



Richness, community structure and distance decay of soil fungi along a sharp aridity gradient in monodominant pine forests

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Abstract

Background and aims Understanding how aridity shapes fungal communities is essential for predicting ecosystem responses to climate change. Monodominant forests of Aleppo pine (*Pinus halepensis*) occurring along a steep precipitation gradient, offer the opportunity to test the effect of aridity on distance decay patterns of soil fungi, without the confounding effects of vegetation.

Methods We conducted nested soil sampling in four Mediterranean, two semi-arid and three arid forests along an aridity gradient (250–800 mm annual precipitation) and examined distance decay patterns of

saprotrophic (SAP) and ectomycorrhizal (ECM) fungi at different spatial scales.

Results ITS2 soil metabarcoding revealed that both fungal richness and diversity increased with precipitation. Fungal communities showed significant spatial autocorrelation at multiple scales, with stronger distance decay patterns in Mediterranean than arid forests. ECM and SAP communities in arid sites were largely subsets of the Mediterranean climate communities. Stochastic assembly processes dominated under mesic conditions, while deterministic processes prevailed in arid regions, particularly for ECM fungi.

Conclusions Our results suggest that aridity can reduce fungal richness and stochasticity in community assembly, and that climate can structure fungal communities independently of vegetation. This study highlights the need to consider scale-dependent ecological processes and emphasizes the role of climate, beyond vegetation, in shaping fungal community assembly in forest soils.

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Introduction

Many natural communities display a distance decay of similarity, with spatially closer communities being more similar to each other compared with communities further away (Ettema and Wardle 2002). Such spatial structure can vary across scales, from a few centimeters to thousands of kilometers (Bahram et al. 2013) as well as between different organismal groups (Nekola and White 1999; Soininen et al. 2007). The extent of such spatial structure is expected to depend on both intrinsic characteristics of the organisms, such as dispersal ability (Gómez-Rodríguez and Baselga 2018) and niche breadth (Nekola and White 1999), as well as extrinsic factors characterizing the environment such as habitat heterogeneity (Kim et al. 2021) and disturbance (Grogan et al. 2000; van der Gast et al. 2011; Pec and Cahill Jr 2019). Understanding the spatial structure of natural communities is critical to improve our understanding of species coexistence (Levin and Paine 1974) and population persistence (Ellner et al. 2001).

While the spatial structure of conspicuous and large organisms has been relatively well studied, processes driving those patterns in cryptic species are not well-understood. Along with the development of molecular methods, such as metabarcoding for species detection, the spatial structure of soil fungi in general and of ectomycorrhizal (ECM) fungi in particular has received increasing attention in recent years. Many of these studies sampled soil at different spatial intervals to estimate the distance decay of similarity based on variation in community composition among samples (Bahram et al. 2013). As a result, ECM fungal communities often showed patterns of spatial autocorrelation both horizontally (Peter et al. 2001; Lilleskov et al. 2004; Carriconde et al. 2008; Pickles et al. 2012; Toju et al. 2014) and vertically (Kadowaki et al. 2014; Bahram et al. 2015).

The spatial structure of fungal communities is expected to result from various extrinsic ecological features of the environment and intrinsic characteristics of the fungi themselves. Spatial heterogeneity is a key driver of spatial autocorrelation that, in the case

of fungi, can originate from changes in soil characteristics (Kivlin and Hawkes 2016), host patchiness in the case of mutualists (Ishida et al. 2007; Teder-soo et al. 2008) and pathogenic fungi (Packer and Clay 2000), and organic matter composition in the case of saprotrophic (SAP) fungi (Huang et al. 2021). For spatial heterogeneity to create spatial structure in fungal communities, some level of variability in habitat specificity must exist between different fungal taxa. However, even in homogeneous environments, spatial structure can still be formed if species differ in their dispersal distances (Peay and Bruns 2014), and/or vectors (Golan and Pringle 2017), propagule or hyphae longevity (Shemesh et al. 2023) or hyphal growth patterns (Dahlberg 1997).

While many of the above mentioned studies looked at the distance decay pattern in soil fungi, other studies examined how fungal communities change along gradients of environmental factors or use proxies such as elevation (Bahram et al. 2012; Rincón et al. 2015; Matsuoka et al. 2016; Truong et al. 2019), nutrients (Lilleskov et al. 2002; Toljander et al. 2006) and climate (Ostonen et al. 2011; Scattolin et al. 2014; Erlandson et al. 2016; Pellitier et al. 2024). Overall, fungal richness was often found to decrease with increasing stress levels (Maestre et al. 2015; Neilson et al. 2017).

Although there is significant knowledge about how fungal communities change along environmental gradients and there is some knowledge about their distance decay patterns, there are only two studies that examined how distance decay patterns change under different environmental conditions. To do so, data must be collected and analyzed at different spatial scales, examining the distance decay patterns in habitats that differ in some environmental characteristic. In their meta-analysis, Bahram et al (2013) looked at distance decay patterns across environments at global scale and showed that distance decay patterns were stronger in tropical habitats. The authors suggested that because ECM hosts make up a relatively small fraction of the trees in tropical forests, the fragmentation of ECM communities led to stronger distance decay patterns (Bahram et al. 2013). Furthermore, distance decay patterns were stronger for both soil fungi in general as well as arbuscular mycorrhizal fungi (AMF), in contaminated sites of the taiga forest (Mikryukov et al. 2015).

Water availability is a strong determinant of plant (Kreft and Jetz 2007) and fungal (Tedersoo et al. 2012) communities. Generally, water scarcity was found to reduce fungal richness (Maestre et al. 2015; Jiao et al. 2021; Wang et al. 2021, 2024; Ding and Eldridge 2022; Dong et al. 2024). However, its effect on distance decay patterns was, to the best of our knowledge, never tested. While it is reasonable to assume that aridity is likely to affect the above-mentioned intrinsic characteristics and extrinsic patterns involved in the distance decay pattern, the extent and even the directions of these effects are practically unknown. On the one hand, aridity is often associated with spatial heterogeneity in water availability (Tongway and Ludwig 1994) that could drive strong distance decay patterns. On the other hand, aridity could implement strong environmental filtering that could result in a relatively fixed fungal community, therefore reducing distance decay patterns. The manner by which intrinsic factors such as spore dispersal ability and longevity are affected by aridity are also unknown. Therefore, characterizing the distance decay patterns of soil fungi along such gradients is an important landmark on the way to testing the role of these different intrinsic and extrinsic characteristics in spatially structuring fungal communities. Understanding these processes increases our ability to predict how fungal community structure changes along environmental gradients and in future climates.

The monodominant forests of Aleppo pine (*Pinus halepensis*) that occur along Israel's steep precipitation gradient enabled us to test the effect of climate on distance decay patterns of soil fungi, while reducing the confounding effects of vegetation. Using soil ITS2 metabarcoding, we conducted nested soil sampling in four Mediterranean forests (500–800 mm of annual precipitation), two semi-arid forests at the aridity limit of pine seedling establishment (~400 mm) and three arid forests where no significant seedling establishment takes place (~300 mm). We then examined the distance decay patterns of both SAP and ECM fungi at different spatial scales. We hypothesized that the Mediterranean climate forests would contain higher fungal richness due to the milder conditions. These more benign conditions should increase the role of stochastic processes in community assembly,

resulting in stronger distance decay in these mesic habitats. In the case of ECM fungi, the greater richness of ECM hosts (like *Quercus* and *Cistus*) in adjacent habitats in the Mediterranean climatic region (Danin 1988) should further strengthen this pattern.

