



Coprophilous fungal spores in modern lowland agropastoral ecosystems of Eastern-Central Europe reflect herbivore grazing pressure

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Abstract

Herbivores play a pivotal role in shaping vegetation dynamics, yet the intensity of past grazing remains difficult to reconstruct. Coprophilous fungal spores (CFS) are widely used to trace grazing by herbivores, but their potential to reflect grazing pressure has been less explored. Here, we present the first systematic assessment of CFS diversity to grazing pressure in European lowland agropastoral ecosystems, based on surface soil and dung samples from diverse habitats in Romania. While a quantitative reconstruction of herbivore density remains challenging due to animal mobility, we identified several CFS types associated with elevated grazing pressure. *Sordaria*, *Podospora*, *Sporormiella*, and *Delitschia*-types emerged as robust indicators of grazing pressure and/or herbivore density, while other taxa, such as *Arnium*, *Bombardioidea*, and *Trichodelitschia*-types, occurred only sporadically. Notably, *Sordaria* and *Sporormiella* abundance commonly peaked under heavy grazing. Additional fungal taxa with occasional coprophilous affinities, including *Apiosordaria*, *Cercophora*, *Chaetomium*, *Coniochaeta*, *Gelasinospora*, and *Neurospora*-types, also tracked grazing activity, and *Glomus* strongly indicated heavily grazed environments. CFS abundance was further elevated in moist habitats, spring samples, and areas where animals congregate. Independent CFS quantification as concentrations or percentages relative to the fungal sum is preferred over a pollen sum approach. Our findings also emphasise the value of plant indicators, such as *Plantago lanceolata* and Asteraceae for strengthening grazing regime reconstructions in Eastern-Central European lowlands. Key suggestions for future studies include broadening the range of fungal spore types used to reconstruct past grazing intensity, identifying CFS typical for certain dung, and standardizing identification and nomenclature protocols to enhance comparability across palaeoecological studies.

Keywords Coprophilous fungal spores · Domestic herbivores · Herbivore density · Grazing regime · Dung surface samples · Romania · Grasslands

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Introduction

Grazing by herbivores impacts vegetation distribution and dynamics and maintain vegetation in a more open state (Bond 2019; Stevens et al. 2022). The magnitude of herbivores' impact largely depends on their density and community composition, i.e. body size and dietary preference (grazers, mixed feeders, and browsers). Wild herbivores, particularly megafauna, have declined in abundance, diversity, and body size, over the past 15 ka, associated with an increase in the abundance of domestic herbivores with more intensive, localised herbivory pressure (Németh et al. 2017; Staver et al. 2021; Pearce et al. 2023; Davoli et al. 2024; Lundgren et al. 2024; Dal Corso et al. 2025). This shift in herbivores' composition led to changes in vegetation composition and reduced diversity raising the question of how herbivory by livestock compensates wild herbivores in sustaining more open ecosystems. Understanding the effect of grazing by domestic herbivores is particularly critical when livestock is introduced for grassland management and restoration (Feurdean et al. 2018; Török et al. 2024).

Herbivores leave coprophilous fungal spores (CFS) in their environments that can be preserved for long periods. CFS are a diverse group; while some species grow exclusively on animal dung, others thrive on a variety of substrates, including soil, charred and saprotrophic substrate (Bell 1983; van Geel 2001; Richardson 2001; Krug et al. 2004; Cugny et al. 2010; van Asperen 2017; Shumilovskikh et al. 2021; Lee et al. 2022; Zimny et al. 2024, 2025). The occurrence of CFS in sediments is therefore interpreted as evidence for past herbivores and their probable impact on the structure and composition of ecosystems (Davis 1987; Gill et al. 2009; Baker et al. 2013; Johnson et al. 2016; Perrotti et al. 2022; Shumilovskikh et al. 2022; Feurdean et al. 2025).

Recent studies have aimed at improving the diagnostic tools of coprophilous fungal spores; however, many aspects remain to be established. First, modern dung contains a diverse range of fungal spores. However, only a comparatively small subset is typically observed in palaeoecological records due to poor preservation in sediments and the difficulties in identifying detailed anatomical features (Richardson 2001; Dietre et al. 2012; Miola et al. 2012; van Asperen et al. 2016; Lee et al. 2022; Wei et al. 2024). While the number of CFS articles and databases used to identify them has grown, research still largely focuses on a few well-known taxa, such as *Sporormiella*-, *Podospora*- and *Sordaria*-type. Second, growing efforts aim to refine the diagnostic significance of CFS for distinguishing dung types and, thus, specific herbivore taxa. While no fungi or CFS are strictly exclusive to one dung type, some types consistently appear in higher abundances on certain dung (Richardson 2001;

Krug et al. 2004; Schlütz and Shumilovskikh 2017; Wei et al. 2024). Moreover, the dung of ruminant taxa (e.g. cattle, deer) tends to be dominated by fungal species with hyaline, thin-walled spores (*Cheilymenia*) that are less likely to be preserved, whereas hind-gut fermenters (e.g. elephants, horses) produce dung richer in thick-walled spores i.e. *Sporormiella* (van Asperen et al. 2016; Lee et al. 2022). These anatomical differences can influence how coprophilous spores are represented in the palaeoecological record, underestimating the livestock density particularly of cattle, sheep and goats. Third, there is no complete agreement between the dung spore abundance and herbivory density, with some studies reporting a strong correlation (Baker et al. 2016; Wei et al. 2024), while other have found that this relationship is strongly influenced by water transportation (Etienne et al. 2013). Fourth, environmental factors such as habitat type and climatic conditions strongly influence CFS production, with moisture availability favouring spore development and preservation (Richardson 2001; van Asperen et al. 2020; see review by Lee et al. 2022). Fifth, CFS are typically deposited close to the fruiting body (a few meters), but transport by wind and water may carry them much farther (Etienne et al. 2013; Baker et al. 2016; Lee et al. 2022 and references therein). Finally, spore taxa with thin-walled or hyaline structures (e.g. some Basidiomycetes) are more susceptible to chemical damage during pollen processing, and small size spores (e.g. *Sporormiella*) may be lost when larger sieve meshes are used during sample preparation, thus affecting both the abundance and composition of spores in palaeoecological records (van Asperen 2017).

A better understanding of how modern CFS assemblages relate to herbivore abundance and type is crucial for refining the use of sedimentary CFS as indicators of past herbivory pressure. In this study, we examine surface samples from lowland agropastoral ecosystems in Romania to improve the interpretation of CFS signals of livestock biomass and type. To our knowledge, this is the first study of its kind conducted in a European lowland agropastoral ecosystem. Specifically, we aim to (i) detect common types or assemblages of CFS indicative of grazing; (ii) identify CFS or assemblages typical for specific herbivore taxa; (iii) investigate the relationship between the abundance of CFS and herbivore biomass, and thereby grazing pressure regime; (iv) assess the influence of environmental drivers (e.g. vegetation types and climate) and transportation processes on the production, preservation and distribution of CFS. Comparison with published records of fossil CFS from Europe evaluates the suitability of CFS assemblages used so far for inferring past grazing regime pressure. Finally, we provide future recommendation on the use of CFS to refine our

capacity to interpret grazing pressure by herbivores and ecosystem dynamics from the palaeoecological record.

