rains, while solar radiation is very high (Goldreich 2012). The soil is clayish and shallow due to large limestone plates. The vegetation cover is of a Mediterranean shrubland (garrigue), with patches of small multi-stem trees (e.g., *Quercus calliprinos* and *Rhamnus lycioides*), shrubs (1–1.5 m high; e.g., *Pistacia lentiscus*, *Rhamnus lycioides*, and *Calicotome villosa*), dwarf-shrubs (≤ 1 m; e.g., *Cistus salviifolius*, *Cistus creticus*, and *Teucrium divaricatum*), and patches of herbaceous vegetation. The main ectomycorrhizal hosts in the study area are (by order of dominance) *C. salviifolius*, *Q. calliprinos*, and *C. creticus*. There is an adjacent planted pine (*P. halepensis* and *P. brutia*) forest uphill of the study area, but there were no mature pine trees and only a few pine seedlings were found in the research plots.

Experimental design

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

Soil sampling

Soil samples were collected at four different sampling periods: (1) pre-fire soil samples (i.e., March 2014), (2) post-spring fire (2 weeks after the spring burns, i.e., June 2014), (3) post-autumn fire (2 weeks after the autumn burns, i.e., Oct. 2014), and (4) post-fires (~ 1 year after the collection of pre-fire samples, i.e., June 2015). This experimental design and sampling scheme (Fig. 1) enabled us to quantify both the immediate changes in the mycorrhizal community composition caused by seasonal fires, as well as those that developed during the first post-fire year. This is because the rate of change in post-fire secondary succession is considered high only during the first years after the fire, decreasing exponentially afterwards (Schiller et al. 1997).

All samples were collected using the following protocol: three soil cores (10-cm depth, ~ 0.5L) were collected from each of the eight subplots located within each experimental plot. Since the field site is characterized by several rocky patches, each soil core was collected from wherever possible within the 5- × 5-m subplot, staying within 0.5 m from a

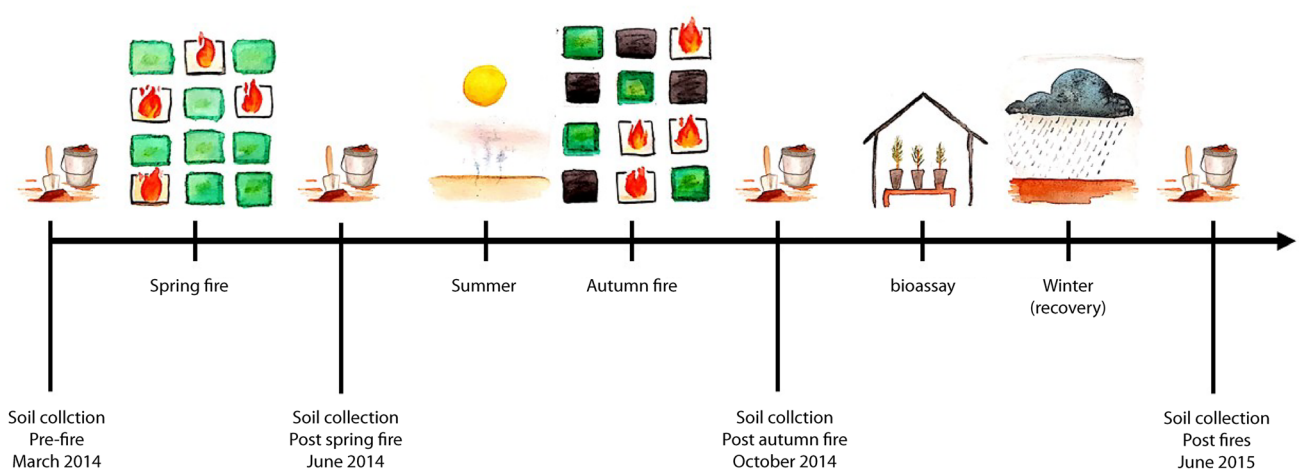


Fig. 1 Experimental timescale and sampling scheme. Soil samples were collected from burned and unburned sites before and after the early- (spring) and late-dry season (autumn) burns. Bioassay samples were collected just before the rainy season, reflecting the soil spore bank that germinating plants encounter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

Cistus shrub (the dominant EMF host in the study area). Each soil core was bagged separately, and all tools were sterilized with 70% ethanol when moving among different subplots to avoid cross-sample contamination. Upon returning to the lab, the three soil cores of each subplot were sieved (2 mm) and homogenized. Then 0.25 g of soil from each sample was directly added to Powersoil DNA tubes (MoBio, Carlsbad, CA USA) and stored at 4 °C up to 1 week before DNA extraction. The remaining soil from the pre-fire (March 2014) and post-fires (June 2015) was kept (4 °C) in a zip-lock bag for later soil property analysis.

