# Soil and climatic characteristics and farming system shape fungal communities in European wheat fields

Krista Peltoniemi [a,*], Sannakajsa Velmala [a], Eva Lloret [b], Irene Ollio [b], Juha Hyvönen [c], Eero Liski [d], Kristian K. Brandt [e], Claudia Campillo-Cora [f], Hannu Fritze [a], Sari Iivonen [g], Simon B. Lassen [e], Kaire Loit [h], Silvia Martínez-Martínez [b], Taina Pennanen [a], Marian Põldmets [h], Stefan Schrader [i], Merrit Shanskiy [h], Raúl Zornoza [b], Lieven Waeyenberge [j], David Fernández Calviño [f]

[a] *Natural Resources Institute Finland (Luke), Natural resources, Soil Ecosystems, Latokartanonkaari 9, Helsinki FI-00790, Finland*
[b] *Department of Agricultural Engineering, Universidad Politécnica de Cartagena, Paseo Alfonso XIII 48, Cartagena 30203, Spain*
[c] *Natural Resources Institute Finland (Luke), Natural resources, Applied Statistical Methods, Ounasjoentie 6, Rovaniemi FI-96200, Finland*
[d] *Natural Resources Institute Finland (Luke), Natural resources, Applied Statistical Methods, Latokartanonkaari 9, Helsinki FI-00790, Finland*
[e] *Section for Microbial Ecology and Biotechnology, Department of Plant and Environmental Sciences, University of Copenhagen, Thorvaldsensvej 40, Frederiksberg DK-1871, Denmark*
[f] *Institute of Agroecology and Food (IAA)/Faculty of Sciences, University of Vigo – Campus Auga, Ourense 32004, Spain*
[g] *Natural Resources Institute Finland (Luke), Finnish Organic Research Institute, Lönnrotinkatu 7, Mikkeli FI-50100, Finland*
[h] *Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, 5 Fr. R. Kreutzwaldi St, Tartu 51006, Estonia*
[i] *Thünen Institute of Biodiversity, Bundesallee 65, Braunschweig D-38116, Germany*
[j] *Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Plant Sciences Unit, Burg. Van Gansberghelaan 96, Merelbeke B-9820, Belgium*

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## ABSTRACT

Fungi play a pivotal role as highly effective decomposers of plant residues and essential mycorrhizal symbionts, augmenting water and nutrient uptake in plants and contributing to diverse functions within agroecosystems. This study examined soil fungi in 188 wheat fields across nine European pedoclimatic zones under both conventional and organic farming systems, utilizing ITS1 amplicon sequencing. The investigation aimed to quantify changes induced by the farming system in soil fungi and their correlation with soil features and climatic factors across these pedoclimatic zones, spanning from northern to southern Europe. The pedoclimatic zone emerged as a key determinant in shaping the overall composition of the fungal community. Zones characterized by moist and cool climates, along with low levels of available phosphorus and carbonate, exhibited higher fungal richness. However, variations in fungal diversity and relative abundances were observed within zones due to farming system-induced changes. Soil pH and bulk density were identified as major factors, for example, they correlate with an increase in potential pathogenic taxa (*Mycosphaerella*, Nectriaceae, *Alternaria*) in two Mediterranean zones and with an increase of potential plant growth promoting taxa (*Saitozyma*, *Solicoccozyma*) in the Boreal zone. Organic farming, in general, promoted elevated fungal richness. The Lusitanian and Nemoral zones under organic farming exhibited the highest fungal richness and diversity. In terms of organic farming, both symbiotrophs and potential pathogens increased in the Lusitanian zone, while pathotrophs were more prevalent in the Central Atlantic and South Mediterranean zones under organic farming. These findings propose potential indicators for organic farming, including fungal endophytes in zones characterized by a moist and cool climate, low available phosphorus content, and low soil pH. Organic farming may favor mycorrhizae and potential pathogens in zones with drier and warmer climates, along with higher soil pH, calcium carbonate content, and bulk density. This study provides novel insights and underscores the significance of regional climatic and edaphic conditions in shaping the soil fungal community in different farming systems within European wheat fields.

## 1. Introduction

Fungi play a critical role in soil ecosystems by contributing to nutrient cycling, soil structure maintenance, and disease regulation (Frąc et al., 2018). The response of diverse fungal taxa with varying ecological functions to changes in arable soil conditions, influenced by climatic factors and management practices, is multifaceted. Saprotrophic soil fungi, functioning as biogeochemical engineers, significantly contribute to soil organic matter decomposition and the recycling of carbon (C) and other essential nutrients (Turbé et al., 2010). Mutualistic arbuscular mycorrhizal fungi (AMF) produce a sticky glycoprotein known as glomalin (Wright and Upadhyaya, 1996), which forms aggregates with soil particles crucial for erosion control (Augé, 2001; Caravaca et al., 2002; Rillig, 2004a, 2004b; Rillig and Mummey, 2006). Moreover, symbiotic AMF enhance plant growth (Gianinazzi et al., 2010; Njeru et al., 2015; Cozzolino et al., 2016), protect plants from diseases (Solaiman et al., 2014), and aid in water deficit tolerance (Bowles et al., 2016). Nevertheless, the soil fungal community also harbors harmful organisms, such as certain soil-borne phytopathogenic *Fusarium* species that produce mycotoxins, thereby compromising crop yield and quality (Ferrigo et al., 2016). Additionally, there exist numerous lesser-known fungi capable of transitioning from free-living soil saprotrophs to endophytic fungi residing within plant hosts, thereby promoting plant growth (Weiß et al., 2016; Grabka et al., 2022). Consequently, besides fostering plant growth and development, these fungi can mitigate oxidative stress in hosts and provide protection against pathogens and pests (White et al., 2019).

In aiming to reduce the use of chemical pesticides and mineral fertilizers by 20% by 2030, the European Union (EU) has set an ambitious target of achieving an average of 25% organically cultivated agricultural land across Europe by the same year. Fungi may play a crucial role in realizing sustainable agriculture objectives by reducing reliance on external inputs. This can be achieved through the implementation of management practices commonly associated with organic farming systems, such as diversified cropping rotations, utilization of leguminous plants, and the application of organic fertilizers. It is widely suggested that management strategies aimed at restoring fungal biodiversity and promoting fungal-mediated energy pathways could effectively diminish chemical, biological and ecological degradation of arable soils and mitigate nutrient and C loss, soil erosion, acidification, pollution and desertification (Morriën et al., 2017; Hannula and Morriën, 2022). Nutrient and C cycling facilitated by the slow fungal cycle would prolong the retention of nutrients and C in the soil (Coleman et al., 1983), consequently enhancing soil structure and functionality, thereby fostering a more sustainable approach to agriculture.

While scientific knowledge addressing the impacts of farming systems, namely organic versus conventional practices, on fungi does exist, these findings primarily rely on biomass estimations or data derived from a limited number of experimental cereal field sites across specific regions of Europe. For example, a long-term field study conducted in Switzerland demonstrated the eventual development of distinct fungal communities under organic and conventional farming systems (Hartmann et al., 2015). Fungi associated with the degradation of complex compounds, such as manure, were characteristic of organic farming systems utilizing organic fertilizers (Hartmann et al., 2015). Similarly, another long-term investigation revealed that many fungal decomposers of organic substrates, such as leaf litter, wood, and manure, were associated with organic farming practices (Harkes et al., 2019). Additionally, fungal abundance, as indicated by fungal ITS copy numbers, was higher under the organic farming system in cereal crop rotations (Peltoniemi et al., 2021). Numerous independent studies have indicated that organic farming techniques, such as reduced tillage and diversified crop rotations, tend to enhance the activity or abundance of AMF (Bedini et al., 2013; Banerjee et al., 2019; Gosling et al., 2006; Säle et al., 2015). Conversely, intensive tillage, commonly employed in conventional farming, has been identified as a significant factor leading to decreased

AMF abundance and diversity in agricultural settings (Kabir, 2005). Moreover, deep tillage practices have been shown to alter fungal community structures (Fritze et al., 2024), diminish fungal hyphal lengths (Oehl et al., 2004), and reduce fungal colonization potential (e.g., Jasper et al., 1991; Usuki et al., 2007; Al-Karaki, 2013). Consequently, conventional agricultural methods may pose a threat to fungi and ecosystem services when management practices exclusively focus on factors such as monocultures without crop rotation, extensive mechanical soil disturbance via deep tillage, and excessive application of synthetic mineral fertilizers or pesticides.