Materials and methods

Modern samples collection

A total of 40 surface samples (soil, moss cushions, dung, and lake sediments) were collected from two lowland regions in Romania: around Lake Oltina in the southeast and the Cristești wetland in the northeast, both subject to significant

livestock grazing (Fig. 1a, b). The southeastern region has a warm-temperate continental climate (Köppen-Geiger Dfa), whereas Cristești experiences a temperate continental climate (Dfb; Peel et al. 2007). The dominant forests in both regions primarily consist of *Quercus* and *Carpinus* species, however much of the natural vegetation has been converted into arable land and pasture (Fig. 1a, b).

The study areas differed in size, spatial variation in sampling effort, and timing of sample collection. In the Oltina region, 28 samples were collected in autumn 2016, including moss pollsters ($n=2$), surface soils ($n=25$), and dry cattle dung ($n=1$). These samples were gathered from various vegetation communities within a 20 km radius ($\sim 2,000$ ha) around the Lake Oltina and were initially used to calibrate pollen–vegetation relationships (Grindean et al. 2019). In July 2024, additional samples were collected from soil ($n=1$), sheep dung ($n=2$; one dry, one fresh), and cattle dung ($n=1$, dry) in a pasture near Strunga village located at the southern Lake Oltina margin. Surface lake sediments (top 1 cm, $n=5$) were also taken with a split soil corer along a transect extending from the lake margin towards the centre, spaced ~ 600 m apart, to examine the CFS transportation within a large lake (Table 1, Fig. 1a).

In the Cristești region, 12 surface samples were collected in April 2024. Ten sampling points extended over ~ 60 ha were taken near Cristești village and distributed as follows: Cristești village pasture in fenced ($n=4$) and non-fenced ($n=3$) areas; samples from the Cristești wetland, including marginal north ($n=1$), central ($n=1$) and marginal south ($n=1$) with points spaced 50 m apart. Three of the 12 surface samples collected from the pasture (samples 4, 5, 6) were taken from the proximity or together with cattle or sheep dry, old droppings (Fig. 2c). Additionally, one moss sample was taken from a sheep corral and one from abandoned pastures with *Elaeagnus* encroachment at Blândești (Fig. 1b). Each sample consisted of material collected from 1 to 3 adjacent points. Samples collected in three seasons, spring (Cristești 2024), summer (Oltina 2024), and autumn (Oltina 2016), allow us to examine the potential influence of moisture seasonality on CFS dung availability and preservation. Samples were collected in plastic bags and stored in a refrigerator. No changes in sample content i.e. fungal growth were detected before chemical preparation, except for cattle dung sample 7 from Oltina.

In the Oltina region, vegetation types were classified based on botanical surveys conducted in the field (Grindean et al. 2019). These included grasslands with varying moisture levels and grazing intensities, abandoned grasslands overgrown with shrubs and former vineyards, as well as *Quercus*–*Carpinus* forests (Table 1). At Cristești, dominant pasture vegetation was categorized as either primarily grasses (Poaceae) or a mixture of Poaceae and forbs.

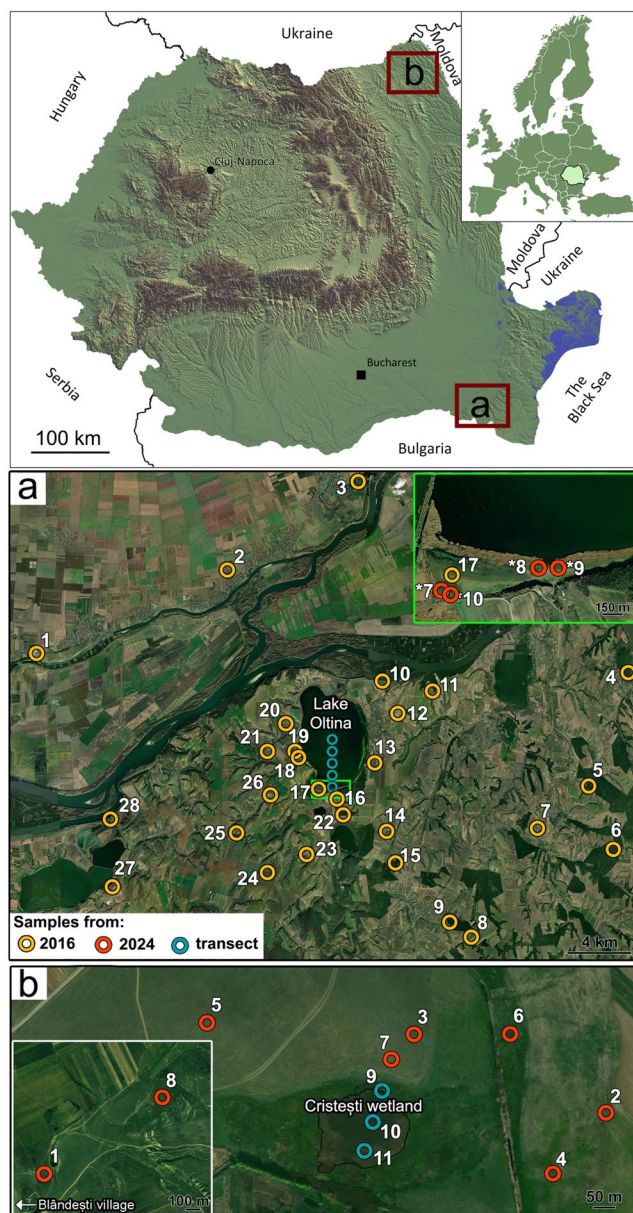


Fig. 1 Location of the two study regions in Romania: Oltina (a) and Cristești (b) and their position within Europe, along with the distribution of surface sampling points in each region

Table 1 The location of the surface samples in dominate vegetation types along with their attribution to a vegetation gradient and grazing intensity in the two regions