Greenhouse bioassays

Fungal DNA extracted from the soil may originate from active hyphae or from the soil EMF spore bank (Lindahl et al. 2013; Taylor and Bruns 1999). While most perennial shrub and tree species characterizing Israel's Mediterranean habitats are post-fire resprouters, both *Pinus halepensis* and *Cistus* are obligatory seeders (Lahav 1989; Naveh 1973; Trabaud 1987). *Pinus halepensis* regenerates from post-fire-dispersed seeds stored in serotinous cones, while *Cistus* regenerates from the soil seed bank. Therefore, the secondary succession of Mediterranean Pine forests usually begins with a massive regeneration of both *Cistus* and Pines. In this current study, we aimed at studying potential mycorrhizal links between the two species. Therefore, to assess the inoculation potential of the post-fire EMF community, we bioassayed the soils collected from the study area with *P. halepensis* using standard protocols (Glassman et al. 2016).

We used the same protocol described above to collect soil samples for the greenhouse bioassay from each of the twelve experimental plots, while distinguishing between two different microhabitats: (1) under a *Cistus* shrub and (2) an open area with no perennial shrub cover (12 plots × 8 subplots × 2 microhabitats = 192 soil samples). Soil samplings were collected in October 2014, 2 weeks after the late-dry season (autumn) burns and ~ 4 months after the early-dry season (spring) burns, i.e., before the rainy season when most fungi are active. 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 6 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 6 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. (2016) 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 respective bioinformatic analyses, see Supplement S1 and Table S1 in online resource 1. Illumina data were processed using a combination of the UPARSE (Edgar 2013) and QIIME (Caporaso et al. 2010) pipelines following the methods of Smith and Peay (2014), and Glassman et al. (2016) with minor modifications related to software updates. High-quality sequences were grouped into operational taxonomic units (OTUs) using USEARCH (Edgar 2013) based on 97% similarity (a lower threshold of 95% similarity generated qualitatively similar results). Taxonomic assignments were made in QIIME based on the UNITE database (Koljalg et al. 2005). FUNguild was then used to parse OTUs into ecological guilds (Nguyen et al. 2016).

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

Statistical analyses

Multivariate analyses were performed in PRIMER v.6 of the Plymouth Marine Laboratory (Clarke et al. 2014). Relative abundances were fourth-root transformed (Clarke et al. 2014, 2008). A permutational MANOVA (PERMANOVA) based

on Bray-Curtis similarity matrix (Anderson 2001) followed by non-metric multi-dimensional scaling (nMDS) ordination was performed to test for the combined effect of fire season (whole plot) and sampling treatments (within plot) on the entire fungal community composition (and on the EMF community) using a split-plot experimental design. A similar analysis was used to examine the combined effects of fire season (whole plot treatment) and microhabitat (*Cistus* vs. Open; within plot treatment) on the fungal community composition associated with pine roots (greenhouse bioassay experiment). We examined the same effects on the relative abundance of each fungal OTU in order to search for specific fungal species that were differentially expressed among these bioassay treatments; the p values obtained from these tests were then corrected for multiple testing using the false discovery rate correction (Benjamini and Hochberg 1995) implemented in the p.adjust function of the R Stats Package (R Development Core Team 2010). To identify the percentage contribution of different fungal OTUs to observed differences in community composition, we used a similarity percentages routine SIMPER (Anderson 2001). In all cases, qualitative similar results were obtained when a square-root or no transformation were applied, as well as when using a Jaccard similarity matrix (Clarke et al. 2014) based on presence/absence data (Clarke et al. 2014), so unless otherwise mentioned all results refer to the fourth-root Bray-Curtis similarity matrix. To test for the combined effect of fire season and sampling period on the ratio of saprotrophic to EM fungi ((saprotrophic/EM)/total OTUs) and on the bioassay OTU richness (and several other diversity indexes), we used split-plot ANOVAs with fire season as the whole-plot factor and sampling period as the within-plot factor. These analyses were performed using STATISTICA v.12 (StatSoft, Inc., Tulsa, OK, USA). A heatmap of the top 30 most abundant putative fungal taxa was created using the pheatmap package implemented in R (R Development Core Team 2010).