A recent continental-scale study, conducted with soil samples gathered from eight European biogeographical regions, has unveiled that specific soil attributes, particularly pH and temperature, exert significant influence on soil fungal communities and their potential functional groups, with variations contingent upon climatic and vegetative contexts (Labouyrie et al., 2023). However, extensive studies exclusively focusing on fungi within agroecosystems cultivated with a specific crop are currently lacking. In this study, we delved into the soil fungal diversity and community composition within European wheat fields under both organic and conventional farming systems across nine distinct pedoclimatic zones, spanning Europe from north to south. These zones encompass diverse climatic conditions, soil types, and physical and chemical soil properties. Fungal diversity and community composition were evaluated using amplicon sequencing of the fungal ITS1 region. The study aimed to test three hypotheses. Firstly, it was hypothesized that fungi exhibit changes along each pedoclimatic zone attributable to climate variables and soil characteristics. Secondly, it was posited that these changes manifest in fungi between farming systems. Lastly, it was proposed that organic farming fosters the presence of fungi beneficial for soil and plant health.

## 2. Materials and methods

### 2.1. Field study sites and sampling

The field study sites were selected from farms with wheat (*Triticum aestivum*) as a cash crop in the following pedoclimatic zones (respective country) arranged from north to south: Boreal (Finland), Nemoral (Estonia), North-Atlantic (Denmark), Continental (Germany), Central-Atlantic (Belgium), Pannonian (Hungary and Serbia), Lusitanian (north-western Spain), Mediterranean North and Mediterranean South (highland and low-altitudinal areas in southern and eastern Spain, respectively) (Fig. S1 in the supplementary material). The definition and descriptions of zones are according to the dataset about the environmental stratification of Europe (Metzger, 2018). Wheat was chosen because it accounts for more than half of all cereals grown in the EU. We sampled at least 10 conventional and 10 organic farming field study sites, respectively, from each of the nine pedoclimatic zones (Table S1). In each country, fields with organic farming fulfilled the EU criteria of organic production (Council regulation, 2018), and 84 out of 95 fields had been under organic farming for at least 5 years (Table S2). According to European Comission regulation, organic crop rotations do not allow cereals to be cultivated for more than three consecutive years, and more than 30% of plants in crop rotations must be legumes. Furthermore, synthetic fertilizers and pesticides are strictly prohibited in organic farming. Multiple correspondence analysis (MCA) illustrates the similarities and dissimilarities between the farming systems (conventional and organic) in the sampled field study sites (Fig. S2). The two-dimensional ordination plots obtained clearly show how the applied management practices (tillage system, crop rotation cycle, type of fertilizer, inclusion of legumes or crop residues in the rotation, use of pesticides) vary. Always, two or three field study sites, representing both conventional and organic farms, were selected from the same locality, so that the farms represented the same edaphic and climatic conditions for correct comparison. Soil textures were mainly sandy or silt loams with a few clay loams. The 2 kg composite sample per field was composed of 60

soil cores sampled using an auger with variable diameter from 13 to 50 mm following a zigzag pattern to cover the entire 1 ha field study site area, at a depth of 0–25 cm, as specified in the SoildiverAgro's Handbook of WP3 (Waeyenberge, 2020). Bulk density samples, from two different depths (0–10 cm and 10–25 cm), were taken with a cylinder (50 mm × 50 mm) to get undisturbed soil for gravimetric method analysis (Waeyenberge, 2020).

In total, we collected 188 composite samples shortly after the wheat harvest and before any management practices to reduce the temporal variability and to avoid soil disturbances. Sampling was conducted in all other except in the Pannonian zone between July and October in 2019. For practical reasons sampling from the Pannonian zone was conducted in July 2020. The summary of the short- and long-term climatic factors (mean temperatures and precipitations for 30 years and 12 months before sampling) in farm locations and the prevalent management practices (tillage method, crop rotation system, type of fertilization, use of legumes, incorporation of crop residues, type of pesticides) applied to the field study sites are summarized in Table S2. More detailed information about the sampled field study sites and their management can be found in the public repository of Zenodo (Fernández-Calviño et al., 2023).

The composite soil samples were stored and shipped in a cool box within eight hours from the field to the laboratory. Upon arrival, they were manually homogenized by mixing the bags gently to break any remaining soil cores and divided into sub-samples for different analyses. Sub-samples consisting of 20 g of soil were stored in 50 ml Falcon tubes frozen at −80 °C and shipped as frozen in a cool box with cool packs within one day using express courier service for DNA extraction, PCR, and sequencing. A part of these frozen samples was then used for organic C and field moisture analyses, and assessments of ammonium, nitrite, and nitrate contents after thawing, sieving with 2 mm sieve and air-drying. Another subset of samples was dried at room temperature for two weeks and sieved before nutrient and elemental analyses, soil texture (sand/silt/clay contents) and soil pH measurements. The final subsamples for aggregate size analyses were not sieved but only dried at room temperature for two weeks. Analyses are described in detail by Soto-Gómez et al. (2020a), (2020b). All information about the measured soil chemical and physical data is deposited in the public repository of Zenodo (Fernández-Calviño et al., 2023). Climatic data, management practices, and soil physical and chemical data used in the analyses are listed in Table S3.

## 2.2. Soil DNA extraction and fungal ITS1 amplicon sequencing

Soil DNA extraction and fungal ITS amplicon sequencing were done according to Lloret et al., (2020). Soil DNA was extracted from the 0.25 g of soil using the DNeasy PowerSoil Pro Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions except for the bead-beating step, which was conducted in a FastPrep-24 instrument (MP Biomedicals, Solon, OH, USA) at 5.5 m s-1 during 45 s for a total of two periods of shaking. The extracted DNA was purified using NucleoMag® NGS Clean-up and Size Select (Macherey-Nagel, Düren, Germany). Quantification and purity assessment of the purified DNA were performed using Qubit® 2.0 Fluorometer (ThermoFisher Scientific, Waltham, Massachusetts, US) and NanoDrop 2000c (ThermoFisher Scientific, Waltham, Massachusetts, US) respectively.

Fungal amplicon sequencing was conducted by AllGenetics & Biology SL (www.allgenetics.eu) using fungal internal transcribed spacer (ITS) region 1 with primers ITS1F (5' CTT GGT CAT TTA GAG GAA GTA A 3') (modified from White et al., 1990) and ITS2 (5' GCT GCG TTC TTC ATC GAT GC 3') (White et al., 1990), including the Illumina adaptor sequence, following the Earth Microbiome Project (EMP) protocol (http://www.earthmicrobiome.org/protocols-and -standards/its/). The EMP is a massively collaborative effort to characterize microbial life on the planet and EMP protocols have been used in thousands of publications. PCRs comprised of 2.5 µl of template DNA,

0.5 µM of the primers, 12.5 µl of Supreme NZYTaq 2x Green Master Mix (NZYTech, Lisbon, Portugal), and ultrapure water up to 25 µl. The reaction mixture was incubated as follows: an initial denaturation step at 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s, 47 °C for 45 s, 72 °C for 30 s, and a final extension step at 72 °C for 10 min.

The oligonucleotide indices required for multiplexing different libraries in the same sequencing pool were attached in a second PCR round with identical conditions, but only five cycles and an annealing temperature of 60 ºC. The libraries were run on 2% agarose gels stained with GreenSafe (NZYTech, Lisbon, Portugal) and imaged under UV light to confirm library size. The libraries were purified using Mag-Bind RXNPure Plus magnetic beads (Omega Bio-tek, Norcross, Georgia) and pooled in equimolar amounts based on the quantification data provided by the Qubit dsDNA HS Assay (Thermo Fisher Scientific, Waltham, Massachusetts, US), and sequenced in a fraction of a NovaSeq PE250 flow cell (Illumina) aiming for a total output of 21 gigabases. The median sample library size was 173,973 reads.