No	Vegetation gradient	Dominant plant taxa	Grazing index	Grazing gradient	Live-stock
<i>Oltina</i>					
26	Xeric <i>Botriochloa</i> grassland	<i>Botriochloa</i> , <i>Bromus</i> , <i>Artemisia</i> , <i>Filago</i> , <i>Chondrilla</i> , <i>Plantago lanceolata</i> , <i>Asperula</i> , <i>Scabiosa</i>	4	Overgrazed	Sheep
23	Xeric <i>Botriochloa</i> grassland	<i>Botriochloa</i> , <i>Thymus</i> , <i>Agrimonia</i> , <i>Scabiosa</i> , <i>Asperula</i> , <i>Galium</i> , <i>Medicago</i> , <i>Poa</i> , <i>Thymelaea</i> , <i>Teucrium</i> , <i>Picris</i> , <i>Daucus</i> , <i>Achillea</i>	4	Overgrazed	
8	Xeric <i>Botriochloa</i> grassland	<i>Botriochloa</i> , <i>Stipa</i> , <i>Secale cereale</i> , <i>Daucus</i> , <i>Agrimonia</i> , <i>P. lanceolata</i> , <i>Picris</i> , <i>Xeranthemum</i> , <i>Scabiosa</i> , <i>Cichorium</i> , <i>Achillea</i> , <i>Hieracium</i>	2	Moderately grazed	
7	Xeric <i>Botriochloa</i> - <i>Festuca</i> grassland	<i>Botriochloa</i> , <i>Festuca</i> , <i>Bromus</i> , <i>Plantago lanceolata</i> , <i>Teucrium</i> , <i>Euphorbia</i> , <i>Medicago</i> , <i>Thymus</i> , <i>Hieracium</i>	3	Intensively grazed	
27	Xeric <i>Botriochloa</i> - <i>Festuca</i> grassland	<i>Botriochloa</i> , <i>Festuca</i> , <i>Bromus</i> , <i>Dactylis</i> , <i>Cynodon</i> , <i>Poa</i> , <i>Daucus</i> , <i>Xeranthemum</i> , <i>Medicago</i> , <i>Cephalaria</i> , <i>Thymus</i> , <i>Scabiosa</i> , <i>Centaurea</i> , <i>Asperula</i> , <i>Lotus</i> , <i>Agrimonia</i> , <i>Crataegus</i>	2	Moderately grazed	
16	Xeric <i>Botriochloa</i> grassland	<i>Botriochloa</i> , <i>Festuca</i> , <i>S. cereale</i> , <i>Poa</i> , <i>Chondrilla</i> , <i>Daucus</i> , <i>Xeranthemum</i> , <i>P. lanceolata</i> , <i>Galium</i> , <i>Bromus</i> , <i>Teucrium</i>	4	Overgrazed	Cattle
1	Xeric <i>Cynodon</i> grassland	<i>Cynodon</i> , <i>Trifolium fragiferum</i> , <i>P. lanceolata</i> , <i>Taraxacum</i> , <i>Agrostis</i>	4	Overgrazed pasture	Sheep
4	Xeric <i>Cynodon</i> grassland on abandoned field	<i>Cynodon</i> , <i>Bromus</i> , <i>Achillea</i> , <i>Poa</i> , <i>Artemisia</i> , <i>S. cereale</i> , <i>Convolvulus</i>	2	Moderately grazed	
20	Xeric <i>Botriochloa</i> grassland on abandoned field	<i>Botriochloa</i> , <i>Cynodon</i> , <i>Poa</i> , <i>Bromus</i> , <i>Aegilops</i> , <i>Daucus</i> , <i>Petrorhagia</i> , <i>Artemisia</i> , <i>Galium</i> , <i>Xeranthemum</i> , <i>Lolium</i>	2	Moderately grazed	
25	Xeric <i>Botriochloa</i> grassland on abandoned field	<i>Botriochloa</i> , <i>Daucus</i> , <i>Stipa</i> , <i>Poa</i> , <i>Crepis</i> , <i>P. lanceolata</i> , <i>Bromus</i> , <i>Galium</i> , <i>Asperula</i> , <i>Agrimonia</i> , <i>S. cereale</i> , <i>Achillea</i>	2	Moderately grazed	
2	Xeric <i>Bromus</i> grasslands on abandoned field	<i>Bromus</i> , <i>Medicago</i> , <i>Setaria</i> , <i>Daucus</i> , <i>Achillea</i> , <i>P. lanceolata</i> , <i>Artemisia</i>	4	Overgrazed	
11	Xeric <i>Cynodon</i> ruderal community next to cultivated fields with <i>Brassica</i> and <i>Cerealia</i>	<i>Cynodon</i> , <i>Poa</i> , <i>Bromus</i> , <i>Geum</i> , <i>Convolvulus</i> , <i>Galium</i> , <i>Setaria</i> , <i>Chenopodium</i>	1	Rarely grazed	
12	Xeric <i>Cynodon</i> - <i>Polygonum</i> ruderal community next to cultivated fields with <i>Brassica</i> and <i>Cerealia</i>	<i>Cynodon</i> , <i>Polygonum</i> , <i>Lolium</i> , <i>Setaria</i> , <i>Chenopodium</i> , <i>Fallopia</i>	0	No obvious grazing activity	
13	Meso-xeric <i>Festuca</i> grassland on abandoned vineyard	<i>Festuca</i> , <i>Stipa</i> , <i>Poa</i> , <i>Thymus</i> , <i>Kengia</i> , <i>Teucrium</i> , <i>Asperula</i> , <i>Eryngium</i> , <i>Scabiosa</i> , <i>Euphorbia</i> , <i>Botriochloa</i> , <i>Achillea</i>	1	Rarely grazed	
24	Meso-xeric <i>Crepis</i> grassland on abandoned vineyard	<i>Crepis</i> , <i>Erigeron</i> , <i>Avena</i> , <i>Daucus</i> , <i>Medicago</i> , <i>Picris</i> , <i>Setaria</i> , <i>Cephalaria</i> , <i>Sorghum</i> , <i>Convolvulus</i> , <i>Lactuca</i> , <i>Artemisia</i> , <i>Agrimonia</i> , <i>Vulpia</i>	1	Rarely grazed	
21	Meso-xeric <i>Botriochloa</i> - <i>Stipa</i> grassland on abandoned vineyard	<i>Botriochloa</i> , <i>Bromus</i> , <i>Stipa</i> , <i>Poa</i> , <i>Agrimonia</i> , <i>Odontites</i> , <i>Rubus</i> , <i>Euphorbia</i> , <i>Scabiosa</i> , <i>Daucus</i> , <i>Galium</i> , <i>Achillea</i> , <i>Vitis</i>	1	Rarely grazed	
14	Mesic <i>Cynodon</i> - <i>Lolium</i> grassland	<i>Lolium</i> , <i>Cynodon</i> , <i>Sambucus</i> , <i>P. lanceolata</i> , <i>Cirsium</i> , <i>Achillea</i> , <i>Crepis</i> , <i>Galium</i> , <i>Trifolium</i> , <i>Carduus</i> , <i>Eryngium</i>	2	Moderately grazed	
*7	Mesic <i>Cynodon</i> - <i>Agrostis</i> - <i>Potentilla</i> grassland (cattle dung)	<i>Cynodon</i> , <i>Agrostis</i> , <i>Potentilla</i> , various forbs	3	Intensively grazed	Mixed (sheep, goat, cattle)
*8	Mesic <i>Cynodon</i> - <i>Agrostis</i> - <i>Potentilla</i> grassland	<i>Cynodon</i> , <i>Agrostis</i> , <i>Potentilla</i> , various forbs	3	Intensively grazed	Mixed
*9	Mesic <i>Cynodon</i> - <i>Agrostis</i> - <i>Potentilla</i> grassland (sheep dung)	<i>Cynodon</i> , <i>Agrostis</i> , <i>Potentilla</i> , various forbs	3	Intensively grazed	Mixed
3	Meso-hygrophilous <i>Agrostis</i> - <i>Potentilla</i> grassland	<i>Potentilla</i> , <i>Agrostis</i> , <i>Cirsium</i> , <i>T. fragiferum</i> , <i>Inula</i> , <i>Xanthium</i> , <i>Mentha</i>	2	Moderately grazed	
17	Meso-hygrophilous <i>Cynodon</i> grassland	<i>Cynodon</i> , <i>Carex</i> , <i>Lolium</i> , <i>T. fragiferum</i> , <i>Taraxacum</i> , <i>Cirsium</i> , <i>Mentha</i> , <i>Ononis</i> , <i>Daucus</i> , <i>Verbena</i> , <i>Pulicaria</i>	3	Intensively grazed	Mixed
*10	Meso-hygrophilous <i>Cynodon</i> grassland (sheep dung)	<i>Cynodon</i> , <i>Agrostis</i> , <i>Potentilla</i> , various forbs	3	Intensively grazed	Mixed