Results

Fire intensity and severity were largely consistent between early-dry season (spring) and late-dry season (autumn) burns (Table S2). Notably, the amount of variation in fire intensity and severity among experimental plots was higher during early- than during late-dry season burns (Fig. S2). The environmental conditions monitored pre- and during the burns, proxies of fire intensity and severity measured during and post the burns, and analyses of these variables are presented as part of 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 × 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 early-dry season (spring) burns, there was a significant reduction in OTU richness and diversity in burned compared with unburned control plots. Approximately 1 year after the early-dry season 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 neither fire season ($F_{2,10.53} = 0.85$, $p = 0.621$) nor the interaction between fire season and sampling period ($F_{5,23.62} = 0.67$, $p = 0.921$) were significant. However, there was higher variation in fungal community composition among plots subjected to early-dry season (spring) burns ($F_{10,249} = 3.42$, $P = 0.0001$). Similar patterns were observed when examining the EMF and saprotrophic fungal community composition (Tables S9 & S10; Fig. S5).

PERMANOVA pair-wise comparisons (Table 1) indicated that there were no significant differences in community composition of the soil fungi among fire seasons during the pre-fire sampling period (i.e., March 2014), and the same holds true when examining the pre-fire EMF and saprotrophic fungal communities. After the early-dry season (spring) burns (i.e., June 2014), community composition of the entire and saprotrophic fungal communities varied significantly between the control and spring burned plots ($t_{11.1} = 1.52$, $p = 0.024$ and $t_{11.1} = 1.59$, $p = 0.020$, for the entire and saprotrophic fungal communities, respectively).

Table 1 Results of permutation pair-wise contrasts, examining differences in the average Bray Curtis similarity of fire seasons within each one of the sampling periods in the entire soil fungal community, the EMF community, and saprotrophic (SAP) community. Significant differences ($p \leq 0.05$) appear in italics

	All fungi	EMF	SAP
Pre-fire (March 14)			
Control, Autumn	$t_6 = 0.40$, $p = 0.999$	$t_6 = 0.39$, $p = 0.994$	$t_6 = 0.46$, $p = 0.855$
Control, Spring	$t_6 = 0.44$, $p = 0.973$	$t_6 = 0.67$, $p = 0.575$	$t_6 = 0.51$, $p = 0.881$
Autumn, Spring	$t_6 = 0.40$, $p = 0.939$	$t_6 = 0.54$, $p = 0.700$	$t_6 = 0.39$, $p = 0.995$
Post-spring fire (June 14)			
Control, Spring	$t_{11} = 1.52$, $p = \mathit{0.024}$	$t_{11} = 1.13$, $p = 0.214$	$t_{11} = \mathit{1.59}$, $p = \mathit{0.020}$
Post-Autumn fire (October 14)			
Control, Autumn	$t_6 = 1.67$, $p = \mathit{0.006}$	$t_6 = 1.53$, $p = \mathit{0.007}$	$t_6 = 1.40$, $p = \mathit{0.025}$
Control, Spring	$t_6 = 1.04$, $p = 0.409$	$t_6 = 1.08$, $p = 0.378$	$t_6 = 1.04$, $p = 0.322$
Autumn, Spring	$t_6 = 1.24$, $p = \mathit{0.058}$	$t_6 = 1.00$, $p = 0.499$	$t_6 = 1.07$, $p = 0.315$
Post-fires (June 15)			
Control, Autumn	$t_6 = 1.39$, $p = \mathit{0.031}$	$t_6 = 0.98$, $p = 0.551$	$t_6 = 1.21$, $p = 0.096$
Control, Spring	$t_6 = 1.25$, $p = \mathit{0.058}$	$t_6 = 0.98$, $p = 0.590$	$t_6 = 1.27$, $p = 0.063$
Autumn, Spring	$t_6 = 1.02$, $p = 0.406$	$t_6 = 0.92$, $p = 0.672$	$t_6 = 1.03$, $p = 0.365$