Methods

To examine the distance decay patterns of soil fungi along an aridity gradient, we surveyed nine coniferous forests along a 200-km transect of Israel's precipitation gradient (Fig. 1A–B) during the rainy season of 2022. All forests were planted approximately 70–50 years ago and are dominated by mature Aleppo pine (*Pinus halepensis*). The forest understory of the forest plots was strongly dominated AMF associated annuals and shrubs. Despite pines being the dominant ECM host in the plots, the understory contained a few numbers of individuals of other ECM hosts (*Quercus* or *Cistus* shrubs in the mesic and semi-arid plots or *Helianthemum* in the arid plots). Therefore, we recorded the number of other ECM hosts in each of the plots. In each of the nine forests, two plots were sampled, for a total of 18 sampled plots. The distance between plots was 0.5–3 km apart. Geographical, climatic and vegetation characteristics of each plot are reported in Table 1. For each plot, we defined the regeneration status at an ordinal scale from “none” for plot with no pine seedlings to “very high” for plots containing dozens of young seedlings. To characterize long-term vegetation greenness and seasonal variability at each plot, we derived a 10-year time series of the red-edge-based MERIS Terrestrial Chlorophyll Index (MTCI) from Sentinel-2A L2A surface reflectance (and VENuS where available), following Dubinin et al. (2023). Then, we calculated the 10-year mean MTCI and its temporal coefficient of variation ($CV = SD/mean$) across monthly composites.

Sampling design and soil collection

Sampling followed a hierarchical structural design of four nested squares emerging from the same vertex (Talbot et al. 2014). All plots had a uniform grid size of 0.16 ha, each consisting of four nested squares with a size ratio of 1:4:16:64 (Fig. 1A). The design of this grid contained 13 different vertices from which

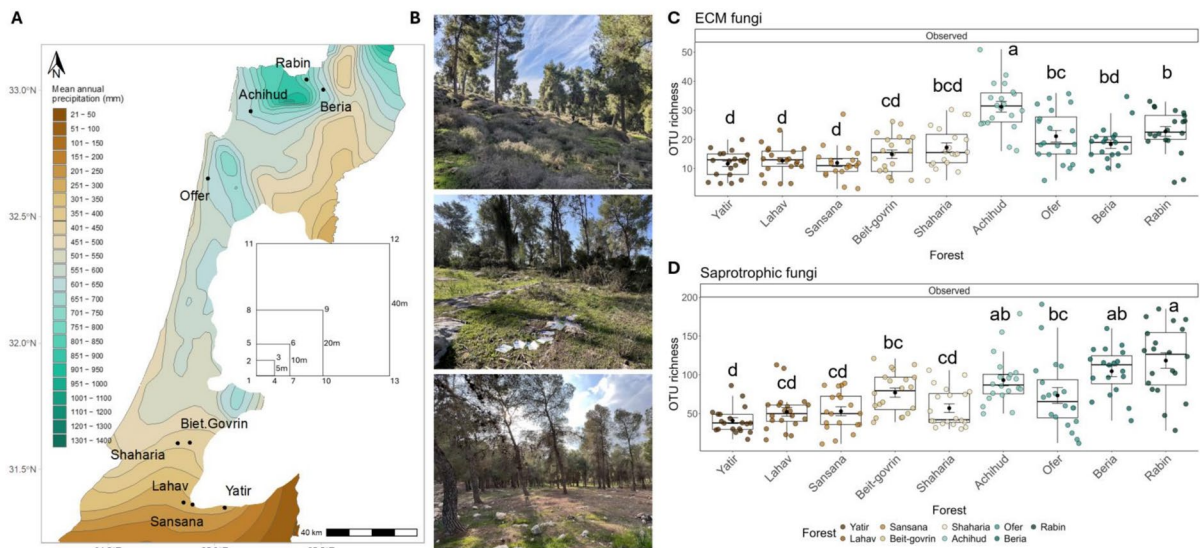


Fig. 1 **A)** Map of the mean annual precipitation gradient in Israel, based on data from 1981–2010, showing the locations of sampling sites (marked with purple stars). The sampling scheme is a hierarchical nested design with 13 points per plot. Each sampling site includes two 40×40 m plots, approximately 2 km apart. **B)** Three representative forest plots: Beria (~680 mm), Beit-Govrin (~400 mm) and Yatir (~280 mm). **C)**

ECM fungi and **D)** Saprotrrophic fungi observed OTU richness across nine different forests along the precipitation gradient. Each forest is denoted by a unique color ranging from brown to green according to the mean annual precipitation. The box plots display the median, interquartile range, and individual data points for each forest. Letters denote significant differences according to the Tukey-HSD test $p < 0.05$

soil samples were collected. The distance between each sampled point and the closest mature pine tree was documented, as well as the tree's Diameter at Breast Height (DBH) (Tabel 1).

Prior to sampling, dry foliage and debris were removed from the soil surface and a sample was collected by digging up soil to 10 cm depth in a 10 cm diameter. The 10 cm depth soil sampling represented well the shallow soil profiles at our sites. For example, in a forest site bordering the Beit Govrin and Shaharia forest sites (15 km NE of them and at similar elevation), soil depth was 10–25 cm (Jakoby et al. 2020). Within this layer, Aleppo pine and accompanying tree species accounted for 85–90% of the roots that were identified with DNA barcoding (Jakoby et al. 2020). Shrub and herb species had only a minor share of the forest biomass. For example, in Yatir forest we quantified the aboveground biomass, with 0.1–1.1% belonging to shrubs and herbs, and the rest to the pine trees (Livne-Luzon et al. 2025).

A small subsample was stored in a 1.5 ml Eppendorf tube and kept frozen in an ice cooler until it reached the laboratory, where it was freeze-dried and stored at -80°C . All the tools used in the

process were sterilized using 70% ethanol to prevent contamination.

Soil chemical analysis

Soil samples collected from each sampling point within the two plots of each of the nine forests ($n=18$) were homogenized to create a composite sample per plot. The following chemical analyses were conducted for each composite sample, as reported in Table 2. A soil sample (8 g) was transferred into a 50 ml tube, then mixed with 32 ml of deionized distilled water to create a soil–water suspension. The tubes were placed horizontally on a shaker at 110 rpm and shaken overnight to promote nutrient extraction. The following morning, the tubes were allowed to stand vertically for 30 min to enable sedimentation of larger particles. Subsequently, 10 ml of the supernatant was carefully extracted and filtered through a $0.22\ \mu\text{m}$ filter, with the filtered solution transferred to 15 ml tubes. The following measurement were taken from the filtered solution. pH measurements were conducted using a pH meter (VWR phenomenal, PH 1100 L, UK). Electrical

Table 1 Characteristics of the 18 study plots across nine mono-specific pine forests along the climatic gradient in Israel