## 2.3. Bioinformatics

Fungal ITS1 sequences were assembled, quality filtered, and preprocessed along with chimera filtering, clustering and taxonomical annotations using the PipeCraft 1.0 pipeline (Anslan et al., 2017), which incorporates several tools including mothur v1.36.1 (Schloss et al., 2009), vsearch v1.11.1, and CD-HIT v4.6 (Fu et al., 2012). Raw sequence reads were processed according to the manual, with slight modifications for demultiplexed sequence data. Specifically, paired-end reads were assembled, and quality filtered using vsearch (v1.11.1; github.com/torognes/vsearch; Rognes et al., 2016) with the following parameters: minimum overlap 15; maximum differences 99; minimum length 150 bp; e_max 1; maximum ambiguous 0; and trunc qual 20. On average, 60% of the raw reads were filtered out after the assembly. Chimera filtering was conducted on the reoriented reads using reference-based filtering with Unite ITS1 ref. v7.1 as the database, and vsearch *de novo* filtering with the following parameters: annotation 0.97 and abskew 2. At this step, primers and primer artifacts were also filtered out from the sequences. Additionally, the fungal ITS1 region was extracted from the reads using ITSx (Bengtsson-Palme et al., 2013). In the subsequent step, sequence reads were clustered, and an operational taxonomic unit (OTU) table was generated using CD-hit with the following parameters: threshold 0.97 and min size 2. Finally, the taxonomic annotation of the OTUs was performed by searching for representative sequences using BLAST with the reference ITS1 database (sh_general_release_dynamic_02.02.2019.fasta) from UNITE (Nilsson et al., 2018). After the initial filtering steps, the sequence data consisted of 11 394 518 reads clustering into 28 027 OTUs. This initial data was used in calculating Alpha diversity measures described below.

Secondary filtering was performed based on the results of the BLAST and the alignments. We filtered out all OTUs that did not match fungal sequences in the databases, those with an identity below 70% to fungi, and extremely rare OTUs that were observed fewer than 11 times in the entire dataset (< 0.001‰ of the total read count). The raw sequence data has been deposited in the sequence read archive (SRA) of the NCBI database under the BioProject PRJNA984266. The accession numbers SAMN35753908 – SAMN35754095 were assigned for the fungal ITS data. Fungal guilds representing ecologically different fungal groups (saprotrophs, pathotrophs, symbiotrophs) of OTUs were detected using FUNGuild (Nguyen et al., 2016).

## 2.4. Community and statistical analyses

Alpha diversity measures describing the fungal diversity, including observed OTU numbers and Chao1 estimates, as well as Shannon and Inverse Simpson diversity indexes, were obtained using the R's microbiome package (Lahti and Shetty, 2017) from the data after the initial filtering step. Firstly, to investigate the effects of pedoclimatic zones and

farming system on fungal diversity and guilds (saprotrophs, pathotrophs and symbiotrophs), diversity measures and relative abundance of guilds were used as response variables, and pedoclimatic zone, farming system (conventional or organic), and their interaction were used as explanatory (fixed) factors in mixed models. Linear mixed models (LMMs) for normally distributed response variables and generalized linear mixed models (GLMMs) for gamma distributed response variables were used in analyses to account for correlation (similarity) of response variable values. Locality was included as a random factor to account for the correlation of field observations. The data had a three-level hierarchical structure, with 9 pedoclimatic zones divided into altogether 82 localities, which were divided into 188 fields in total (1–8 fields per locality). Secondly, to investigate the link between climatic and soil features and fungi, pedoclimatic zone was moved to the random factor together with locality to identify more precise explanatory factors, while farming system remained as a fixed factor with the measurements of climate, soil properties and field management (listed in Table S1) as the potential explanatory variables. The Tukey-Kramer method was used for pairwise comparisons of model predicted class means. The mixed model analyses were performed using the GLIMMIX procedure of SAS 9.4 software. Additionally, a SAS macro was used to display the correct results with letters in pairwise comparisons (Piepho, 2012).

To investigate the effect of pedoclimatic zone and farming system and on fungal community composition (beta diversity) non-metric multidimensional scaling (NMDS) based on the relative abundance of reads (proportions relative to the total read count in each sample library) was assessed. Soil variables from soil chemical and physical data, data from management practices, and climatic factors listed in supplementary Table S3 were included as environmental factors in the NMDS analysis to investigate their effects on community composition. For the analysis of AMF data, we employed the geometric mean of pairwise ratios normalization (GMPR) (Chen et al., 2018), which accounts for zero-inflated community data and preserves differences in relative taxonomic abundances. NMDS was conducted using the metaMDS function in the vegan package 2.5–5 (Oksanen et al., 2019), with 999 bootstraps and the Bray-Curtis dissimilarity index. Function stat_ellipses was used to draw a 95% confidence level for a multivariate t-distribution between zones. PERMANOVA, implemented with the adonis function in the vegan package, was used to test the effect of pedoclimatic zone and farming system (conventional vs. organic) on fungal community composition. Homogeneity of variances was assessed separately for zone and farming system using the betadisper function.

We examined the impact of climatic variables, soil characteristics, and farming systems on changes in fungal communities within each pedoclimatic zone individually, employing cluster analysis. This approach was adopted because the zone itself emerged as the most influential factor affecting a specific cluster group. Analyzing each zone separately was essential to avoid its potential to obscure the effects of other variables within the analysis. First, OTUs with the same species hypothesis in the UNITE databases were combined, resulting in 3226 OTUs. Due to the n < p problem (number of observations less than the number of predictors), our initial task was to summarize the OTU information to reduce its size (Koch, 2014). OTUs have a specific structure, with components consisting of weights that are either zero or positive, summing up to a constant and carrying only relative information. Consequently, OTU data are compositional, and specialized methods applicable to compositions are required (for detailed reasoning, see Aitchison, 1986, and Supplementary Material A). In compositional data analysis, only strictly positive values are allowed. However, the majority of the OTU data points (509 606 of 606 488) were zero. Therefore, we employed zero imputation, using a very small nonzero value of 1–10.

In the cluster analysis OTUs were identified as two distinct groups. The cluster analysis was performed on clr-transformed OTU data using the Ward method. By summarizing the OTU information into a single variable with two groups, the modeling scenario was significantly

simplified. Our objective was to use potential predictors to model the probability of belonging to a specific cluster group using a logistic regression model. Our modeling scenario followed a machine learning approach, aiming to maximize the predictive ability of the model. We defined a set of potential predictors from the soil chemical and physical data and management practices listed in Table S3. We employed a grid search approach, considering every predictor combination. For each predictor combination, we applied leave-one-out cross-validation to calculate the cross-validation (CV) accuracy. The best model was determined based on the predictor combination that yielded the highest CV accuracy. For the best model, we performed bootstrapping (using the full data) and out-of-bag prediction to obtain error bounds for accuracy.

To investigate fungal representatives associated with farming systems in different zones, a differential abundance analysis was performed using the ancombc function in the ANCOM-BC package (Lin and Peddada, 2020) for each zone. The analysis was conducted at the OTU level with the default settings, except for the following: zero_cut=0.75, lib_cut=1000, struc_zero=TRUE, conserve=TRUE. The results are presented as paired comparisons between farming systems for each pedoclimatic zone.

The analyses were conducted using R software versions 3.6.0, 3.6.1, and 4.2.2 (R Core Team, 2018, 2022) and Rstudio (versions 1.2.5001 and 2022.07.2). All figures were created using R packages phyloseq (McMurdie and Holmes, 2013) or ggplot2 (Wickham, 2016). The compositional analysis utilized the compositions package (van den Boogaart et al., 2022).

## 3. Results

### 3.1. Overview about the obtained fungal sequences and taxa

The quality filtered fungal ITS data consisted of 11 047 626 fungal reads affiliated with 7750 OTUs. The final dataset consisted of 188 separate sample libraries with a median library size of 60 198 reads, ranging from 26 771–85 131 reads. Most of the dominant reads (99%) at fungal phyla level were identified as Ascomycota (62%), Basidiomycota (16%), Mortierellomycota (15%), Chytridiomycota (0.4%) and 5% were left without identification to any known phylum (supplementary figure S3a). Furthermore, the majority of the dominant reads at class level were identified as Sordariomycetes (37%), Mortierellomycetes (15%), Tremellomyces (14%) and Dothideomycetes (11%) (Fig. S3b). Most of the reads, 11% from all reads, showed the closest match to ascomycete *Acremonium furcatum* and to a six species of the genus *Mortierella* (9% of all reads, data not shown).

### 3.2. Impacts of pedoclimatic zones on fungal community

The pedoclimatic zone had a significant effect on fungal richness (observed OTU numbers; LMM, $P < 0.0001$), Chao1 estimates ($P < 0.0001$) and Shannon ($P = 0.004$) and Inverse Simpson indices ($P = 0.0001$). Richness was generally higher in the Nemoral zone compared to Pannonian, Continental and Mediterranean zones (Table 1). Mediterranean zones had the lowest soil fungal richness. The Pannonian zone had a higher Shannon index compared to the Nemoral and North Mediterranean zones. Similarly, Pannonian had a higher Inverse Simpson index compared to all other zones. Fungal richness correlated positively with high long-term precipitation ($P = 0.01$), and negatively with lowest mean temperatures within 12 months prior sampling ($P = 0.03$), calcium carbonate ($CaCO_3$) content ($P = 0.02$) and available phosphorus ($P_{av}$) ($P = 0.004$) (Fig. 1).