However, after the late-dry season (autumn) burns (i.e., October 2014), these differences disappeared ($t_{6.14} = 1.04$, $p = 0.409$), probably due to the large difference in community composition between the control and recently burned autumn plots ($t_{6.14} = 1.67$, $p = 0.006$; $t_{6.14} = 1.53$, $p = 0.007$; and $t_{6.14} = 1.40$, $p = 0.025$, for the entire, EM and saprotrophic fungal communities, respectively). This strong effect of late-dry season burns on the entire soil fungal community translated into nearly significant differences between the autumn and spring (early-dry season) burned plots ($t_{6.05} = 1.24$, $p = 0.058$). In the post-fires sampling period (i.e., June 2015), we observed a significant difference between the control and late-dry season (autumn) burned plots ($t_{6.06} = 1.39$, $p = 0.031$), but only when examining the entire fungal community. In addition, there was a minor, nearly significant difference between the control and early-dry season (spring) burned plots ($t_{6.08} = 1.25$, $p = 0.058$), but not between autumn and spring burned plots ($t_{6.1} = 1.02$, $p = 0.406$).When 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; Fig. S6b). In particular, the soil fungal communities varied between the late-dry season (autumn) burned and unburned control plots (PERMANOVA pair-wise contrast: $t_{6.05} = 1.38$, $p = 0.030$; Table S14). SIMPER analysis (Fig. 2b) illustrated that numerous OTUs 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 OTUs) 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 ($t = 0.39$, $p = 0.415$). Early-dry season (spring) burns led to a reduction in the ratio of saprotrophic fungi, but this pattern was not significant ($t = -0.64$, $p = 0.520$). Late-dry season (autumn) burns led to a significant reduction in the ratio of saprotrophic fungi compared with that of unburned control plots ($t = 3.12$, $p = 0.002$), and this reduction resulted in a significant difference between the early- and late-dry season burned plots ($t = 2.66$, $p = 0.008$). Approximately 1 year after the early-dry season (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 early- and late-dry season burned plots ($t = 2.14$, $p = 0.032$).