Forest	Plot	Climatic region	Latitude	Longitude	Elevation	Mean annual precipitation (mm)	Mean DBH (cm)	Mean pine distance to nearest pine (m)	Number of mature pines	Regeneration status	Number of ECM host species	Shrub cover	Mean MTCI	MTCI coefficient of variation
Rabin	1	Mediterranean	33.04333	35.43056	710	715	81.3	3.8	44	High	2	0	0.445	0.436
Rabin	2	Mediterranean	33.04389	35.43222	719	715	132.0	2.8	26	High	2	0	0.431	0.478
Beria	1	Mediterranean	33.00306	35.50889	679	688	153.1	4.8	12	Low	2	2	0.340	0.762
Beria	2	Mediterranean	33.00417	35.47361	696	688	174.1	5.0	8	None	2	3	0.258	0.864
Ofer	1	Mediterranean	32.65111	34.96778	113	644	99.1	5.1	5	None	2	5	0.495	0.495
Ofer	2	Mediterranean	32.65111	34.975	144	644	85.0	7.0	8	Very High	1	2	0.270	0.501
Achihud	1	Mediterranean	32.91778	35.16833	84	622	94.9	3.0	54	Moderate	1	4	0.585	0.433
Achihud	2	Mediterranean	32.91833	35.18556	132	622	96.9	4.4	32	Moderate	1	3	0.373	0.581
Shaharia	1	Semi-Arid	31.60389	34.82611	196	425	93.7	3.2	23	Very High	1	2	0.352	0.757
Shaharia	2	Semi-Arid	31.60389	34.82972	217	425	94.9	2.6	32	Low	1	1	0.357	0.670
Beit-govrin	1	Semi-Arid	31.60611	34.88306	265	403	109.6	3.5	15	Very High	1	3	0.328	0.781
Beit-govrin	2	Semi-Arid	31.60528	34.87944	284	403	111.7	4.0	10	High	1	4	0.406	0.543
Sansana	1	Arid	31.36056	34.89583	510	302	86.4	2.3	50	None	1	0	0.245	0.815
Sansana	2	Arid	31.35972	34.89944	518	302	88.6	3.1	25	None	1	0	0.183	0.691
Lahav	1	Arid	31.36889	34.85333	474	302	79.3	2.9	33	Low	1	0	0.180	0.631
Lahav	2	Arid	31.37278	34.85194	486	302	72.3	2.7	27	Low	1	1	0.192	0.688
Yatir	1	Arid	31.34861	35.04667	650	280	77.1	3.8	25	None	1	0	0.157	0.801
Yatir	2	Arid	31.3425	35.04167	620	280	81.8	2.2	40	None	1	0	0.196	0.609

Table 2 Soil chemical properties of the 18 study plots across nine mono-specific pine forests along the climatic gradient in Israel

Forest	Plot	pH	EC (dS/m)	Cl (mg/kg soil)	N-NH ₄ (mg/kg soil)	N-NO ₃ (mg/kg soil)	PO ₃ (mg/kg soil)
Rabin	1	7.30	5.15	734.20	10.49	15.82	1630.40
Rabin	2	7.51	4.88	804.90	14.78	142.94	887.23
Beria	1	7.45	4.96	1029.86	18.32	166.96	1825.80
Beria	2	7.53	4.02	392.01	16.39	95.97	1259.86
Ofer	1	7.49	4.75	665.64	10.24	37.89	177.53
Ofer	2	7.76	2.96	856.46	7.06	54.36	116.32
Achihud	1	7.51	4.20	626.90	9.28	151.82	1525.45
Achihud	2	7.44	4.16	566.80	10.20	93.97	428.58
Shaharia	1	7.77	3.30	314.93	2.05	60.87	1310.09
Shaharia	2	7.66	3.69	986.77	3.02	28.83	304.48
Beit-govrin	1	7.67	3.81	700.56	5.56	13.08	642.29
Beit-govrin	2	7.52	4.22	374.22	8.64	37.18	483.88
Sansana	1	7.84	4.40	2281.50	5.45	54.75	284.61
Sansana	2	7.87	3.18	482.83	4.00	62.59	925.22
Lahav	1	7.71	3.97	1340.69	2.89	66.81	1309.55
Lahav	2	7.84	3.32	1219.98	6.07	52.41	889.17
Yatir	1	7.83	2.33	1411.31	1.26	29.77	104.55
Yatir	2	7.79	3.01	506.96	2.81	14.52	195.80

conductivity (EC) was measured using an EC Meter (Model 4510, Jenway, England; $n = 104$). Major anions, including chloride (Cl[−]) and nitrate (NO₃[−]), as well as cations such as ammonium (NH₄⁺), were analyzed using the Gallery Analyzer (Thermo Scientific, Germaring, Germany). The fully automated discrete analyzer provided consistent, high-quality results with minimal errors, ensuring reliable analytical data. Phosphorus (PO₄^{3−}) was analyzed separately from the original soil samples using the method described by Olsen (1954).

Molecular identification of fungal species

Fungal DNA was extracted from 0.25 g of each of the soil samples using the NucleoSpin® Soil kit (MACHEREY–NAGEL GmbH & Co. KG, Dueren, Germany) according to the manufacturer's protocol, all extractions were run in one batch. Extraction blank (no-soil) controls were included through DNA extraction and library prep/sequencing and processed alongside samples, which yielded approximately 50 ng/μl of DNA quantified using a NanoDrop spectrophotometer. Next, a two-step polymerase chain reaction (PCR) was performed to target the internal

transcribed spacer 2 (ITS2) region of the rRNA using the fungal-specific forward primers 5.8S-Fun (5'-AAGTTTYRRCAAYGGATCWCT-3'), and reverse primers with linker-primers RD2-ITS4-Fun (5'-AGA CGTTGCTCTTCCGATCT-AGCCTCCCTTATTGA TATGCT-3'). The reaction mixture contained 25 μl of KAPA HiFi Hot Start Ready-mix DNA polymerase (Hoffmann-La Roch, Basel, Switzerland), 2 μl of diluted DNA, 1 μl of each primer and 21 μl of ultrapure water. The thermocycling conditions were: initial denaturing at 98 °C for 2 min, 35 cycles at 98 °C for 10 s, 48 °C for 15 s, 72 °C for 35 s, and the final extension at 72 °C for 5 min. For the second PCR, we used adaptor-linker-forward primers P5-RD1-5.8S-FUN (5'-AATGATACGGCGACC ACCGAGATCT-ACACTCTTTCCCTACACGACG CTCTTCCGATCT- AAGTTTYRRCAAYGGATC WCT-3') and reverse linker-barcode primers RD2-Barcode (5'-AGACGTGTGCTCTTCCGATCT-BARCODE-3'). The reaction mixture contained 10 μl of 5×Phusion HS HF Buffer, 1 μl 10 mM dNTPs, 0.5 μl of Phusion DNA pol, 1 μl of each primer, 5 μl of the amplicon from the first PCR and 31.5 μl UPW. The thermocycling conditions were as the first PCR, but with 6 cycles instead of 35. Finally, all amplicons

were quantified using Qubit dsDNA HS kit (Life Technologies Inc., Gaithersburg, MD, USA), pooled at equimolar concentration and purified using Qiaquick PCR purification kit (Qiagen, Hilden, Germany). Amplicon size selection was performed using solid-phase reversible immobilization (SPRI) with Agencourt AMPure XP beads kit (Beckman Coulter Inc., Brea, CA, USA) following the manufacturer's instructions. High-throughput sequencing was carried out using Illumina MiSeq 2×300 bp (PE300_V3), at the Grand Israel National Center for Personalized Medicine (Weizmann institute of science, Rehovot, Israel).