Based on PERMANOVA analysis, the pedoclimatic zone explained 40% ($R^2 = 0.404$, $P = 0.001$) of the variation in soil fungal community composition (Table 2). Betadisper analysis indicated that the variance in the OTU data was not homogeneous across zones, i.e., the dispersions of different groups differ, suggesting caution when interpreting the results from PERMANOVA, especially for zones with overlapping communities

**Table 1**

Average alpha diversity measures from fungal OTU data in pedoclimatic zones with standard error of means in parentheses. Letters represent statistically significance between zones according to linear mixed model and paired comparisons with Tukey-Kramer method. Differences are considered significant if $P \leq 0.05$.

| Zone | Observed | Chao1 | Shannon | InvSimpson |
|---|---|---|---|---|
| boreal | 861 (126) ab | 1051 (138) ab | 3.7 (0.6) b | 17.5 (10.4) b |
| nemoral | 962 (130) a | 1194 (143) a | 3.9 (0.5) ab | 15.0 (10.3) b |
| atl_north | 826 (156) ac | 1030 (178) ab | 3.6 (0.8) b | 16.1 (6.9) b |
| cont | 804 (97) bcd | 983 (125) bc | 3.8 (0.4) b | 14.8 (8.4) b |
| atl_cent | 872 (122) ab | 1066 (145) ab | 3.8 (0.3) b | 15.6 (5.9) b |
| pann | 776 (103) bcd | 1056 (145) ab | 4.4 (0.4) a | 30.4 (13.4) a |
| lusit | 855 (175) ab | 1040 (209) b | 3.9 (0.4) b | 17.1 (6.8) b |
| med_north | 669 (122) cd | 811 (138) cd | 3.9 (0.5) ab | 19.5 (8.6) b |
| med_south | 625 (118) d | 742 (140) d | 3.7 (0.4) b | 15.7 (9.2) b |

(Fig. 2). However, NMDS shows clearly distinct separation in fungal OTU composition, particularly between the Lusitanian and Mediterranean zones compared to the other zones. Thirteen soil chemical and physical parameters were identified as significant environmental factors influencing the variation in fungal community composition in NMDS, including $P_{av}$, organic matter (OM), $CaCO_3$, sum of base cations (Bs), bulk density in the depth of 0–10 cm (BD), soil pH, particulate organic C (POC), nitrate ($NO_3$), (C:N), field moisture (Fma), mean weight diameter of soil aggregates (AMWD), and sand and silt content. The community of

AMF did not show any clustering according to pedoclimatic zone (Fig. S4). AMF abundances varied across zones, and the presence of rare OTUs likely correlated slightly with sequencing depth, which could partly explain the limited observations in Pannonian zone (Fig. S5).

FunGuild analysis identified matches for 65% of the fungal OTUs, which were categorized into seven fungal guilds (Table S4). Here we investigate in more detail the impacts on three different guilds with major ecological role (saprotrophic, pathotrophic or symbiotrophic) according to Funguild. The pedoclimatic zone ($P=0.09$) did not influence the number of saprotrophs. However, more pathotrophs were recorded in the Central Atlantic zone compared to the Continental zone (Table S4). The Nemoral zone exhibited a higher relative abundance of symbiotrophs compared to other zones, except for the Boreal, Pannonian, and South Mediterranean zones (Table S4).

**Table 2**

Results of PERMANOVA analysis to test the effect of pedoclimatic zone, farming system (conventional or organic farming systems) or their interactions on fungal OTU composition. Differences are considered significant if $P \leq 0.05$.

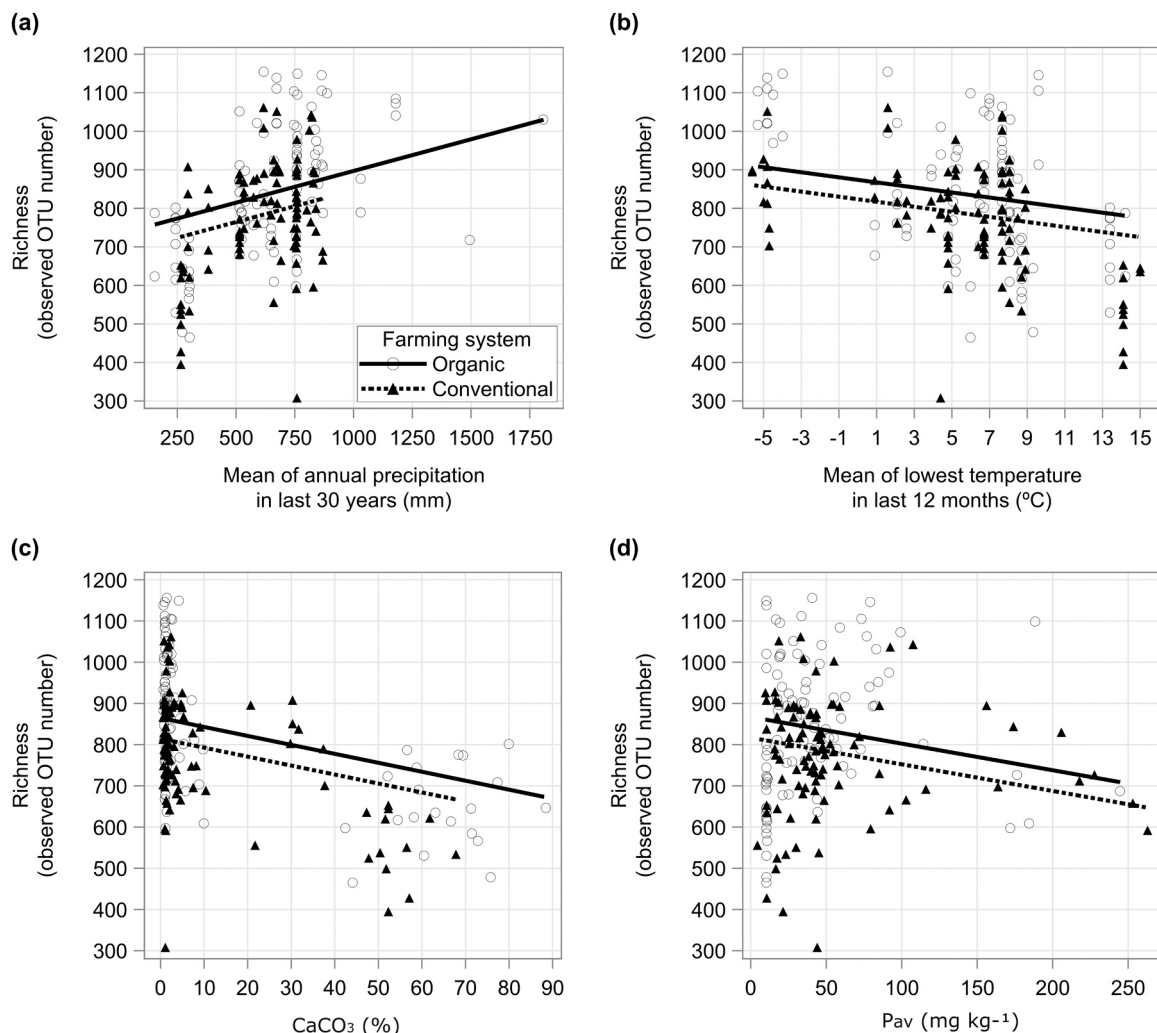| Variable | F | $R^2$ | P |
|---|---|---|---|
| Zone | 16.5529 | 0.40413 | 0.001 |
| Farming system | 4.9691 | 0.01516 | 0.001 |
| Zone & farming system | 2.5357 | 0.06191 | 0.001 |
| Residuals | | 0.51880 | |



**Fig. 1.** The average predictions for soil richness (observed OTU number) by the explanatory variables of the linear mixed model.
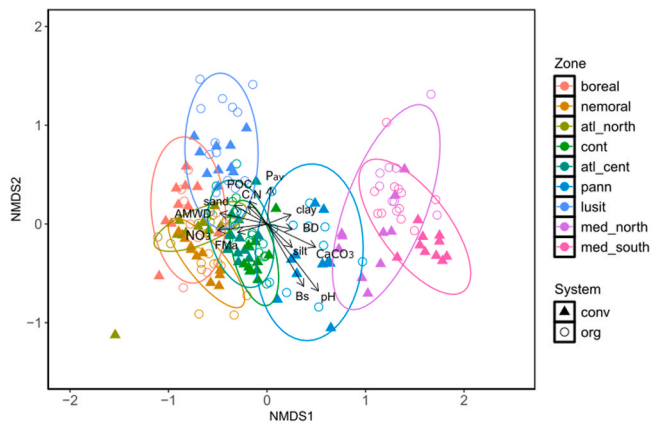
**Fig. 2.** NMDS ordination from the fungal ITS derived OTU data. Different symbols separate OTUs by farming systems (conv, conventional; org, organic) and colors by pedoclimatic zones. Ellipses show 95% confidence level for a multivariate t-distribution according to zones. Vectors represent significant environmental variables affecting the composition (*P*<0.05). Explanations of the abbreviations for soil chemical and physical data behind the environmental vectors is listed in the supplementary Table S3.