Greenhouse bioassay EMF community

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

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

Description of the bioassay EMF community

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

Discussion

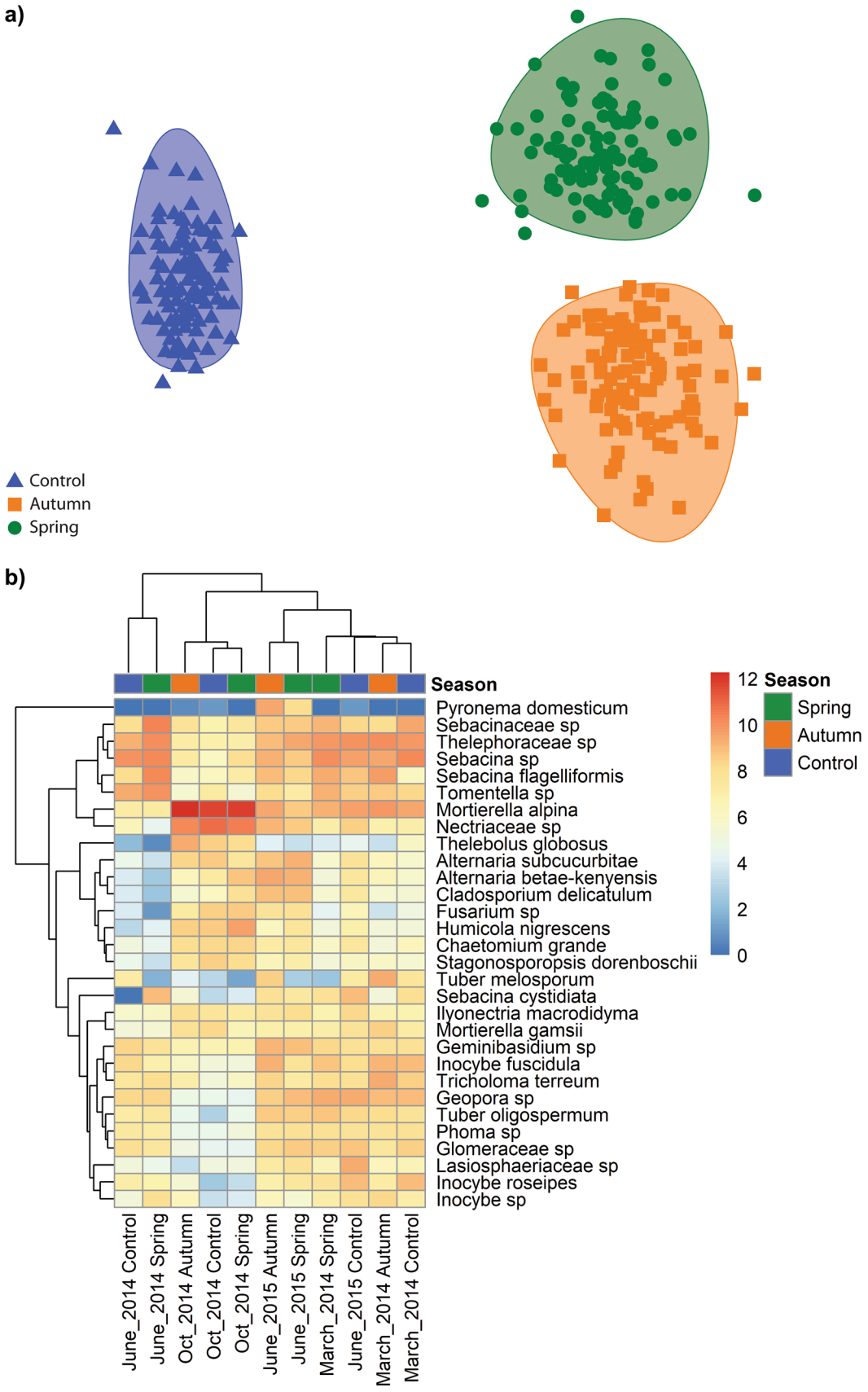
We report here the results of the first comprehensive field experiment quantifying the effects of seasonal fires on the soil fungal community in the eastern Mediterranean basin. Fire season caused differential effects on the community composition of soil fungi (Fig. 2), driven by alterations within the saprotrophic fungal community (Fig. 3), with the EMF community demonstrating high resilience.

Differential fire season effects on soil fungi

Prescribed burns are very different from wildfires in the sense that the environmental conditions required to authorize them by governmental/forest authorities are usually in total contradiction to the naturally occurring conditions characterizing the outburst of wildfires (Allen et al. 1968). While both fires require low precipitation and rather dry conditions, the first requires stable conditions with no wind, and wildfires usually increase in windy conditions. Wildfires in the eastern Mediterranean region evolve under different conditions during early- (spring) and late-dry season (autumn). Early-dry season (spring) wildfires occur during heatwaves characterized by high temperatures, low humidity, and strong winds, when both the fuel and soil moisture contents are high. In contrast, late-dry season (autumn) wildfires usually occur during similar or even more extreme heatwaves, when both the fuel and soil moisture contents are low. Owing to the constraints associated with executing prescribed burns, the environmental conditions during our seasonal burns were very similar (Fig. S1). Yet they led to differential modifications in the soil fungal community. Specifically, early-dry season (spring) burns caused reductions in soil OTU richness and diversity. But ~ 1 year after the burns, these differences disappeared. Moreover, fire season induced changes in the community composition of soil fungi were mostly driven by alterations within the saprotrophic fungal guild (Figs. 2 and 3). Examining fire-related temporal changes among the most abundant (top 30) fungal taxa (Fig. 2b) indicated that EMF taxa, including *Geopora*, *Tuber*, *Sebacinaceae*, and *Tomentella* spp., were hardly affected by our prescribed burns. The most abundant fungal taxa detected in our soil samples pertained to the *Thelephoraceae* family, but we failed to further identify it to the genus or species levels. Consequently, we had no other choice but to classify it as either EMF or saprotrophs. Furthermore, the abundance of this *Thelephoraceae* sp. varied over time, irrespective of the burnings. Notably, *Pyronema domesticum* was the only species to exhibit increased abundance in the burned plots. *Pyronema* spp. are known for their pyrophilic characteristics and often occur in burned sites (Bruns et al. 2020).