Table 1 (continued)

No	Vegetation gradient	Dominant plant taxa	Grazing index	Grazing gradient	Live-stock
18	Hygrophilous <i>Cynodon</i> grassland	<i>Cynodon</i> , <i>Calamagrostis</i> , <i>Erigeron</i> , <i>Daucus</i> , <i>Potentilla</i> , <i>T. fragiferum</i> , <i>Ononis</i> , <i>Sonchus</i> , <i>Taraxacum</i> , <i>Metha</i> , <i>Lolium</i>	3	Intensively grazed	Mixed
19	Hygrophilous <i>Phragmites</i> - <i>Cynodon</i> - <i>Agrostis</i> grassland	<i>Phragmites</i> , <i>Agrostis</i> , <i>Cynodon</i> , <i>Agropyron</i> , <i>Calystegia</i> , <i>Carex</i> , <i>Lactuca</i>	3	Intensively grazed	
28	Meso-hygrophilous <i>Echinochloa</i> grassland within riparian <i>Populus</i> forest	<i>Echinochloa</i> , <i>Xanthium</i> , <i>Helianthus</i> , <i>Polygonum</i> , <i>Chenopodium</i> , <i>Amorpha</i> , <i>Aster</i> , <i>Populus canadensis</i> , <i>Sycios</i>	0	No obvious grazing activity	
10	Open riparian <i>Populus</i> forest with tall herb community	<i>Populus canadensis</i> , <i>Amorpha</i> , <i>Glycyrrhiza echinata</i> , <i>Brachypodium</i> , <i>Poa nemoralis</i> , <i>Daucus</i> , <i>Aster</i> , <i>Sorghum</i> , <i>Aristolochia</i> , <i>Agrimonia</i>	1	Rarely grazed	
5	Young <i>Carpinus orientalis</i> - <i>Quercus cerris</i> forest	<i>Carpinus orientalis</i> , <i>Quercus cerris</i> , <i>Fraxinus ornus</i> , <i>Acer tataricum</i> , <i>Geum</i> , <i>Viola</i>	0	No obvious grazing activity	
6	Young <i>Carpinus orientalis</i> - <i>Quercus cerris</i> forest	<i>C. orientalis</i> , <i>Q. cerris</i> , <i>F. ornus</i> , <i>Piptatherum</i> , <i>Viola</i> , <i>Alliaria</i>	0	No obvious grazing activity	
9	<i>Quercus cerris</i> - <i>Carpinus orientalis</i> forest	<i>Q. cerris</i> , <i>C. orientalis</i> , <i>F. ornus</i> , <i>Acer campestre</i> , <i>A. tataricum</i> , <i>Piptatherum</i> , <i>Viola</i> , <i>Alliaria</i> , <i>Crataegus</i> , <i>Rosa</i> , <i>Geum</i>	0	No obvious grazing activity	
15	<i>Quercus cerris</i> - <i>Carpinus orientalis</i> forest (moss)	<i>Q. cerris</i> , <i>C. orientalis</i> , <i>F. ornus</i> , <i>A. campestre</i> , <i>Polygonatum</i> , <i>Viola</i> , <i>Fallopia</i> , <i>Prunus mahaleb</i> , <i>Crataegus monogyna</i>	0	No obvious grazing activity	
22	<i>Quercus cerris</i> - <i>Carpinus orientalis</i> forest	<i>Q. cerris</i> , <i>C. orientalis</i> , <i>F. ornus</i> , <i>A. campestre</i> , <i>Geum</i> , <i>Viola</i> , <i>Brachypodium</i>	0	No obvious grazing activity	
<i>Cristești</i>					
1	Xeric grasslands (sheep dung)	Forbs and Poaceae	4	Overgrazed	
2	Xeric grasslands	Mainly Poaceae	4	Overgrazed	
3	Xeric grasslands	Mainly Poaceae	4	Overgrazed	
5	Xeric grasslands	Mainly Poaceae	3	Intensively grazed	
8	Xeric grasslands	Mainly Poaceae encroached by <i>Elaeagnus</i>	2	Moderately grazed	
4	Meso-xeric grasslands	Forbs and Poaceae	4	Overgrazed	
6	Meso-xeric grasslands	Forbs and Poaceae	3	Intensively grazed	
7	Mesic grasslands	Mainly forbs	3	Intensively grazed	
9	Hygrophilous wetland margin north	<i>Phragmites</i>	0	No obvious grazing activity	
10	Hygrophilous wetland central	<i>Phragmites</i>	0	No obvious grazing activity	
11	Hygrophilous wetland margin south	<i>Phragmites</i>	0	No obvious grazing activity	

Laboratory preparation and quantification of coprophilous fungal spores

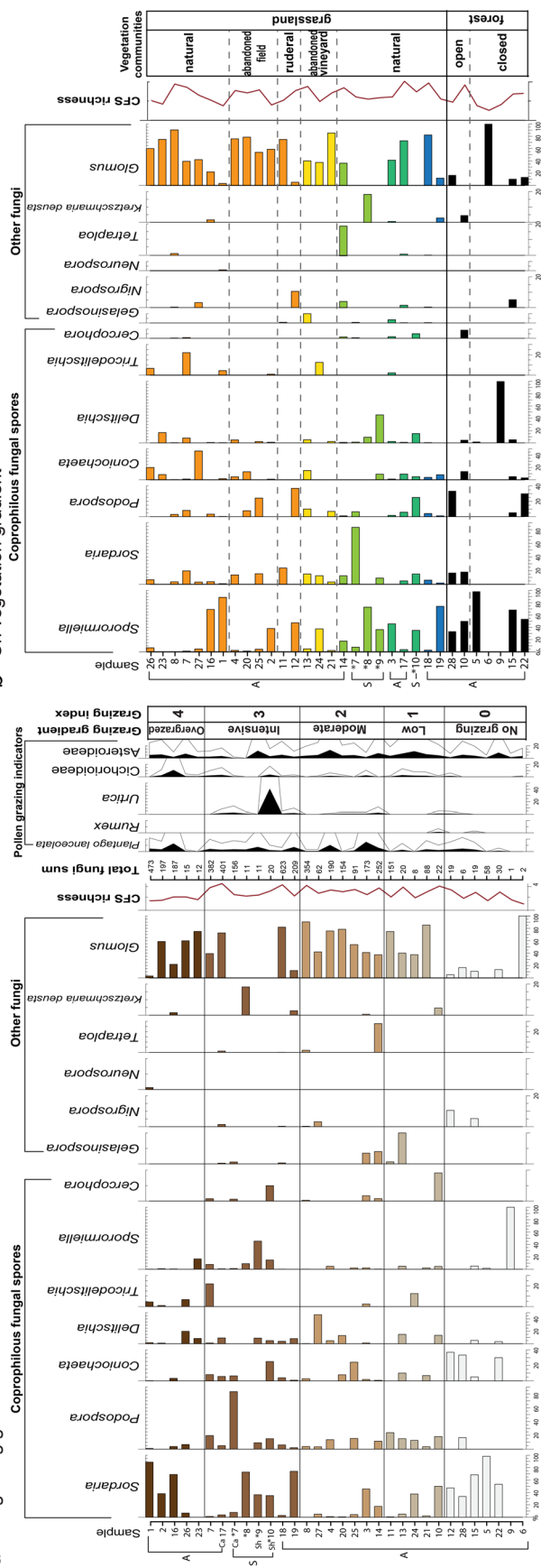
For the 2016 Oltina samples, we followed standard pollen analysis protocols, including treatment with 10% HCl and 10% NaOH, sieved through a 250 µm mesh, and heavy liquid separation using ZnCl₂ (Faegri and Iversen 1989). A similar protocol was applied to all 2024 samples, with slight modifications: sodium polytungstate was used for density separation, and an additional sieving step with a 10 µm mesh was introduced to remove clay (soil samples only). Dung samples were treated only with 10% NaOH and 10% HCl. This protocol minimises the effect of chemical treatment i.e.