### 3.3. Impacts of farming system on fungal community

The farming system alone had a significant effect on fungal richness (*P*=0.0008) and Chao1 estimates (*P*=0.002), but not on the diversity indices (*P*=0.16 for Shannon, *P*=0.48 for Inverse Simpson). Organic farming generally increased fungal richness (*P*<0.0001, with estimates for conventional and organic farming being 840 and 772 OTUs, respectively). Together, the pedoclimatic zone and farming system had significant effects both on richness (*P*=0.009) and Chao1 estimates (*P*=0.007) and diversity values (*P*=0.03 for Shannon, *P*=0.04 for

Inverse Simpson). Richness, Chao1 estimate, and Shannon index were higher under organic farming in Lusitanian and Nemoral zones compared to other zones (Fig. 3 and S6a). In turn, Inverse Simpson index was higher under conventional farming in Pannonian zone compared to other zones (Fig. S6b).

Based on PERMANOVA analysis, the farming system (conventional or organic farming) alone explained 1.5% ($R^2$= 0.0151, *P*=0.001) and with the zone 6% ($R^2$= 0.0619, *P*=0.001) of the variation in soil fungal community composition (Table 2). The community composition of AMF did not show clustering according to farming system (Fig. S4). In addition, relative abundances of AMF community did not differ between farming systems in any zone (Fig. S5).

In general, the farming system (*P*=0.08) individually did not influence the number of saprotrophs. Nevertheless, the farming system together with the pedoclimatic zone significantly affected the relative abundances of all investigated guilds, including saprotrophs (*P*=0.02), pathotrophs (*P*=0.03), and symbiotrophs (*P*=0.04) (Figs. 4–6). In the Lusitanian zone, there were more saprotrophs in the conventional farming system compared to organic farming (Fig. 4). Conversely, organic farming in the Continental and Lusitanian zones had a higher relative abundance of pathotrophs compared to conventional farming (Fig. 5). Additionally, organic farming in the Lusitanian and South Mediterranean zones exhibited a higher relative abundance of symbiotrophs compared to conventional farming (Fig. 6).

### 3.4. Environmental factors predicting fungal OTUs in pedoclimatic zones

Using the logistic regression machine learning, we identified six potential models for the pedoclimatic zones. Except for the Mediterranean North, the cross-validated accuracy point estimate exceeded the no information rate, indicating predictive potential for most zones. However, the 95% confidence intervals for each model included values lower than the no information rate, suggesting some noise in the results.
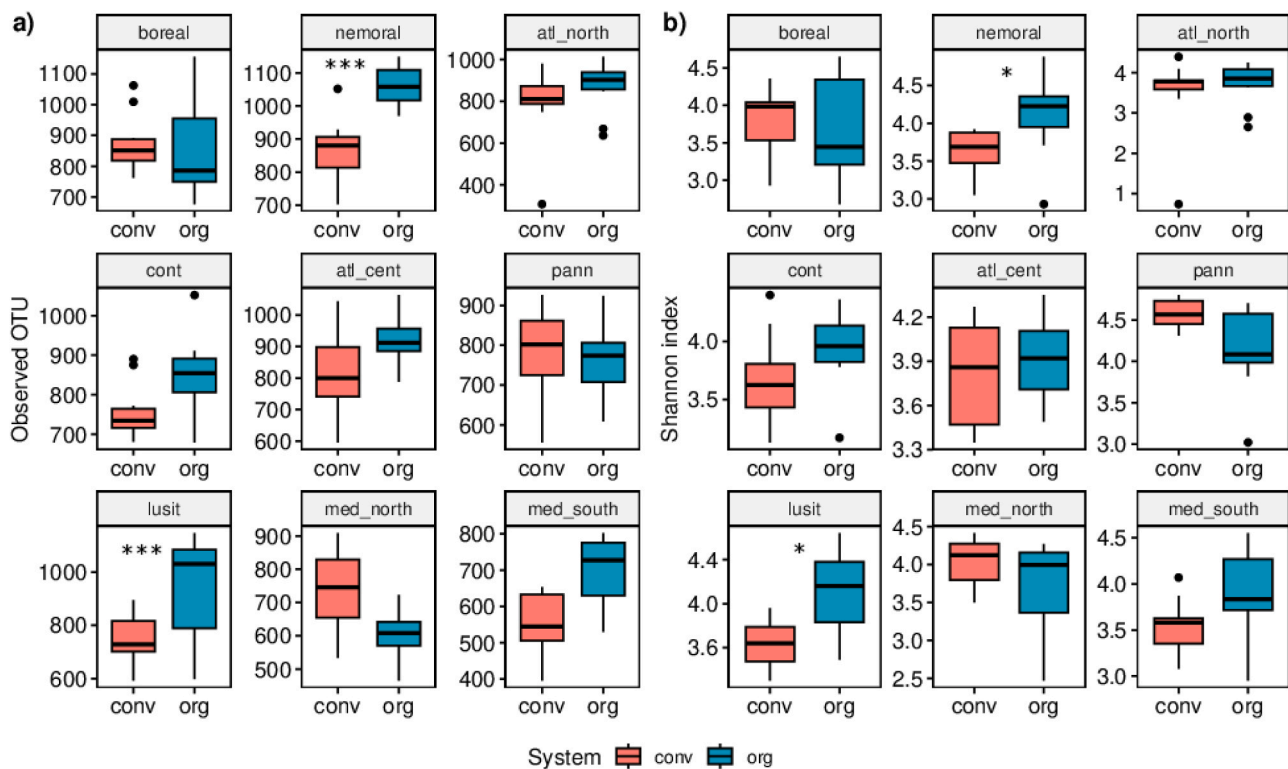