Bioinformatics and data processing

In total, 234 soil samples were collected (13 per plot×2 plots per forest×9 forests). Due to sequencing capacity constraints, 178 samples were selected for sequencing to maintain balanced representation across all plots and forests. Raw ITS2 sequence data were denoised and chimera-filtered using LotuS2 v2.34 (Özkurt et al. 2022) with default parameters except for *-derepMin 10:1,5:2,3:3*. Sequences were clustered into operational taxonomic units (OTUs) at 97% similarity, as recommended for fungal metabarcoding of the non-coding ITS region (Tedersoo et al. 2022), using vsearch (Rognes et al. 2016) in LotuS2 and the taxonomic identity of OTUs was assigned using BLASTn (Camacho et al. 2009) against UNITE v10.0 (Abarenkov et al. 2024). Putative contaminants were filtered from the OTU table using the *decontam* package (Davis et al. 2018) with default parameters in R v4.3.3 (R Core Team, 2024). OTUs with less than 0.01% of read counts per sample and samples with less than 1000 reads were removed. These low-read samples were distributed across different climatic regions and did not cluster within a specific region or treatment group; thus, their exclusion is not expected to introduce bias in downstream analyses. To account for differences in sequencing depth, OTU read counts were normalised into relative abundance per sample before performing downstream analyses (McMurdie and Holmes 2014; Tedersoo et al. 2022). Functional guilds were assigned to OTUs at generic level using FungalTraits (Pölme et al. 2020), based on “primary_lifestyle”. Downstream analyses were performed using R version 4.4.2 (R Studio Team 2020; R Core Team 2024).

Statistical analysis

Richness and diversity

Fungal richness and diversity (Observed richness and Shannon index) were calculated using *plot_richness* from the *phyloseq* package (McMurdie and Holmes 2013). Relationships with precipitation were assessed using generalized (family = poisson) and linear mixed-effects models (*glmer* and *lmer* from *lme4* (Bates et al. 2015)), with nested random effects for Forest, Plot, and Point.

Mantel correlograms and spatial structure

To assess the spatial structure of fungal communities, we calculated Mantel correlograms using the *mantel.correlog* function from the *vegan* package (Oksanen et al. 2001). Community dissimilarities were based on the Jaccard index (*vegdist*) based on presence absence data, and geographic distances were computed using the Haversine formula (*dism*, *geosphere* (Bergman et al. 2025)). Analyses were conducted both on the full dataset and separately by climatic region, with significance assessed via 999 permutations and Holm corrections for multiple testing. Data processing and plotting were performed using *phyloseq*, *dplyr* (Wickham 2015), and *ggplot2* (Wickham 2016). We used a general linear model (GLM) to test the effects of guild identity (ECM vs. SAP), the mean annual precipitation, and their interaction on Mantel correlation coefficients (*r*) across spatial scales (region, forest, and plot). To account for small sample sizes and avoid inflated Type I error, we assessed the significance of each model term via permutation tests (*n* = 999), shuffling the ECM/SAP labels while preserving precipitation values.

RDA, PERMANOVA, and β -dispersion

Spatial structure was modeled using distance-based Moran's eigenvector maps (dbMEM) from the *adespatial* package (Dray et al. 2018). Significant eigenvectors (based on Moran's I with 999 permutations via *spdep*) and environmental predictors (all the parameters from Tables 1,2: e.g., precipitation, vegetation metrics, pH, nutrients) were included in redundancy analyses (RDA; *rda*, *vegan*). Community

composition differences among regions and forests were evaluated using PERMANOVA (using 999 permutations, *adonis2*, *vegan*) on Jaccard dissimilarities (presence-absence). Similar qualitative results were obtained using relative abundance data and Bray–Curtis dissimilarities. β -dispersion was assessed with *betadisper* and tested using ANOVA and Bonferroni-corrected pairwise t-tests. All dissimilarities were calculated using *phyloseq::distance*.

OTU overlap and community assembly

Euler diagrams (*euler*, *eulerr* (Larsson et al. 2024)) were used to visualize fungal OTU overlap among climatic regions. We tested whether observed OTU overlaps between regions exceeded chance expectations using Fisher's exact tests on 2×2 contingency tables built from each pair's overlap, uniques, and the OTU universe (union across regions), with a one-sided ("greater") alternative. P-values were FDR corrected across pairs, and we report effect sizes (Jaccard index, odds ratio) alongside adjusted significance. Community assembly processes were inferred using the Taxonomic Normalized Stochasticity Ratio (tNST, NST (Ning et al. 2019)), with 1000 randomizations and Jaccard dissimilarity, focusing on regional-scale variation. Because ITS2 lacks a robust phylogeny, instead of β NTI we complemented tNST with a phylogeny-free, incidence-based Raup–Crick null model (*vegan::raupcrick*, 999 randomizations). This tests whether between-sample dissimilarity is lower or higher than expected by chance while preserving each sample's richness and each OTU's overall frequency; we report RC scaled to $[-1, 1]$, with $RC < -0.95$ = strong homogenization, $RC > +0.95$ = strong dispersal limitation, and intermediate values indicating drift/weak selection (Stegen et al. 2013).

Differential abundance analysis

Differential abundance of ECM fungal taxa at the genus level were tested with ANCOM-BC (*ancombc*, ANCOMBC (Lin and Peddada 2020); no random effects included) using a two-level climate factor (Mediterranean vs. Semi-arid + Arid); low-depth samples were filtered at 1,000 reads. Results were visualized using a bar plot of log-fold changes, highlighting top taxa showing significant differences. Multiple

testing was controlled using Benjamini–Hochberg FDR (q-values). To remain consistent with our hypothesis-driven approach, differential abundance analyses were conducted only for EMF taxa, as we did not have a priori hypotheses regarding climate-related shifts in specific SAP taxa (for SAP community composition, see Fig. S5).

Results

Richness of saprotrophic and ECM fungi along the precipitation gradient

Overall, the richness (Fig. 1C,D) and Shannon diversity index (Fig. S1) of both SAP and ECM fungi increased along the precipitation gradient with SAP displaying higher richness than ECM in all regions. The most arid forest (Yatir) showed the lowest richness (11 ECM and 40 saprotroph OTUs, respectively; Fig. S1), while the lowest α -diversity was recorded in Sansana and Lahav forests in the arid region for both ECM and SAP fungi. In the Mediterranean climatic region, Achihud forest showed the highest ECM richness (31 OTUs) and α -diversity. The forest with the highest precipitation (Rabin) had the highest richness of SAP fungi (118 OTUs), although it was not significantly more diverse than the other Mediterranean climate forests (Fig. S1).

Spatial autocorrelation of saprotrophic and ECM communities

Overall, a significant distance decay pattern of both ECM and SAP fungal communities was observed along the aridity gradient (Fig. 2A).

Mantel correlograms (Fig. 2B) show that samples were more similar than expected by chance within plots and forests, and more dissimilar than expected by chance between forests for both ECM and SAP fungi. This pattern was maintained when analysing the data of the different regions separately (Fig. S2). At the regional scale, SAP fungi displayed a stronger distance decay than ECM fungi (Guild: $p = 0.047$, Fig. 2C). At the forest scale, distance decay of SAP and ECM fungi was positively correlated with precipitation (Precipitation: $p = 0.010$, Fig. 2D), while at the plot scale, no clear pattern of autocorrelation was identified (Table S1, Fig. 2E).