acetolysis on spore preservation. However, this mesh size could affect the proportion of very small CFS types such as *Sporormiella*. For each sample, 1 cm³ of sediment was processed with the addition of one *Lycopodium* tablet. The batch number and spore concentration of the tablets are provided in ESM 1 Tables S1, S2.

Fungal spore identification was conducted using several identification keys and databases, including van Geel and Aptroot (2006), Shumilovskikh and van Geel (2020); Shumilovskikh et al. (2021, 2022), van Asperen et al. (2016, 2020, 2021), and Cugny et al. (2010). The fungal spore terminology uses the fungal genus to which these spores belong. *Sordaria*-type include HdV-55A, HdV-55B, and sordariaceous ascospores (van Geel et al. 1978, 1979, 2003; HdV=Hugo-de-Vries

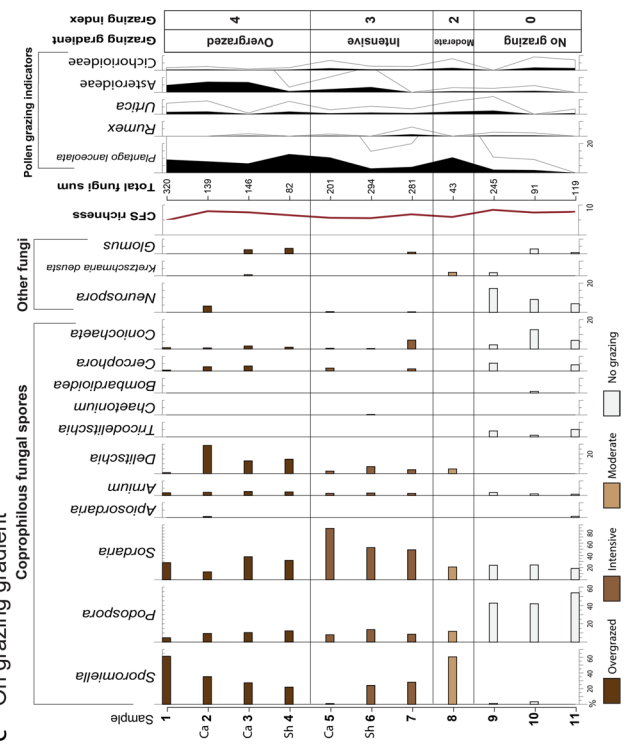
OLTINA

a On grazing gradient

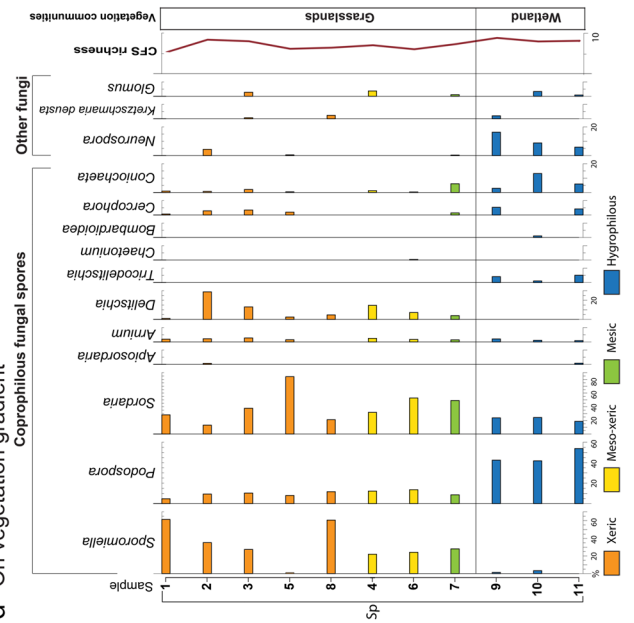


CRISTEȘTI

c On grazing gradient



d On vegetation gradient



b On vegetation gradient

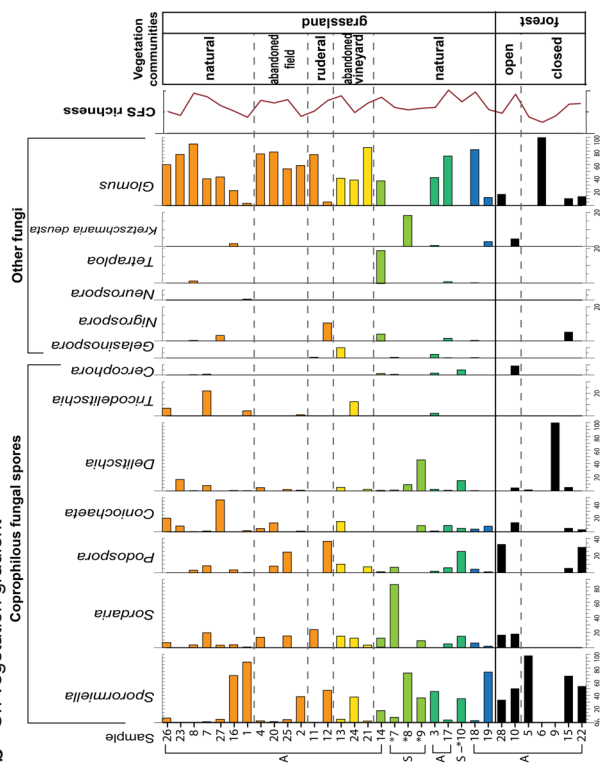


Fig. 2 Composition and richness of coprophilous and other fungal spore types (percentages) shown across grazing and vegetation gradients in the Oltina (a, b) and Cristești (c, d) regions. Pollen percentages of typical grazing indicator plants are also shown. Colour bars within vegetation communities indicate a moisture gradient, ranging from xeric to hygrophilous vegetation types, while brown shades on the grazing gradient from no grazing to overgrazing. A=autumn samples, S=summer samples, * 2024 dung samples from Ca=cattle and Sh=sheep. Note that samples 4, 5, and 6 at Cristești are not exclusively dung samples but were collected very near or together with drooping. Grey curves represent $\times 5$ exaggeration of pollen percentages