**Fig. 3.** Average a) richness (observed OTU number) and b) Shannon index values in pedoclimatic zones between conventional (conv) and organic (org) farming systems. Asterisks show the statistical significance between farming systems according to linear mixed model and pairwise comparison with Tukey-Kramer methods and *P*-values: **** <0.0001, *** <0.001, ** <0.01, * <0.05.
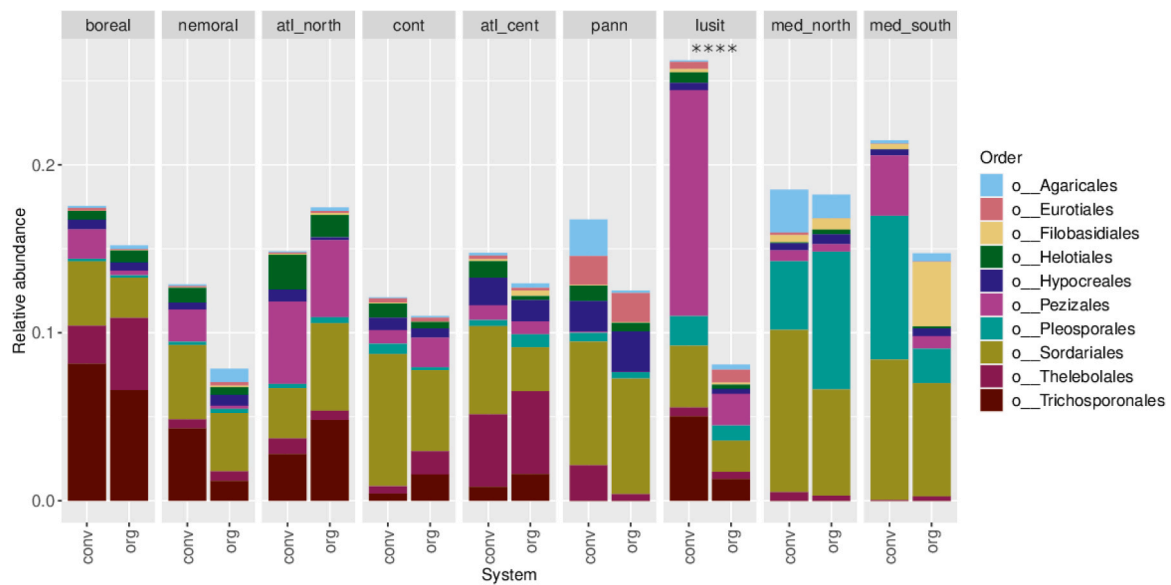
**Fig. 4.** The relative abundance of the most dominant saprotrophic orders in the pedoclimatic zones between conventional (conv) and organic (org) farming systems. Asterisks show the statistical significance in the relative abundance of saprotrophs between systems according to GLMM. *P*-values: **** <0.0001, *** <0.001, ** <0.01, * <0.05.
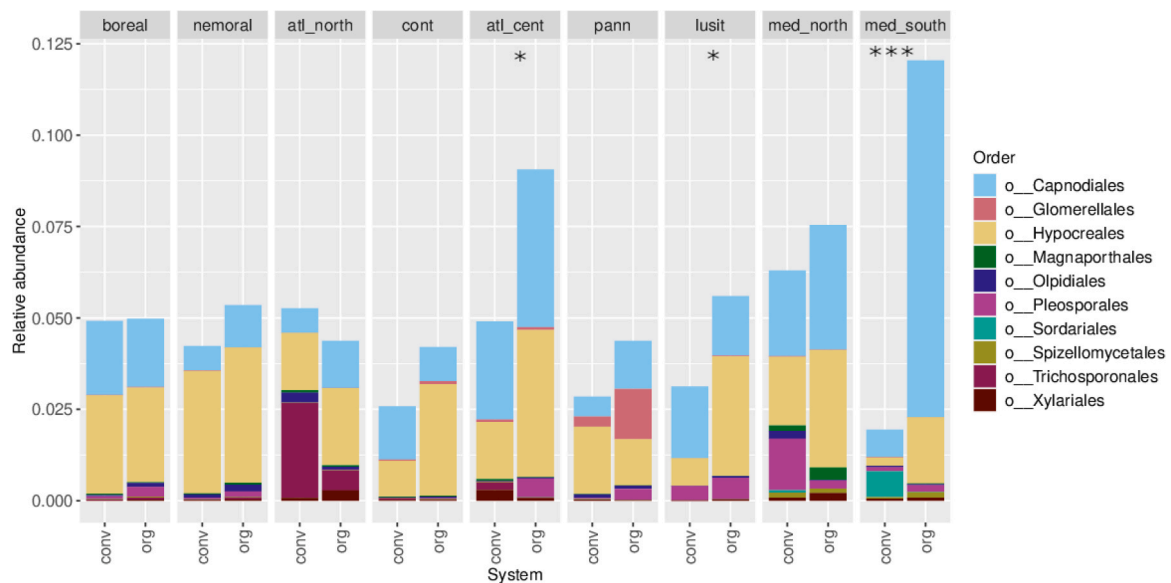


**Fig. 5.** The relative abundance of the most dominant pathotrophic orders in the pedoclimatic zones between conventional (conv) and organic (org) farming systems. Asterisks show the statistical significance in the relative abundance of pathotrophs between systems according to GLMM. *P*-values: **** <0.0001, *** <0.001, ** <0.01, * <0.05.

Consequently, we conclude that while the models hold potential, further improvement could be achieved by gathering more data and constructing more sophisticated models.

Bulk density in the depth of 0–10 cm and soil pH and were the most common explanatory factors predicting relative OTU abundances in many zones (Table S5). However, the same explanatory factors did not consistently predict the relative abundance of the same fungal OTUs. For instance, in both the Central Atlantic and Pannonian zones, increasing soil pH predicted increased relative abundance of *Acremonium furcatum* and decreased relative abundance of *Fusarium acutatum* and *Mortierella elongate*. Conversely, in the Boreal zone, increasing soil pH predicted increased relative abundance of *Saitozyma podzolica* and *Solicoccozyma terricola*, whereas in the South Mediterranean zone, it predicted increased relative abundances of *Mycosphaerella tassiana*, Nectriaceae,

and *Alternaria chlamydosporigena*. Similarly, higher bulk density in the Central Atlantic and Boreal zones predicted increased relative abundance of *S. podzolica*, whereas in the Mediterranean zones, it predicted increased relative abundance of Nectriaceae and *A. chlamydosporigena*, and in the Nemoral zone, it predicted increased relative abundance of *A. furcatum*.

Moreover, increasing $P_{av}$ in both the Lusitanian and Nemoral zones predicted increased relative abundance of *A. furcatum*, but in the Lusitanian zone, it also predicted increased relative abundance of *Pseudaleuria* and *Humicola grisea*. Higher clay content in the Lusitanian zone predicted increased relative abundance of *F. acutatum* and *M. elongata*, while in the South Mediterranean zone, it predicted increased relative abundance of *Botryotrichum atrogriseum*. Additionally, certain explanatory factors were specific to particular zones. For example, higher soil
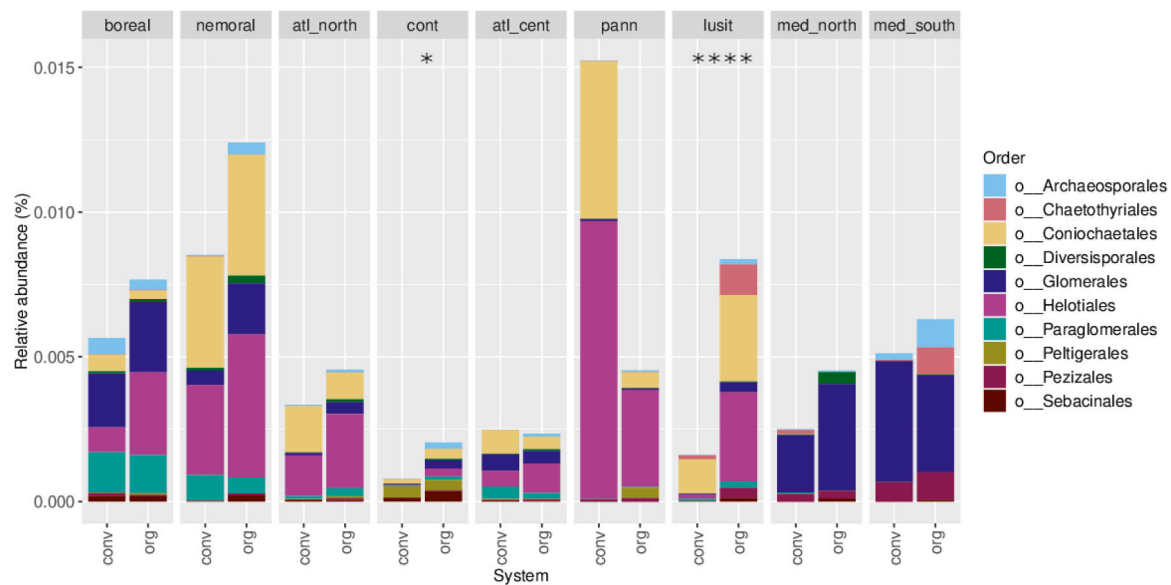
**Fig. 6.** The relative abundance of the most dominant symbiotrophic orders in the pedoclimatic zones between conventional (conv) and organic (org) farming systems. Asterisks show the statistical significance in the relative abundance of symbiotrophs between systems according to GLMM. *P*-values: **** <0.0001, *** <0.001, ** <0.01, * <0.05.

aggregate size in the South Mediterranean zone predicted increased relative abundance of *M. tassiana*, Nectriaceae, and *A. chlamydosporigena*. In the Boreal zone, soil moisture predicted increased relative abundance of the same OTUs as soil pH and bulk density, and the organic farming system predicted increased relative abundances of *Mortierella horticola, Solicoccozyma terrea,* and *Apiotrichum dulcitum*. In the Continental zone, higher soil organic matter content and the absence of legumes in crop rotation predicted increased relative abundance of *A. furcatum*.