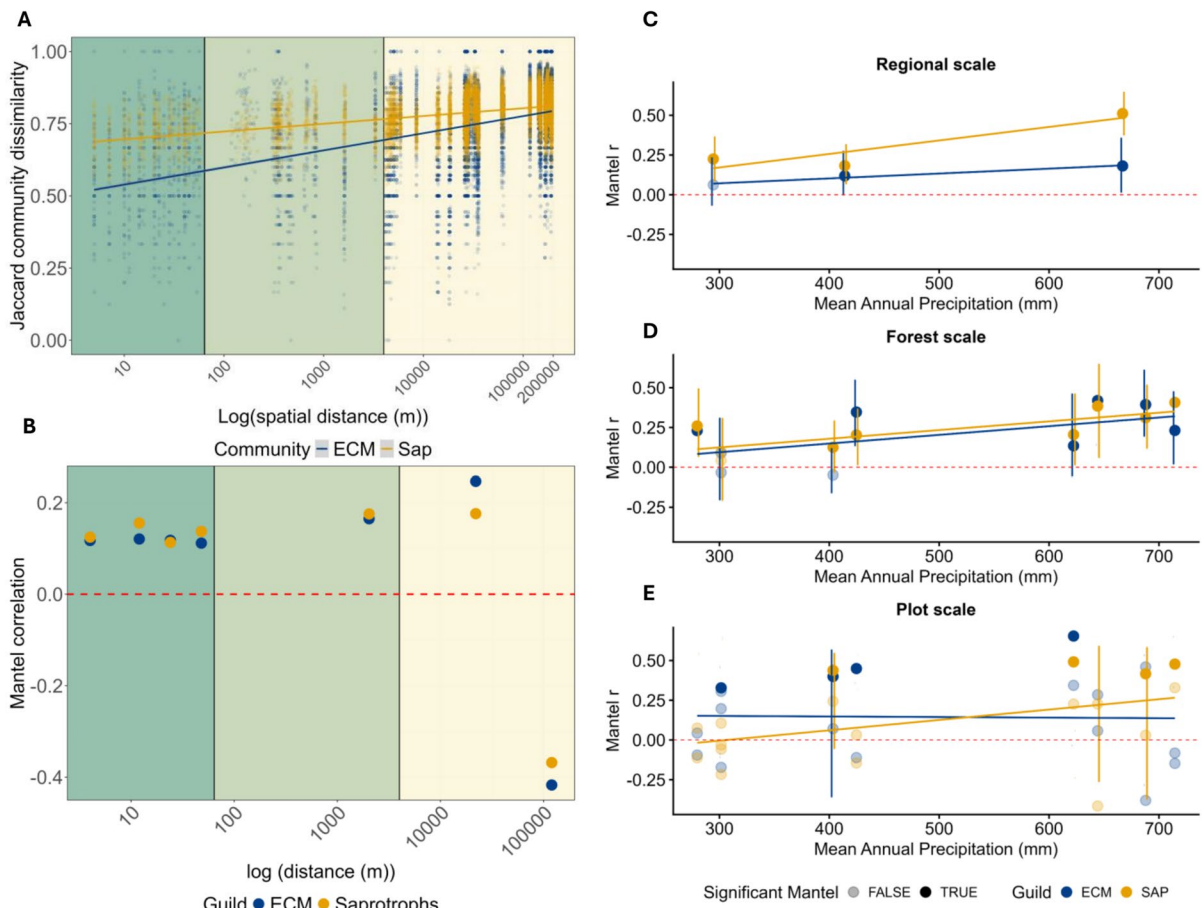


Fig. 2 **A**) Mantel test of fungal Jaccard community dissimilarity against log-transformed spatial distance (m). There is a significant effect of distance decay along the climatic gradient for ECM (Mantel $r=0.29$, $P<0.001$) and Saprotophths (Mantel $r=0.14$, $P<0.001$). **B**) Mantel correlograms for ECM and Saprotophths (different color lines) show that samples are more similar than expected by chance within 100 km, and more dissimilar than expected by chance at scales above 100 km. Solid

points indicate significance at $P<0.05$. Panels **C**, **D**, and **E** display the relationships between Mantel r and Mean Annual Precipitation across different spatial scales. In each panel, the points represent the observed Mantel r values for ECM (blue) and SAP (orange) taxa, with the solid lines showing linear fits. Error bars correspond to the 95% bootstrap-permutation confidence intervals. The dashed red line represents no correlation (Mantel $r=0$)

Fungal community variability along the precipitation gradient

Multivariate analyses showed that fungal community composition differed significantly among forest sites and across climatic regions for both ECM and saprotrophic fungi. PERMANOVA indicated significant differences among forests for ECM (Forest: $F_{8,162}=8.28$, $p<0.001$, $R^2=0.30$) and saprotrophic communities (Forest: $F_{8,162}=4.42$, $p<0.001$, $R^2=0.18$; Fig. 3A). Similar patterns were observed at the regional scale, where climate explained significant

compositional variation in both ECM (Climate: $F_{2,162}=19.75$, $p<0.001$, $R^2=0.19$) and saprotrophic fungi (Climate: $F_{2,162}=6.51$, $p<0.001$, $R^2=0.07$).

Redundancy analysis further identified the main environmental gradients associated with these compositional differences. For ECM fungi, the RDA explained 24.5% of the total community variation (adjusted $R^2=0.245$; permutation test $p=0.001$), with RDA1 and RDA2 accounting for 17.3% and 3.9% of the constrained variance, respectively. ECM community variation along RDA1 was primarily associated with precipitation, distance to pine, and