Laboratory, University of Amsterdam, Netherlands). However, Schlütz and Shumilovskikh (2017) connected some of *Sordaria* HdV-55B to *Podospora inflatula*-type. *Podospora*-type fall into HdV-368 (van Geel et al. 1980), *Podospora decipiens*-type (Schlütz and Shumilovskikh 2017), and *Podospora polysporus* TM-110 (Cugny et al. 2010). *Chaetomium*-type refers to HdV-7A (van Geel et al. 1978), and HdV-1009 (van Geel et al. 2011), *Delitschia*-type to 023A-B and TM-006 (Cugny et al. 2010), *Apiosordaria*-type to HdV-169 (van Geel et al. 1983), *Sporormiella*-type to HdV-113, *Arnium*-type to HdV-261, HdV-55B-1 and HdV-55B-2 (van Geel et al. 2003). *Trichodelitschia*-type to HdV-546 (van Hofnegen 1983). *Cercophora*-type is associated with HdV-112 (van Geel et al. 1978) and HdV-1013 (van Geel et al. 2011). The *Coniochaeta*-type is primarily linked to *Coniochaeta* cf. *lignaria* (HdV-172, van Geel et al. 1983), as well as TM-6 and 211 (Cugny et al. 2010).

Counting the CFS and pollen in all samples continued until at least 300 pollen grains were identified, whereas for the 2024 samples, counting continued also until at least 100–150 *Lycopodium* spores were detected (van Asperen 2017). The percentages of CFS and of other fungal spore types (*Nigrospora*, *Neurospora*, *Gelasinospora*, *Glomus*, *Kretzschmaria deusta* and *Tetraploa*) were expressed as a percentage of the total fungal spores as well as a percentage of the total fungal spores added to the terrestrial pollen sum (ESM 2 Fig. S1). CFS concentrations were calculated relative to the number of *Lycopodium* spores added and the sediment volume (cm^3).

Grazing pressure quantification from vegetation composition and livestock density

To assess grazing intensity at a local scale, we developed a semi-quantitative estimate based on field-recorded vegetation composition within a 100 m radius around each sampling point and dung presence, resulting in a grazing intensity scale ranging from 0 to 4 (Table 1). To determine the relationship between fungal spore composition and abundance in surface samples and dominant livestock density (animals/ha), we obtained livestock numbers and species from the National Sanitary Veterinary and Food Safety Authority of Romania (ANSVSA). We divided their number

to each pasture area (ha) derived from the Copernicus Land Monitoring Service (2024). Our analysis focused on surface samples collected from pastures, yielding data from seven villages in the Oltina region and one from Cristești. We used census data from the two years preceding each sampling period: 2015 and 2016 for Oltina (2016), 2023 and 2024 for Oltina (2024), and 2022 and 2023 for Cristești (2024) (ESM 1 Table S3). The livestock statistics reflect the total number of grazing animals (cattle, horse, sheep, goats) per village; however, not all animals are sent to graze on communal pastures. As a result, the exact number of grazing animals on the sampled pastures remains uncertain and is likely overestimated. Due to this uncertainty, and the dominance of sheep and goats, we did not convert the animal numbers into biomass per unit area (e.g. kg or t/ha).

Numerical analysis

We applied principal component analysis (PCA) to investigate the relationships between fungal spore assemblages and grazing pressure at each site. Fungal spore percentages were square root-transformed, and data were centred by sample, as implemented in Canoco version 5.1 software (ter Braak and Šmilauer 2002). Coprophilous richness was determined via rarefaction analysis (Birks and Line 1992), implemented in the „vegan” package in R (Oksanen et al. 2024).

To visualize the composition and abundance of fungal spores along gradients of grazing intensity from ungrazed to intensively grazed habitats and vegetation composition from xeric to mesic and hygrophilous grasslands and grasslands to forests, we aggregated their percentages and displayed them as box plots.

Scattered plots were used to explore the relationship between the abundance of total CFS (concentration), and selected taxa, *Sordaria*, *Podospora*, *Sporormiella*, and *Delitschia*, and the grazing intensity scale from 0 to 4. For the eight grazed areas with known livestock density, we additionally explore the link between the abundance of total CFS (concentration), and livestock density (animals/ha).

Results

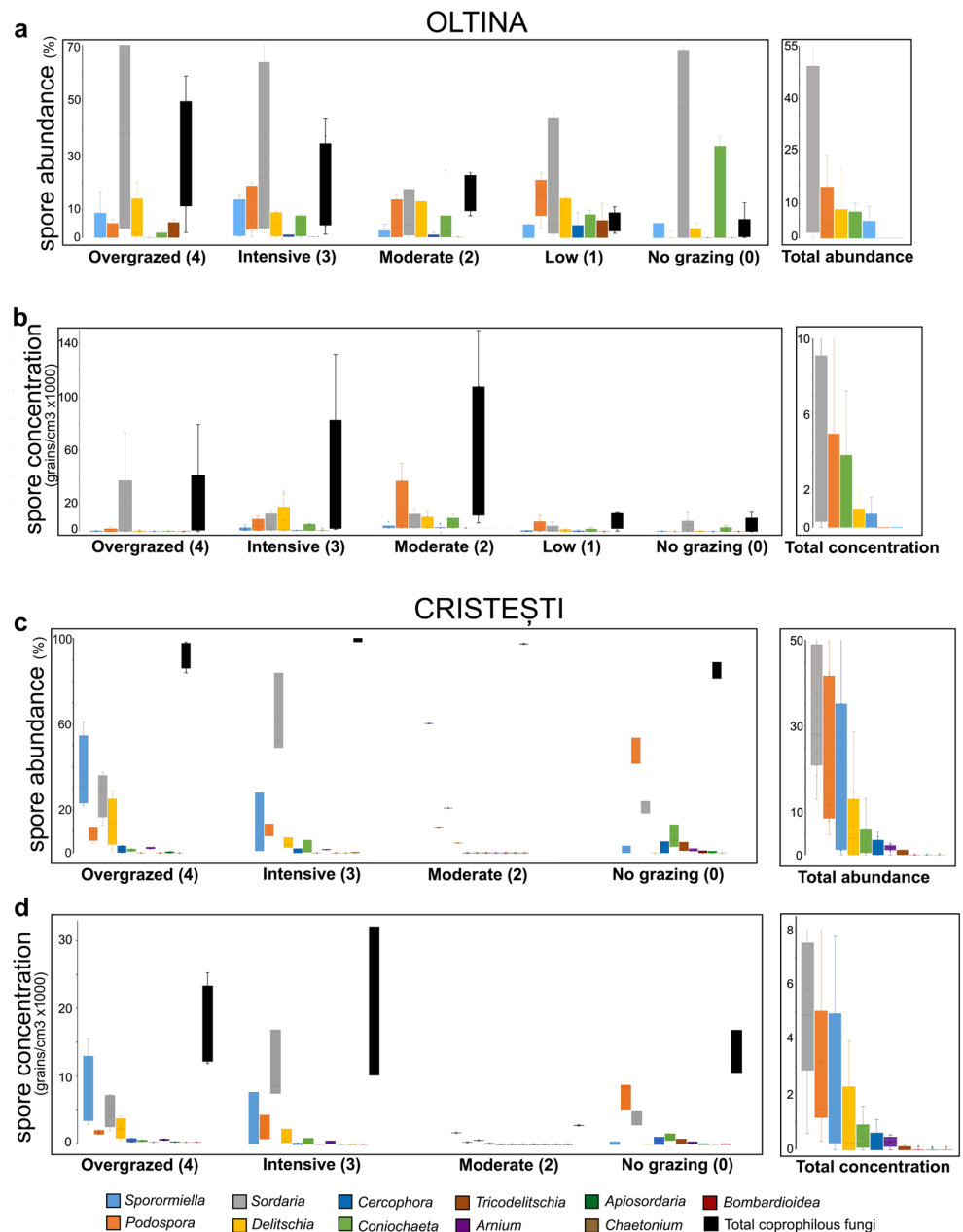
Coprophilous fungal spore diversity and abundance in agropastoral systems