### 3.5. Differentially abundant fungal OTUs between farming systems

The Atlantic Central and Boreal zones exhibited the fewest differentially abundant OTUs between farming systems (Table S6). In the Central Atlantic zone, the only differentially abundant OTU for organic farming belonged to the order Xylariales, while the Boreal zone had two differentially abundant OTUs for organic farming belonging to the genus *Leptodiscella* and the class Sordariomycetes. In the Nemoral zone, the differentially abundant OTUs for organic farming included the genera *Podospora* and *Rhizopydium*, as well as the order Sebacinales. Similarly, the Pannonian zone had differentially abundant OTUs for organic farming, including genera *Trichoderma* and *Fusarium*.

In the North Atlantic zone, there were over twice as many differentially abundant OTUs indicative of organic farming (26 OTUs) compared to conventional farming (12 OTUs). The differentially abundant OTUs for organic farming belonged to the ascomycetes classes Eurotiales, Dothideomycetes, Leotiomycetes, and Sordariomycetes. They included the orders Hypocreales (genera *Acremonium, Dactylonectria, Fusarium, Metarhizium*), Sordariales (genus *Remersonia*), Glomerellales (*Plectosphaerella*), Pleosporales (genera *Boeremia, Didymella, Paraphaeosphaeria, Torula*), Helotiales (genus *Cadophora*), and Eurotiales (genera *Aspergillus* and *Penicillium*). In the Continental zone, the differentially abundant OTUs for organic farming belonged to the genus *Dactylonectria* and the order Pleosporales. On the other hand, the differentially abundant OTUs for conventional farming included the genera *Sclerostagonospora* (Pleosporales) and *Byssochlamys* (Eurotiales) of the Ascomycota phylum, as well as the genus *Sporobolomyces* of the Basidiomycota phylum.

Significantly more differentially abundant OTUs were detected in the Lusitanian and Mediterranean zones for both farming systems compared to other zones. In the Lusitanian zone, 68 differentially abundant OTUs

were detected for conventional farming, while 16 were detected for organic farming. Most of the differentially abundant OTUs for organic farming belonged to the ascomycetes classes Eurotiomycetes, Dothideomycetes, Leotiomycetes, and Sordariomycetes. They included taxa such as *Cadophora, Chaetomium, Metarhizium, Neonectria, Tolypocladium*, and *Dichotomopilus*. Conversely, the differentially abundant OTUs for conventional farming included the genera *Fusarium, Apiotrichum, Articulospora, Cladophialophora, Emericellopsis, Gymnoascus,* and *Petriella*. In the North Mediterranean zone, almost all the differentially abundant OTUs (90% of them) were detected for organic farming. Many of these belonged to ascomycetes, including the genera *Arachnomyces, Chaetomium, Coprinus, Podospora, Stillbella,* and *Thielavia*, as well as the glomeromycetes *Diversispora* and *Claroideoglomus*. In the South Mediterranean zone, there were 68 differentially abundant OTUs for conventional farming and 28 for organic farming. Many differentially abundant OTUs for organic farming were not identified at the genus level but showed closest relation to the order Pleosporales, class Sordariomycetes, and family Nectriaceae. However, they included OTUs belonging to the genera *Alternaria, Gibberella, Lophiotrema, Mycosphaerella, Nectria,* and *Schizothecium*. Conversely, differentially abundant OTUs for conventional farming included the genera *Alternaria, Aspergillus, Botryotrichum, Fusarium, Chaetomium, Iodophanus, Parascenodosporium, Pithoascus, Preussia, Rhizophlyctis,* and *Trichophaeopsis*.

## 4. Discussion

Our objective was to examine soil fungal biodiversity in European agroecosystems characterized by varying pedoclimatic and soil features under both conventional and organic farming systems. Consistent with our first hypothesis, notable disparities in soil fungi were observed across the pedoclimatic zones. As anticipated, these variations correlated with distinct climatic attributes and soil characteristics typical of each zone. For instance, higher fungal richness was predicted in areas with high long-term precipitation and low minimum temperatures in the 12 months preceding sampling. This phenomenon likely contributed to the highest fungal richness observed in the Nemoral zone, characterized by the lowest minimum temperature, while the South Mediterranean zone, with the highest minimum temperature and lowest long-term precipitation, exhibited the lowest fungal richness. These findings echo those of a recent study where water stress emerged as a primary

factor influencing fungal diversity across diverse climatic and vegetation settings (Zhang et al., 2023). Elevated temperatures are known to favor slow-growing fungal species over their fast-growing counterparts, potentially impacting the richness of various functional groups (Wesener et al., 2023).

The distinct fungal community compositions observed in the most northern (Boreal, Nemoral, North-Atlantic) and two southern (south and north Mediterranean) zones underscore significant differences between fungi inhabiting cooler northern climates and warmer southern climates. Nevertheless, our results underscore the undeniable influence of climatic factors, including temperature and water availability, in regulating fungal biomass, diversity, and community composition (Siles et al., 2023; Zhang et al., 2023).

In support of the first hypothesis, soil characteristics exerted a significant influence on both the diversity and composition of soil fungi. Generally, lower levels of $CaCO_3$ and $P_{av}$ were indicative of higher fungal richness. Moreover, $P_{av}$ levels exhibited an impact on the relative abundance of specific fungal taxa. At the regional scale, elevated $P_{av}$ content in the Lusitanian and Nemoral zones emerged as a potential factor associated with the proliferation of the fungal root endophyte and potentially pathogenic *Acremonium furcatum*. Concurrently, higher $P_{av}$ levels were correlated with decreased occurrences of several species within the genus *Mortierella* in these zones. *Mortierella* fungi are typically characterized as widespread saprotrophs capable of surviving diverse environmental conditions and utilizing various C sources containing cellulose, hemicellulose, and chitin. Certain strains also function as plant growth-promoting fungi, aiding plants in accessing available forms of phosphorus and iron, producing phytohormones, and shielding them from pathogens (Ozimek and Hanaka, 2021).

In numerous other pedoclimatic zones, increased bulk density in the 0—10 cm depth and soil pH emerged as significant predictors affecting changes in the relative abundances of *Mortierella*. Cluster analysis further unveiled that soil pH exerted an influence on fungi at the individual (OTU) level, contingent upon the zone. Mediterranean soils stood out from others due to their elevated pH levels, $CaCO_3$ content, and base cation content. These pronounced disparities in soil pH-related properties likely account for the divergent outcomes observed in fungal community composition. For instance, fungal genera capable of producing plant hormones (*Saitozyma*, *Solicoccozyma*) were more prevalent in the Boreal zone, while common pathogenic taxa (*Mycopshaerella*, *Nectriaceae*, *Alternaria*) exhibited increased occurrence in the South Mediterranean zone with rising soil pH levels.

Consistent with the second hypothesis, disparities in soil fungi among zones are also influenced by the farming system. According to our findings, organic farming generally enhances fungal richness. A study in Germany reported an increase in fungal taxa in winter wheat fields under organic farming (Garnica et al., 2020). However, upon separate examination of farming systems within different zones, organic farming exhibited an increase in both richness and Shannon diversity only in the Lusitanian and Nemoral zones. These zones displayed lower $P_{av}$ levels under organic farming, and a negative correlation between fungal alpha diversity and $P_{av}$ was observed in wheat fields (Wu et al., 2022). In line with the second hypothesis, the distinct fungal community composition in the Lusitanian zone can also be explained by the content of POC under organic farming. POC is known to be the predominant form of accumulated C under organic farming practices (Chan, 2001).

Soil pH can indirectly be associated with the farming system, as organic fertilizers may decrease soil pH in alkaline soils and increase it in acidic soils (Wei et al., 2017). Moreover, higher soil pH and bulk density in the surface soil of Mediterranean zones may potentially account for the abundance of fungal taxa, including certain pathogens. Larger soil aggregate size, associated with organic farming (Williams, et al., 2017), has been linked to increased soil aggregate stability and soil organic C concentration in bulk soils (Zhou et al., 2020). However, the rotation cycle exhibited only a weak association with organic farming in our fields. Additionally, soil type significantly impacts soil aggregate classes, although long-term cultivation may lead to soil structure deterioration and clod formation (Ciric et al., 2012). Challenging climatic conditions, particularly the warm and dry conditions in Mediterranean zones, may hinder the positive effects of organic farming management practices on soil physical and chemical properties. This can influence the abundance of both potential pathogens and beneficial fungi, partly supporting the second and third hypotheses.