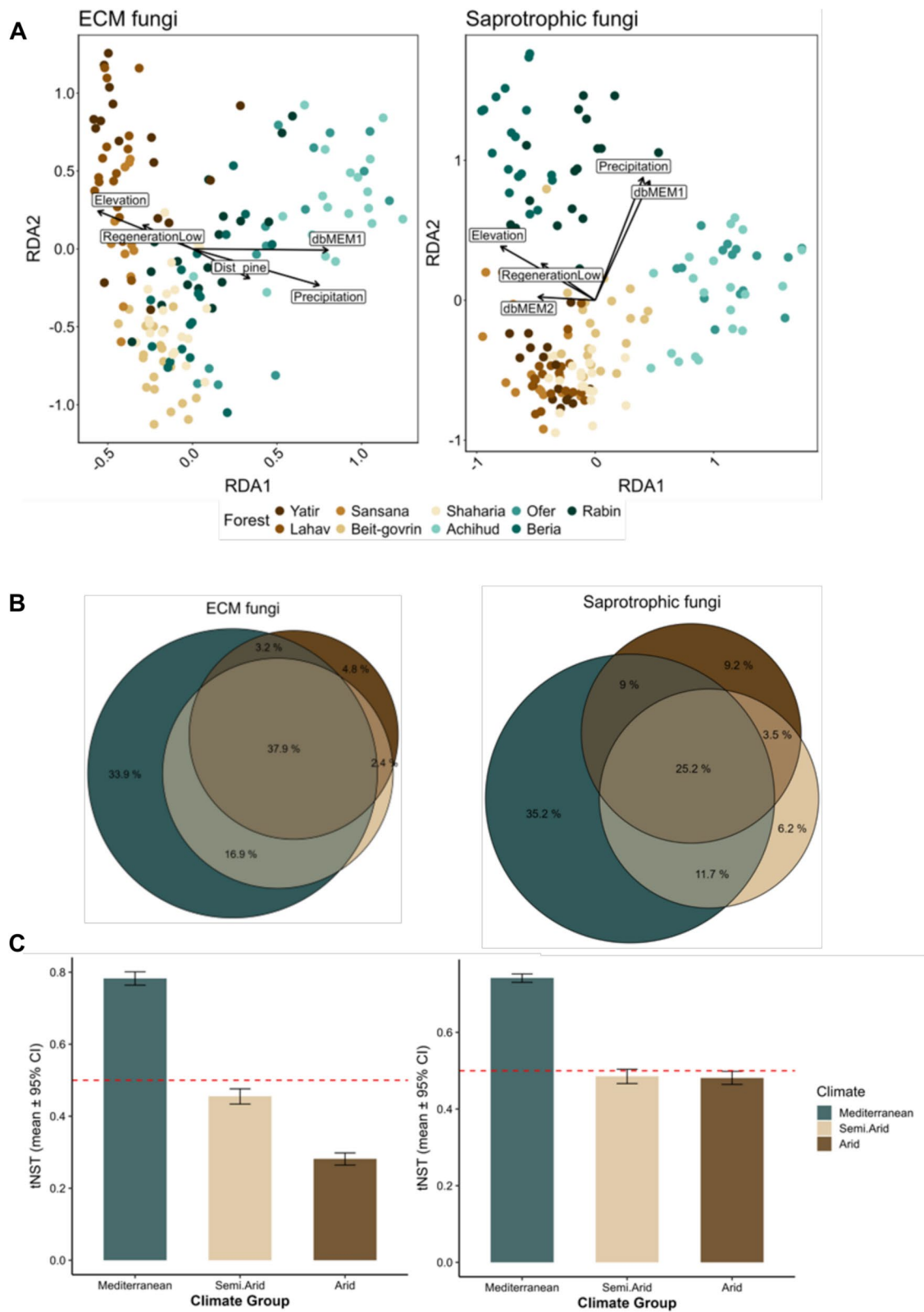


Fig. 3 **A)** Redundancy Analysis (RDA) plot based on ECM fungi (left) or saprotrophic fungi (right) presence/absence data showing the relationship between environmental variables and dbMEM eigenvectors across different forest sites. Each point represents a sample site colored by its respective forest. Arrows indicate the direction and magnitude of environmental variables, relative to the axes of the RDA. **B)** Venn Diagrams of unique OTUs are shown for fungal communities in Mediterranean, Semi-Arid, and Arid climate groups. **C)** Mean Taxonomic Normalized Stochasticity Ratio (tNST) values ($\pm 95\%$ confidence interval) across climatic regions. The red dashed line at tNST=0.5 indicates the threshold between deterministic (<0.5) and stochastic (>0.5) community assembly processes

spatial structure (dbMEM1), whereas elevation and low-regeneration plots loaded negatively and were associated with more arid sites. For saprotrophic fungi, the RDA explained 12.7% of the total variation (adjusted $R^2=0.12$; permutation test $p=0.001$), with RDA1 and RDA2 accounting for 6.1% and 4.4% of the constrained variance, respectively. Saprotrophic communities were structured along a similar environmental gradient, with RDA1 loading positively on precipitation and dbMEM1 and negatively on elevation, low-regeneration, and dbMEM2. None of the other vegetation indices, including shrub cover, number of ECM hosts, and MTCL, were retained in the final ordinations. Spatial structure was evident in both guilds, with one significant dbMEM axis for ECM and two for saprotrophs, capturing a gradient from mesic Mediterranean forests toward drier sites. RDA loadings and correlations among environmental variables are provided in the supplementary materials (Fig. S4, Table S2).

At the regional scale, β -dispersion (i.e., the distance of each sample community from the regional centroid) differed significantly among climates for both ectomycorrhizal (ECM) and saprotrophic (SAP) fungi. For ECM fungi, β -dispersion varied by climate (ANOVA $F_{2,160}=16.06$, $p<0.001$), with lower dispersion in the arid region compared to both the semi-arid ($p<0.001$) and Mediterranean ($p<0.001$) regions, while the Mediterranean and semi-arid regions did not differ significantly ($p=0.758$). For SAP fungi, β -dispersion also differed by climate (ANOVA $F_{2,160}=5.67$, $p=0.004$), with communities in the Mediterranean region being more dispersed than those in the semi-arid ($p=0.026$) and arid ($p=0.011$) regions, whereas the semi-arid and arid regions did not differ ($p=1.000$)

(Fig. S3). Venn diagrams demonstrated that most of the ECM and SAP fungal OTUs from the semi-arid and arid regions mostly represented a subset of the fungal diversity detected in the Mediterranean region, though the SAP fungal community had a slightly higher percentage of unique OTUs in the arid/subarid regions (Fig. 3B). Pairwise enrichment tests (Fisher's exact, one-sided 'greater'; FDR-corrected) supported these patterns, revealing a significant enrichment of shared OTUs only between the Semi-Arid and Arid regions for both ECM (Jaccard=0.61, OR=9.34, $q=6.6\times 10^{-8}$) and SAP fungi (Jaccard=0.44, OR=3.11, $q=3.1\times 10^{-9}$), whereas overlaps involving the Mediterranean region were not greater than expected by chance.

The taxonomic Normalized Stochasticity Ratio (tNST) across climatic regions revealed that ECM fungal communities in the Mediterranean region were predominantly shaped by stochastic processes (tNST >0.5), whereas ECM communities in Semi-Arid and Arid regions showed increasingly deterministic assembly (tNST <0.5 ; Fig. 3C). SAP fungal communities exhibited a similar trend, though the tNST values in the semi-arid and arid regions were closer to the stochastic-deterministic threshold (tNST ~ 0.5), suggesting a more balanced influence of random and deterministic processes on community structure (Fig. 3C).

Consistent with these patterns, within-region Raup-Crick (RC) values skewed negative-indicating frequent homogenizing dispersal rather than dispersal limitation—especially in drier climates. For ECM fungi, median RC values were -0.802 (Semi-Arid), -0.708 (Mediterranean), and -0.615 (Arid), with 25.1%, 21.0%, and 20.2% of within-region pairs, respectively, showing strong homogenization (RC <-0.95); dispersal limitation was rare (RC $>+0.95\sim 0-1\%$). For SAP fungi, median RC values were -0.908 (Semi-Arid), -0.802 (Arid), and -0.624 (Mediterranean), with 40.2%, 29.0%, and 20.7% of pairs, respectively, exhibiting RC <-0.95 and similarly low rates of RC $>+0.95$. These Raup-Crick results validate the tNST inference. For SAP fungi, stronger negative RC in Semi-Arid and Arid aligns with tNST <0.5 , indicating deterministic, homogeneous selection intensifies with aridity; the Mediterranean shows weaker homogenization, consistent with tNST >0.5 (more stochasticity). For ECM fungi, homogenization

peaks in Semi-Arid and is comparable between Arid and Mediterranean, still consistent with the direction of tNST (<0.5 in drier climates, >0.5 in Mediterranean), with the nuance that extreme aridity may increase micro-habitat heterogeneity/low richness, tempering within-region similarity.

ECM Fungal community composition and differential abundance across forest sites

The relative abundance of ECM fungal genera varied significantly across the forests sampled along the

aridity gradient. The ECM genera *Geopora* and *Inocybe* were prevalent in most forests (Fig. 4A). Some ECM genera, such as *Suillus*, *Tuber* and *Sebacina*, were more abundant in sites from Mediterranean forests, while others like *Helvellosebacina* and *Tomentella* showed significantly higher differential abundance in semi-arid/arid forests (Fig. 4B).