In the 32 analysed surface samples from Oltina (Fig. 2a) and 12 samples from Cristești (Fig. 2c), the number of fungal spores, including coprophilous and other fungi, ranges between 1 and 623 spores at Oltina autumn, 5 and 156 at Oltina summer, and 43 and 320 at Cristești spring. Overall, the Cristești area shows a higher total coprophilous fungal spore abundance than Oltina

(Fig. 3a, c). *Sordaria*-type (HdV-55A, HdV-55B, and sordaria-ceous ascospores) emerged as the most common and abundant CFS type. *Sordaria*-type abundance varied as followed: Oltina (autumn) mean=27%, range=0–98.3%, Oltina (summer) mean=38%, range=8–73%, Cristești (spring) mean=35%, range=13–84.5%. *Podospora*-type (*Podospora* spp., *P. decipiens*-type and *P. polysporus*-type) present at almost all sampling points, shows the following values: Oltina (autumn) mean=6.5%, range=0–23%, Oltina (summer) mean=27%, range=0–83%; Cristești mean=21%, range=4.6–54%, along with *Sporormiella*-type: Oltina (autumn) mean=5.6%, range=0.5–20%; Oltina (summer) mean=18%, range=0.1–45%; Cristești mean=24%, range=0–61.2%). Other frequently identified CFS were *Delitschia*-type: Oltina (autumn)

mean=5.6%, range=0–48%; Oltina (summer) mean=3.5%, range=0–9%; Cristești mean=7%, range=0–29%), and *Coniochaeta* cf. *lignaria*-type: Oltina (autumn) mean=6.5%, range=0–37% Oltina (summer) mean=8%, range=0–25%; Cristești mean=3.3%, range=0–15%. Less common or less abundant spores included *Trichodelitschia*-, *Apiosordaria*-, *Arnium*- and *Cercophora*-type. Additional fungal spores found were *Neurospora*, *Gelasinospora*, and *Kretzschmaria deusta*. *Glomus* shows high abundance in the Oltina region.

Fig. 3 Box plots showing percentages (a, c) and concentration (b, d) of coprophilous fungal spore types under the four grazing intensities at Oltina and Cristești



Coprophilous fungal spore variability along a gradient of grazing intensity

When grouped by grazing intensity, samples from overgrazed pastures (4) in the Oltina region exhibited the highest total fungal spore abundance but had lower diversity, with *Sordaria*-type (1.5–89%) as the dominant taxon (Figs. 2a, 3a). Intensively (3) and moderately (2) grazed grasslands showed high total CFS abundance and diversity, predominantly of *Sordaria*- (0–74%), *Podospora*- (0–83%) and *Sporormiella*-type (0–45%), and notable contribution from *Delitschia*- (0–46%), and *Coniochaeta*-type (0–25%) more abundant in moderately grazed habitats. Lightly grazed habitats had lower total CFS abundance but showed a more even distribution of CFS. Ungrazed areas were characterized by low total CFS and diversity, dominated by *Sordaria*- (0–98%) and *Coniochaeta*- (0–37%), and of *Sporormiella*- (0–100%) and *Delitschia*-type (0–5.2%) in isolated samples.

In the Cristești area, where CFS assemblages reflect a smaller spatial scale, intensively grazed spots exhibited the highest total dung spore abundance, predominantly dominated by *Sordaria*-type (13–84%) (Figs. 2c, 3c). Overgrazed and moderately grazed areas showed a high total spore abundance and diversity, with *Sporormiella*-type (21–61%) as the dominant taxon, along with high proportions of *Sordaria*-, *Delitschia*- and *Podospora*-type. The abundance of other CFS, including *Cercophora*-, *Arnium*- and

Coniochaeta-type, did not show significant variation among the sampled pasture spots.

Trends in fungal spore concentrations along the grazing gradient mirrored percentage-based patterns at both sites (Figs. 3, 5b, d). At Oltina, concentrations were highest in moderately and intensively grazed areas, followed by overgrazed plots (Fig. 3b), whereas at Cristești they peaked in intensively and overgrazed habitats (Fig. 3d). Scattered plots including all samples confirm a positive association between total CFS concentration and grazing intensity, with CFS peaking in intensively (3) grazed habitats (Fig. 4a). *Sordaria*-, *Sporormiella*- and *Podospora*-type were most strongly linked to intensive and overgrazed (4) areas, while *Delitschia*-type was more frequent in moderately (2) and intensively grazed habitats (Fig. 4b–e). Notably, *Podospora*-type also occurred abundantly in ungrazed (0) habitats such as wetland and forests.

Among the five typical grazing indicator plants considered, *Plantago lanceolata*, *Urtica*, *Rumex*, Asteraceae Cichorioideae, and Asteraceae Asteroideae, we found the strongest association between CFS abundance and the pollen abundance of *Plantago lanceolata* and Asteraceae Cichorioideae in the Oltina region, while at Cristești, the strongest associations were with *Plantago lanceolata* and Asteraceae Asteroideae (Fig. 2a, c).