Differential fire season effects observed 1 year after the burns (Fig. 2a) were mainly driven by a large number of OTUs, characterized as unidentified saprotrophs (each contributing < ~ 1% of the dissimilarity among fire treatments, see SIMPER results in the "Results" section), the most abundant saprotrophs were mostly unaffected by fire season (Fig. 2b). This is an important point, since while each one of this less abundant species has a minor contribution to the general pattern, their cumulative contribution within the entire functional guild might have greater consequences for ecosystem functioning.

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



◂**Fig. 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** Heatmap of community composition at the putative fungal species level, for the most abundant (i.e., top 30) species, by sampling dates. Dendrograms are constructed with Bray-Curtis distances. The highest and lowest relative abundance of putative species are in color gradient. The vertical annotation colors denote fire season treatments

Experiencing no significant change due to disturbance (i.e., resistance) and 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 California's conifer forests (Glassman et al. 2016), and in various conifer wildfires in the west-Mediterranean basin (Buscardo et al. 2012, 2015, 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, late-dry season (autumn) burns affected EMF community composition, but these differences between the control and autumn (late-dry season) burned plots faded quickly and had 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. 4), 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. (2016) 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 suggests that fires occurring at different seasons are impacting different pre-fire fungal communities.

Furthermore, EM and saprotrophic fungi compete for limited 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 (Figs. 2b; 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 ± 1SE; Fig. S3). Conversely, 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 the late-dry season (autumn) burns (25.00 ± 0.91 before autumn vs. 17.35 ± 1.64 before spring burns, mean ± 1SE). Even though soil moisture was only slightly lower during late- (autumn; 3.3 ± 0.22, mean ± 1SE) than during early-dry season burns (spring; 7.69 ± 0.39, mean ± 1SE), plant water content during autumn burns (0.18 ± 0.01) was half of that measured during spring burns (0.28 ± 0.02, mean ± 1SE). This suggests that both temperature and precipitation can influence water availability, thus playing an important role in shaping fungal communities in the semi-arid Mediterranean ecosystem.

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

In the eastern Mediterranean ecosystem, *Cistus* shrubs are often the main EMF hosts at early successional stages, followed by later successional species such as *Pinus halepensis*. Our data represent the first comprehensive description of the EMF community associated with the understudied *Cistus*-dominated eastern Mediterranean ecosystem. We hypothesized that *P. halepensis* colonization should be facilitated by the