Discussion

Examining the effect of aridity on natural fungal communities in soils is a complex task due to the high number of confounding effects that correlate with climate, particularly host diversity and density in the case of ECM association. Specifically, when examining forests along aridity gradients there is often a change in the forest's dominant tree species which complicates causal inference regarding the effects of aridity. This complexity is often confronted with correlative statistical methods (Maestre et al. 2015; Ding and Eldridge 2022; Wang et al. 2024) in an attempt to isolate main effects from complex interactions. In our system we took advantage of monodominant planted pine forests that extend along a sharp climatic gradient, enabling us to characterize soil fungal communities under different climate conditions without changes in the identity of the dominant tree species. Overall, we found that, as hypothesized, richness and stochastic processes in community assembly increased with precipitation for both ECM and SAP fungi. Moreover, for both fungal groups, distance decay was positively correlated with precipitation at the forest scale. However, contrary to our hypothesis, this pattern was not stronger in ECM compared to SAP fungal communities.

Fungal species richness and composition

Fungal species richness was higher in samples originating from the Mediterranean climate at all spatial scales. These findings are in line with previous studies conducted along aridity gradients around the world (Maestre et al. 2015; Jiao et al. 2021; Wang et al. 2021, 2024; Ding and Eldridge 2022; Dong et al. 2024), illustrating the negative effect of extreme physical conditions of the arid habitats on fungal diversity. The reduced amounts of organic matter in the soil of arid habitats (Crowther et al. 2019), was

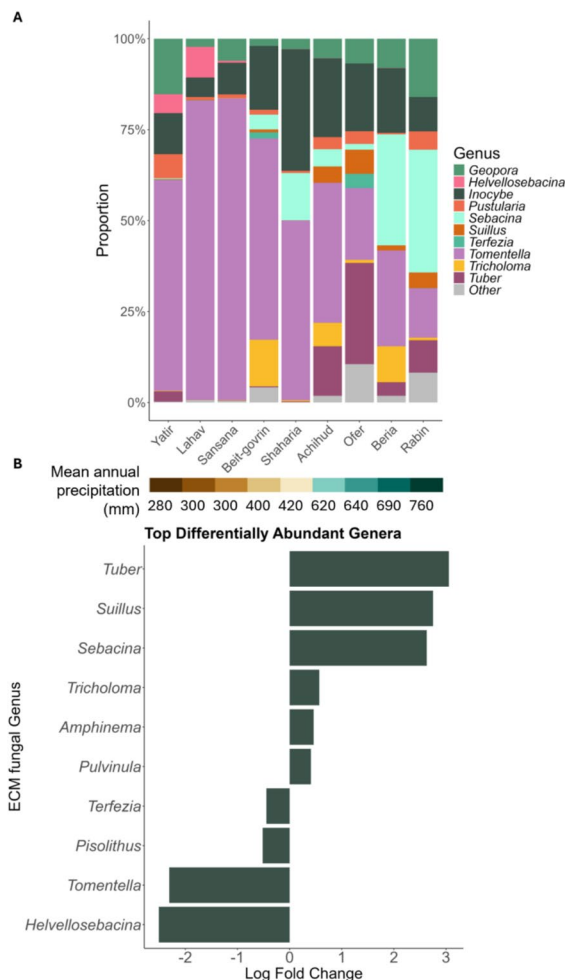


Fig. 4 A) ECM fungi read abundance at the genus level, in soil samples from monodominant *Pinus halepensis* forests along a steep climatic gradient. Forests are ordered according to mean annual precipitation (color coded between panels). B) bar plot of the top 10 significantly differentially abundant taxa between the Mediterranean and Semi-arid/arid climatic regions

suggested as a mechanism limiting microbial richness (Maestre et al. 2015; Ding and Eldridge 2022).

Changes in natural communities along environmental gradients are expected to result from the combination of nestedness (one community is a sub-group of the other) and species turnover (communities have unique species). In our system, the drier regions displayed a small number of unique fungal species, representing only 8% and 19% of the total species pool for ECM and SAP fungi respectively. This suggests the prevalence of nestedness, with fungal communities in arid/semi-arid regions being mostly a subset of the Mediterranean fungal communities (Fig. 3B). These results contrast with previous findings that showed a reduced overlap between soil fungal communities along aridity gradients (Chen et al. 2017; Wang et al. 2021) or following water manipulations (Lopez et al. 2024), where unique species made up a significant portion of the community under arid conditions (high species turnover). The reduced uniqueness of the arid fungal communities found in our study might be related to the confounded effects of aridity and vegetation in previous studies. As different climatic zones host different plant communities with unique fungal communities (but see Lopez et al. 2024), our current understanding of the independent role of climate *per-se* on fungal communities could be biased. In addition to the plant community, soil chemical characteristics can also covary with climate and are known to influence fungal communities. However, in our dataset many soil variables were highly collinear and were therefore excluded from the RDA as redundant predictors, preventing us from evaluating their independent effects. Consequently, precipitation remained the only climatic variable that explained a substantial portion of the variation in fungal community structure (Fig. S4, Table S2). While our study focused on the effects of climate alone, further studies incorporating controlled manipulations of both soil properties and climate conditions would be necessary to more conclusively attribute the observed patterns to specific environmental drivers.

In the current study, the dominant plant species was kept constant along the stress gradient, putatively resulting in the survival of only a subset of the fungal species under the harsh conditions of the arid/semi-arid regions. The fact that ECM fungi displayed a smaller percentage of unique species compared to SAP fungi could result from their greater dependence

on their host plant. Previous studies showed that *P. halepensis* harbor a very constant ECM fungal community in the rhizosphere, regardless of inoculum source, water stress (Herol et al. 2025) or genetic ecotype (Centenaro et al. 2025). Alternatively, the paucity of unique ECM fungal species in the arid region could result from the distribution these planted pine forests outside of the regeneration niche of the species (Pozner et al. 2022), thereby limiting the ECM fungal diversity to species that colonized the saplings prior to planting or species dispersing from Mediterranean sites.

When looking at the ECM fungal species composition in our dataset (Fig. 4A), our findings align with previous studies from the Mediterranean region (Livne-Luzon et al. 2016, Livne-Luzon et al. 2017, Rog et al. 2022), with a high relative abundance of the genera *Tomentella*, *Inocybe*, *Sebacina*, *Tuber*, and *Tricholoma*. More broadly, several of these genera are also commonly reported from other Mediterranean and semiarid ectomycorrhizal systems, including water-limited pine forests (Gehring et al. 2020; Bowman and Arnold 2021), suggesting that the dominant genera detected here are characteristic of dry ECM communities. Interestingly, other important ECM genera such as *Russula* and *Helvella*, that are known to colonize *Quercus calliprinos* roots (Rog et al. 2022) were extremely rare, appearing in only two samples at very low relative abundance, supporting the view that the potential effect of other hosts was negligible in explaining the patterns that we observed here. Functional traits of ECM fungi, including drought-associated traits such as exploration types and gasteroid versus non-gasteroid fruiting bodies (animal-dispersed versus wind-dispersed) did not show a clear and meaningful link to the climatic gradient. The facts that the arid communities were subsamples of the mesic ones and the absence of trait-based patterns along the gradient, suggest that the traits tested might not be the ones most relevant to aridity.