To further support our third hypothesis, many symbiotrophs detected from the Lusitanian zone belong to the orders Chaetothytriales, Coniochaetales, and Helotiales, containing beneficial dark septate endophytic (DSE) fungi, as identified in the differential abundance analysis. DSE fungi provide nutrients for plants from soil organic matter (SOM) and shield plants from pathogens and harsh soil conditions (Berthelot et al., 2019). Due to their capacity to produce highly melanized and resistant hyphae, DSE fungi may play a crucial role in sequestering C into more stable SOM (Siletti et al., 2017). The presence of DSE fungi may also indicate root presence, thereby contributing to the observed accumulation of POC. Furthermore, the soil in Lusitania exhibited the lowest pH (5.5). Hence, it is plausible that endophytes have replaced AMF at low pH, mitigating some of the adverse effects of high soil acidity on plants (Postma et al., 2007).

In support of the third hypothesis, the endophytic group Sebacinales, commonly encountered in agricultural soils, exhibited higher abundance under organic farming in the Nemoral zone. Sebacinales are recognized for their beneficial effects on plant health, including enhanced resistance to abiotic and pathogenic stresses (Weiß et al., 2016). Consequently, Sebacinales could potentially serve as indicative taxa for organic farming, as they have been reported to be prevalent in wheat roots from fields managed organically in previous studies conducted in Central Europe (Riess et al., 2014; Verbruggen et al., 2014). Moreover, recent research on strawberry roots indicated that five OTUs belonging to Sebacinales exhibited sensitivity to glyphosate, a herbicide prohibited in organic farming (Mathew et al., 2023). Sebacinales have also been identified in plots receiving organic amendments in conventionally farmed experimental field sites in Finland, suggesting an association with improved soil quality (Rasa et al., 2021). Additionally, the endophytic genus *Chaetomium* may experience an increase in Mediterranean zones under organic farming, alongside fungi associated with dung saprotrophs. Coprophilous fungi are likely to benefit from the manure fertilization commonly employed in organic farming.

AMF are considered crucial in organic farming as biofertilizers, potentially increasing plant productivity by 16—78% (Ebbisa, 2022; Ryan and Tibbett, 2009). They can also serve as bio-controllers, substituting pesticides (Wang et al., 2019). Furthermore, AMF secrete the glycoprotein glomalin, a significant pool of soil C linked to soil C storage through its influence on soil aggregate stabilization (Rillig et al., 2004b). Although the study did not detect an overall effect of zone or farming system on the symbiotic AMF community, the differential abundance analysis unveiled numerous symbiotrophic fungal taxa, supporting the third hypothesis. For example, the AMF genera *Diversispora* and *Claroideoglomus* potentially increase in organic farming systems in Mediterranean zones, as reported by Cuartero et al. (2021). Reduced tillage or non-tillage practices are known to favor AMF abundance (Kabir, 2005; Dai et al., 2015). However, all fields in the Mediterranean zone were conventionally plowed, suggesting that differences in tillage practices cannot explain our results.

Organic farming potentially increased symbiotic fungal taxa in the Continental zone as well, particularly those belonging to the AMF orders Glomerales and Archaeosporales, along with DSE fungi containing Helotiales. The absence of pesticides was the most closely associated management practice in organic farming, possibly explaining the increased abundances of certain symbiotic taxa. The reported effects of pesticides on AMF vary widely, often yielding contradictory results influenced by factors such as soil type, crop plant, cropping history, fungal species, experiment duration, and application mode and volume (reviewed in Hage-Ahmed et al., 2019). Organic fertilization, commonly

employed in organic farming, is another factor likely explaining some results from the differential abundance analysis, as manure has been demonstrated to be the most significant factor impacting the AMF community (Zhu et al., 2016), benefiting, for instance, the *Funneliformis* genus of AMF (Cuartero et al., 2021).

Organic farming in the North Atlantic zone also increased Pleosporales, which fulfill multiple roles, from saprotrophs to parasites and epiphytes. Previous studies have indicated that practices commonly applied in organic farming augment the abundance and species diversity of plant-associated endophytic fungi, such as those belonging to Pleosporales, which have the potential to enhance plant yields and health (Xia et al., 2019). Conversely, organic farming may promote the abundance of *Dactylonectria* in the Continental zone. This taxon encompasses representatives capable of acting in various roles as pathogens, endophytes, and saprotrophs. On the other hand, a higher soil organic matter content or the absence of legumes in crop rotation predicted an increase in the abundance of *Acremonium furcatum,* known for its capability to produce antimicrobial metabolites against bacteria and phytopathogenic fungi (Gallardo et al., 2006).

It is imperative to acknowledge inherent limitations when conducting continental-scale sampling across the EU. When sampling diverse locations spanning wide biogeographical regions, local weather conditions remain beyond control. Additionally, sampling may not always occur under optimal conditions due to practical constraints. Consequently, the interpretation of results is influenced by various abiotic and biotic factors, along with the history of farming practices, such as the duration of a field under a particular farming system. Moreover, the soil type can impact the yield and quality of extractable DNA (Dequiedt et al., 2011). Nonetheless, the findings of this study, which encompassed commercial farms across Europe and thus encompassed diverse climatic and environmental conditions, serve to complement and broaden the insights gained from numerous single-site controlled field experiments conducted across the continent.

## 5. Conclusions

This study provides novel insights into soil fungi in European wheat fields spanning from northern to southern latitudes under both conventional and organic farming systems. We observed that endophytes with potential beneficial roles as symbiotrophs and plant-growth promoters tend to predominate under organic farming in cooler and moister climates, or in soils with lower $P_{av}$ and pH levels. Conversely, pathotrophs may become more prevalent under organic farming in zones characterized by drier and warmer climates, higher bulk density, $CaCO_3$ content, base cation content, and soil pH. These findings align with those of a European long-term experiment, indicating site- and soil-specific links between fungal groups and management practices (Hannula et al., 2021).

Our study demonstrates the significant influence of regional climatic and edaphic conditions on shaping fungal diversity, community composition, and fungal groups. Furthermore, our results suggest that variations in soil fungi between farming systems are influenced by the pedoclimatic zone, which is defined by specific climatic and soil characteristics. We echo previous findings emphasizing the importance of considering both farming-system-independent factors, such as changes in climatic conditions, and system-dependent factors, including alterations in preceding crops and farmyard manure, when comparing fungal communities between systems (Schneider et al., 2010).

Moreover, our results underscore the necessity of investigating fungal groups with different ecological roles, as this factor profoundly impacts the stability of terrestrial ecosystems (Liu et al., 2022). Overall, our study highlights the importance of gaining a deeper understanding of the linkage between abiotic climate and soil-induced factors and the fungal community in European arable soils. Given the potential variability of climate warming effects across different pedoclimatic zones, it is imperative to comprehensively explore the various factors influencing soil fungi in agroecosystems, as well as the role of management practices in promoting their growth.

## CRediT authorship contribution statement

**Lieven Waeyenberge:** Investigation, Funding acquisition, Data curation, Conceptualization. **Hannu Fritze:** Writing – review & editing, Funding acquisition. **David Calviño:** Resources, Project administration, Investigation, Funding acquisition, Data curation, Conceptualization. **Sari Iivonen:** Writing – review & editing, Data curation. **Simon Lassen:** Investigation, Data curation. **Kaire Loit:** Investigation, Funding acquisition. **Krista Peltoniemi:** Writing – review & editing, Writing – original draft, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Silvia Martínez-Martínez:** Funding acquisition, Data curation, Conceptualization. **Sannakajsa Velmala:** Writing – review & editing, Writing – original draft, Methodology, Funding acquisition, Formal analysis. **Taina Pennanen:** Writing – review & editing, Funding acquisition. **Eva Lloret:** Writing – review & editing, Investigation, Data curation. **Marian Põldmets:** Investigation. **Irene Ollio:** Writing – review & editing, Investigation, Data curation. **Stefan Schrader:** Investigation, Funding acquisition, Data curation, Conceptualization. **Juha Hyvönen:** Writing – review & editing, Methodology, Formal analysis. **Eero Liski:** Writing – review & editing, Methodology, Formal analysis. **Merrit Shanskiy:** Investigation, Funding acquisition, Data curation, Conceptualization. **Kristian Brandt:** Writing – review & editing, Investigation, Funding acquisition, Data curation, Conceptualization. **Raúl Zornoza:** Investigation, Funding acquisition, Data curation, Conceptualization. **Claudia Campillo-Cora:** Investigation, Funding acquisition, Data curation, Conceptualization.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data Availability

Data will be made available on request.

## Acknowledgements

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2024.109035.

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