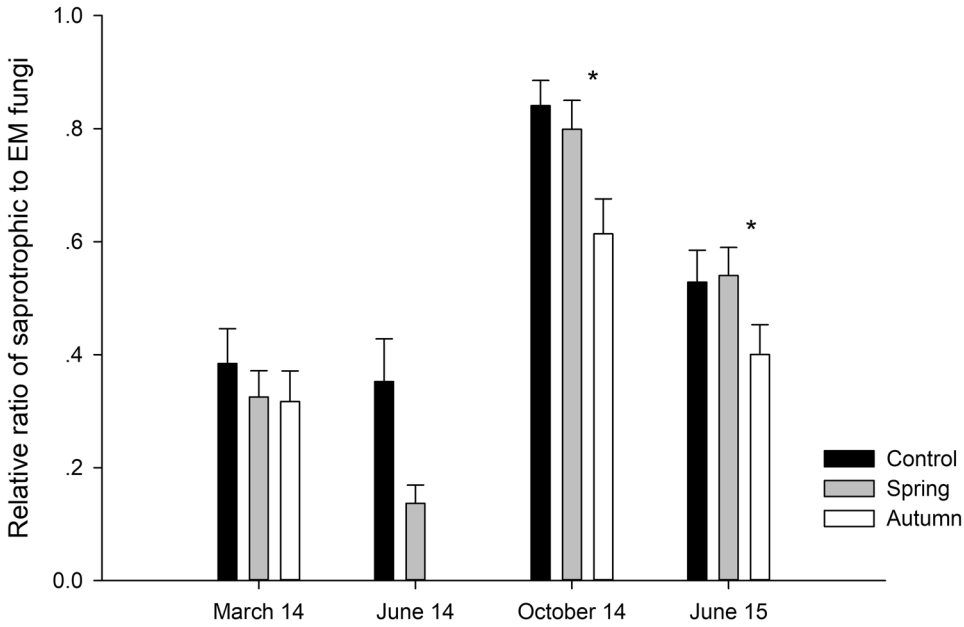


Fig. 3 The ratio between OTUs identified as putative saprotrophic to putative EM fungi out of all OTUs identified ((saprotrophic/EMF)/total OTUs in each sample. Asterisks denote significant differences ($p < 0.05$) between the early- (spring) and late-dry—(autumn) season burns within sampling periods

EMF community characterizing *Cistus*. We observed a high abundance of *Tuber oligospermum* associating with pines grown on soils collected underneath *Cistus* shrubs (Fig. 4), suggesting a newly described link between *T. oligospermum*, *P. halepensis* and the local *Cistus* shrubs (*C. salviifolius*, *C. creticus*). This finding is in agreement with other studies of *Cistus* dominated ecosystems in the western Mediterranean basin (Comandini et al. 2006), describing the association between *Cistus* and various *Tuber* species. Similarly, but in a conifer-dominated ecosystem, Buscardo et al. (2012) and Glassman et al. (2016) found *Rhizopogon* (a Pinaceae specific false truffle) increasing in abundance after fire, possibly indicating a pre-adaptation of these hypogeous fungi to fire survival.

Mycorrhizal facilitations (i.e., facilitation of plants mediated by mycorrhizal symbiosis) can be mediated via three non-mutually exclusive pathways: (i) increased spore density—resident plants can increase spore density of mycorrhizal fungi in their vicinity, thereby improving the inoculation chances of adjacent germinating seedlings (Peay et al. 2012; Teste et al. 2009a, 2009b), (ii) increased hyphal density—the hyphal density of mycorrhizal fungi in the vicinity of living mature plants is higher, therefore increasing the probability of nearby seedlings to become inoculated (Fleming 1983), and lastly by (iii) support via common mycorrhizal networks (CMN)—the existing hyphae of mycorrhizal fungi not only inoculate seedlings but can also create a CMN that results in the net movement of carbon from mature plants to seedlings (Beiler et al. 2015; Booth 2004; van der Heijden and Horton 2009). While our data does not necessarily exclude the last two pathways, it does show increased spore density of *T. oligospermum* on pines, growing where there used to be *Cistus* shrubs. This may suggest that interspecific mycorrhizal facilitation is possible between *Cistus* and pine seedlings. Such facilitation processes can play an important role in shaping plant community dynamics, vegetation structure, and ecosystem functioning (Hayward et al. 2015; Horton et al. 1999). However, the mechanisms governing such facilitation processes are yet to be unravelled.