Distance decay of fungal communities at different scales

As expected, when examining distance decay at the scale of the entire gradient, both ECM and SAP fungi displayed increased dissimilarity with distance (Bahram et al. 2013, Talbot et al. 2014, Glassman

et al. 2015; positive spatial autocorrelation; Fig. 2A). Specifically, given the geographic range of our study (~200 km), samples within 100 km of each other were more similar than expected by chance (Fig. 2B). In our sampling design, such distances only exist between climate regions, i.e. Mediterranean versus arid/semi-arid regions. The differences between these two regions is likely to arise from the clear difference in climatic conditions characterizing each of them. Furthermore, dispersal limitation has been suggested to affect ECM fungal species composition at similar scales (Lilleskov and Bruns 2005; Peay et al. 2010; Policelli et al. 2022). Similarly, spore dispersal by animals is expected to be limited by animal movement. In Israel, wild boar (*Sus scrofa*) are possibly the largest spore dispersal agents (Livne-Luzon et al. 2016) and their movement distances (Miettinen et al. 2023) are probably limited to within each climate region.

Estimating distance decay patterns at different spatial scales enabled us to test for correlations between precipitation and spatial structure. We hypothesized that the more benign conditions and greater richness of ECM hosts in the Mediterranean climatic region, should strengthen spatial structure of the ECM fungal community compared to that of the SAP fungal community. However, our results did not support this hypothesis. SAP fungi demonstrated stronger distance decay pattern across the entire gradient and at the regional scale. At the regional scale SAP fungi showed significant distance decay within each of the three regions, while for ECM fungi this pattern was significant only in the semi-arid and Mediterranean regions (Fig. 2C). Dispersal at regional scale (ca. 40 km) may therefore be somehow limited, not permitting fungal communities to fully homogenize, or that environmental conditions change at such a scale, i.e. a gradient exists within the regional scale. Overall, SAP fungi showed stronger distance decay patterns than ECM fungi (Table S1), suggesting that SAP fungi change more directionally with distance within each region. This difference can arise from differences in dispersal capabilities as well as differences in the environmental heterogeneity experienced by the fungi. While the dispersal capabilities of SAP fungi have been suggested to be limited (Norros et al. 2012; Bässler et al. 2015), we did not observe a clear difference in the dispersal mechanisms between the two groups. We therefore find it more likely that

while EMF experience relatively homogenous conditions because of the uniform pine distribution within the forests, the SAP fungi experience more spatial heterogeneity of resources resulting in a stronger distance decay pattern.

At the forest scale (up to 3 km), both the SAP and ECM fungi showed significant distance decay in most forests (Fig. 2D). This suggests that ECM and SAP fungal distribution show some level of directional change within forests. Such changes could result from the patchy distribution of organic matter in the case of SAP fungi, or secondary ECM hosts in the understory in the case of ECM fungi. Furthermore, the distance decay of both ECM and SAP fungi significantly increased with precipitation, suggesting that resources in the arid forests are more homogenous, and/or that dispersal plays a stronger role in homogenizing fungal communities in arid forests compared to forests from the Mediterranean region. For example, aridity can increase the percentage of exposed soil, thereby enhancing dust formation and spore dispersal by wind (Golan and Pringle 2017). On the other hand, aridity can greatly reduce sporing body formation (De Aragón et al. 2007; Boddy et al. 2014) and spore densities, resulting in reduced dispersal. More research is needed on the effect of aridity on fungal dispersal to fully understand these processes.

When examining fungal species composition at the smallest scale (40×40 m plots), we only found significant distance decay patterns in 22% of the plots for both ECM and SAP fungi (Fig. 2E). Additionally, we did not detect any correlation between precipitation and Mantel-r, putatively as a result of random dispersal processes homogenizing the fungal community at small spatial scales, regardless of precipitation (Lilleskov and Bruns 2005; Bahram et al. 2015).

Community assembly processes

We found relatively high similarity between arid communities while mesic communities showed greater variability. These patterns suggest that deterministic processes played a significant role in community assembly in arid habitats, while stochastic processes increased in importance with precipitation. This was particularly prominent for ECM fungal communities, which showed a low variability in arid habitats, possibly due to the lack of ECM host diversity. These findings contrast with previous studies in

which aridity tends to increase stochastic processes in community structure. Although environmental filtering is hypothesized to be prevalent in arid habitats, most studies on soil fungi found that stochasticity processes drive community assembly regardless of aridity (Jiao et al. 2021; Chen et al. 2022; Wang et al. 2023, 2024; Addison et al. 2025; Li et al. 2025), suggesting that environmental filtering is less important than random processes in these habitats. However, most of these studies used large-scale gradients ranging thousands of kilometers and harboring a range of plant communities across the gradient (but see Addison et al. 2025). These patterns are also likely to be scale dependent, since two arid sites thousands of km apart are expected to be much more different than two arid sites a dozen km apart. Large inter-site distances within the same aridity zone should therefore result in species turnover due to dispersal limitations and even different evolutionary histories of the fungal communities. Therefore, it is possible that dispersal limitations due to distance and histories can mask the deterministic effects of climate on community assembly at large scales. When comparing sites that are separated by distances greater than practical fungal dispersal distances, similarity in species characteristics is a more reasonable expectation than similarity in species identity (Liu et al. 2013). The observational nature of our study, limits our ability to draw causal conclusions regarding the role of aridity in the balance between deterministic and stochastic processes in community assembly. Direct measurements of the effect of aridity on fungal traits such as spore dispersal and longevity as well as competitive ability and survival, should enable a more robust understanding of such community assembly processes.

Future directions

Our findings suggest that the composition of the ECM community of Aleppo pines is highly stable, regardless of climate. The fact that previous studies found significant changes in the ECM communities along climatic gradients could be the result of changes in the dominant forest tree species. This further highlights the need to disentangle the effects of aridity *per-se* on fungal communities from those of vegetation, to better understand the processes determining fungal community assembly along environmental gradients. Due to the observational nature of

such studies, which arises from the long time scales involved in tree maturation and fungal community assembly, detailed planning of sampling schemes and site selection are required to minimize the often existing correlations between climate and the plant community. Focusing on different tree species with different levels of dependency on ECM could further improve our understanding of the links between plants climate and the fungal community. Such an approach will enhance our understanding about the effect of future climates on soil fungal communities and ecosystem functions. Such knowledge is critical to inform forest management by selecting tree species that support and rely on diverse and resistant fungal communities.

For each plot, the table reports geographical coordinates (latitude, longitude), elevation, mean annual precipitation, mean pine DBH, pine density (expressed as the mean distance to the nearest pine tree (m)), number of mature pine trees, regeneration status, mean ten-year MTCI (Meris Terrestrial Chlorophyll Index), and the coefficient of variation of MTCI over ten years. Forests are ordered by decreasing precipitation.

Values represent soil pH, electrical conductivity (EC, dS/m), chloride concentration (Cl, mg/kg soil), ammonium nitrogen (N-NH₄, mg/kg soil), nitrate nitrogen (N-NO₃, mg/kg soil), and phosphate (PO₃, mg/kg soil) measured in the upper soil layer of each plot.

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Author contributions SLL led the study under the supervision of TK and HS. AH and LH collected the data, and AH performed the DNA extractions. SLL prepared the amplicon library for sequencing and conducted the data and statistical analysis; CT instructed the data and statistical analyses; SLL, and HS wrote the manuscript with help from all authors.

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Data availability Sequence data was submitted to the National Center for Biotechnology Information Sequence Read Archive with the accession codes: Bioproject PRJNA1298548.

Declarations

Competing interests The authors declare no conflict of interest.

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