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

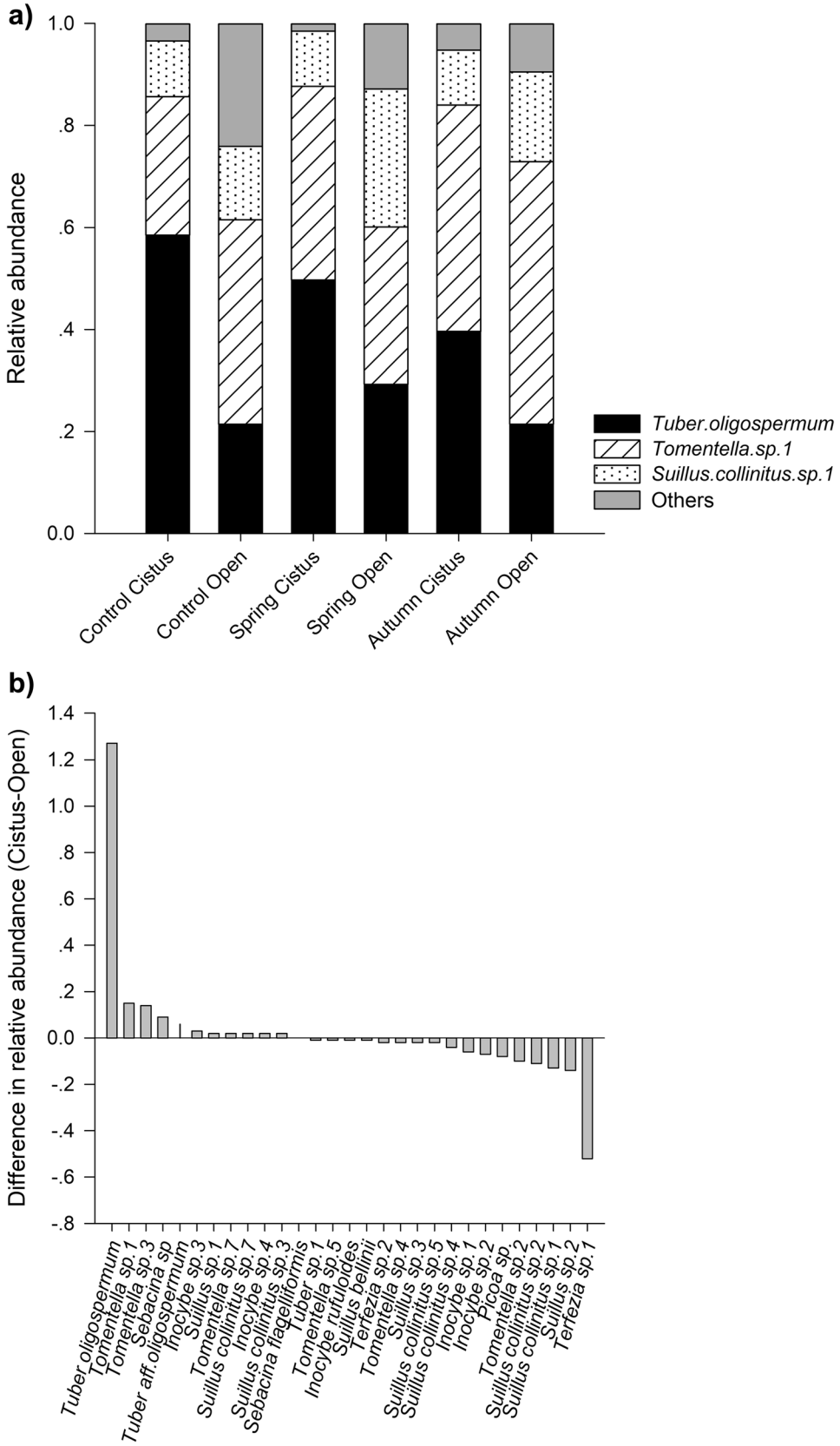


Fig. 4 **a** Relative sequence abundance of the OTUs representing root-associated EMF species in each of the six treatment combinations of fire treatment and microhabitat. **b** Differences in the relative read abundance of the various OTUs representing root-associated EMF species between the two microhabitats (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

Conclusions

We observed the largest differences due to fire season in the total soil fungal community, rather than in the EMF community, and this effect was largely driven by changes within the saprotrophic fungal guild. Most data on fungal response to fire comes from northwestern USA (Taudière et al. 2017), where fires are typically of higher severity than in the eastern Mediterranean basin. Such fires, as those attained in our study, are less likely to lead to host dieback, or to inflict direct damage to soil microorganisms. Therefore, Mediterranean fires might induce different selection pressure on the soil biota. Even though the EMF community appeared to be resilient to fire, saprotrophic and EM fungi were documented to compete over similar niche requirements in many ecosystems (Leake et al. 2002). Therefore, these changes in the saprotrophic community composition might have an additional indirect effect on the EMF community composition. Since small-scale changes in carbon inputs can cascade to affect decomposition rates and carbon emissions (Hawlena et al. 2012; Schmitz et al. 2014), these small yet distinct differences in the soil fungal community composition can further affect ecosystem functioning.

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

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Data availability Sequences were submitted to the National Center for Biotechnology Information Sequence Read Archive under accession numbers SAMN17278264 - 17278556.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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