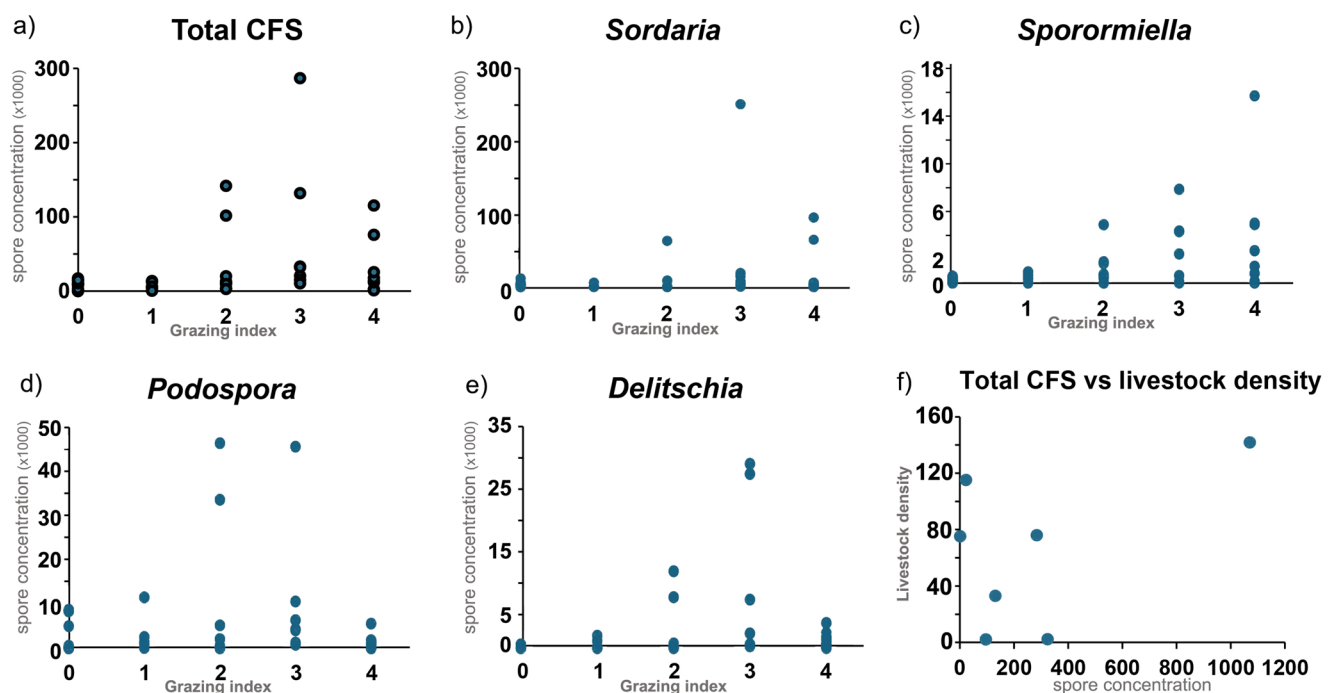


Fig. 4 Relationship between **a** total CFS concentrations (grains/ $\text{cm}^3 \times 10^3$), selected types: **b** *Sordaria*, **c** *Sporormiella*, **d** *Podospora*, **e** *Delitschia* and a grazing index in all samples; and **f** between total CFS

concentrations and livestock density ($\text{nr/ha}10^3$) based on a restricted sample set from seven villages in the Oltina region and one village in the Cristești region

Relationship with dung type and livestock density

In the four analysed dung samples from Oltina, the number of coprophilous fungal spores ranged between 9 and 130 spores, whereas in the Cristești sample (moss from sheep corral), 320 spores. Cattle dung samples ($n=2$) from Oltina were dominated by *Podospora*- (5–83%) and *Sordaria*-type (8–69%), with *Sporormiella*-type displaying only 1.2%. Sheep dung /moss from sheep corral samples from both sites ($n=3$) exhibited high abundances of *Sporormiella* (11–61%) and *Sordaria* (35–89%; Fig. 2a, c). Soil samples collected near cattle and sheep dung ($n=6$) were consistently dominated by *Sordaria*- and *Sporormiella*-type. Livestock census data from seven villages in the Oltina region where our sampled pastures originate, indicate that sheep and goats constitute 75% of the livestock population, with most pastures hosting over 90% ovicaprines, while cattle rarely exceed 5% (ESM 1 Table S3). In contrast, Cristești's livestock consists of 68% sheep and goats and 32% cattle and horses. Scattered plots of livestock density (animals per hectare) and total dung spore concentration showed that sampling points with high dung spore concentrations were found in both areas with low and high livestock density (Fig. 4f).

Coprophilous fungal spore variability along vegetation types

When grouped by vegetation type, total CFS abundance in the Oltina region showed a declining trend from grasslands to abandoned grasslands, vineyards and forest (Figs. 2b, 5b). Within grasslands, mesic and hygrophilous types exhibited the highest abundance (percentages and concentration) and diversity of CFS, with *Sordaria*-type as the dominant taxon in all grassland-types, alongside notable contributions from *Podospora*-, *Sporormiella*-, *Coniochaeta*- and *Delitschia*-type (Fig. 5b). *Sordaria*-type was also the dominant taxon in *Quercus*–*Carpinus* forests (Fig. 5d). In the Cristești area, total CFS percentages and concentrations are highest in meso-xeric grasslands. Hygrophilous grasslands displayed higher CFS diversity, with assemblages dominated by *Podospora*-type (Fig. 5d).

Coprophilous fungal spore variability along wetland and lake transects

At Cristești, the three surface samples collected along a 300 m transect on the wetland revealed *Podospora*-type as the dominant taxon across all points (margin north 42%, centre 41%, margin south 53%; Fig. 6). *Sordaria*-type followed with abundances of 23.7%, 24%, and 18.5%, while *Sporormiella*-type was present in low amounts (1.2%, 3.3%,

and 0%, respectively). Other commonly recorded taxa included *Coniochaeta*-, *Neurospora*-, *Trichodelitschia*-, *Cercophora*- and *Chaetomium*-type (Figs. 2c, 6).

At Lake Oltina, surface lake samples collected along a 3 km transect showed low total spore abundance, with notable variations in composition and diversity from the lake edge to its centre. *Sordaria*- (14–78%), along with *Coniochaeta*- (0–37.5%) and *Delitschia*-type (0–28.6%) were the most common CFS across all coring points. *Podospora*-type (18–28.6%) was more abundant at the lake margin and intermediate points (Fig. 6). *Sporormiella*-type was absent in all surface lake samples.

Principal component analysis

PCA of all samples from the Oltina region shows that the first two axes explain 72% of the variance in fungal spore composition (48% by axis 1 and 24% by axis 2; Fig. 7a). The first axis separates primarily dung-related fungal spores (*Sordaria*-, *Podospora*- and *Delitschia*-type) and erosion (*Glomus*-type) (positive load), which are abundant in heavily grazed habitats, from fungal spore types that are primarily (*Trichodelitschia*), rarely or only occasionally associated with dung (*Apiosordaria*, *Arnium*, *Cercophora*, *Neurospora*, *Gelasinospora*), or erosion (*Kretzschmaria deusta*) (negative load). However, primarily CFS, *Sporormiella*-type groups with the latter. On the second PCA axis, *Sordaria*-type (positive load), the most abundant type at overgrazed sites separates from all other CFS (negative load) found in a range of habitats (dung, charred, soil substrate). The PCA at Cristești indicates that the first two axes explain 88% of the variance in fungal spore composition (69.5% for the first axis and 18.5% for the second; Fig. 7b). The first axis distinguishes primarily dung-associated types (*Sporormiella*, *Sordaria*, *Podospora* and *Delitschia*; positive load) from other fungal types, including *Glomus* (negative load). The second axis separates samples abundant on the wetland (*Podospora*-, *Coniochaeta*-, *Arnium*-, *Cercophora*- and *Bombardioidia*-type; positive load) from those occurring in pastures, which includes a variety of fungal spore types, most abundantly coprophilous fungal types (negative load).

Discussion

Diversity of coprophilous fungal spore assemblages

Our analysis of coprophilous fungal spores in surface soil and dung samples from two agropastoral regions in Romania reveals that *Sordaria*- and *Podospora*-type were the most abundant followed by *Sporormiella*-, *Delitschia*- and *Coniochaeta*-type (Figs. 2, 8). Less frequent taxa in our

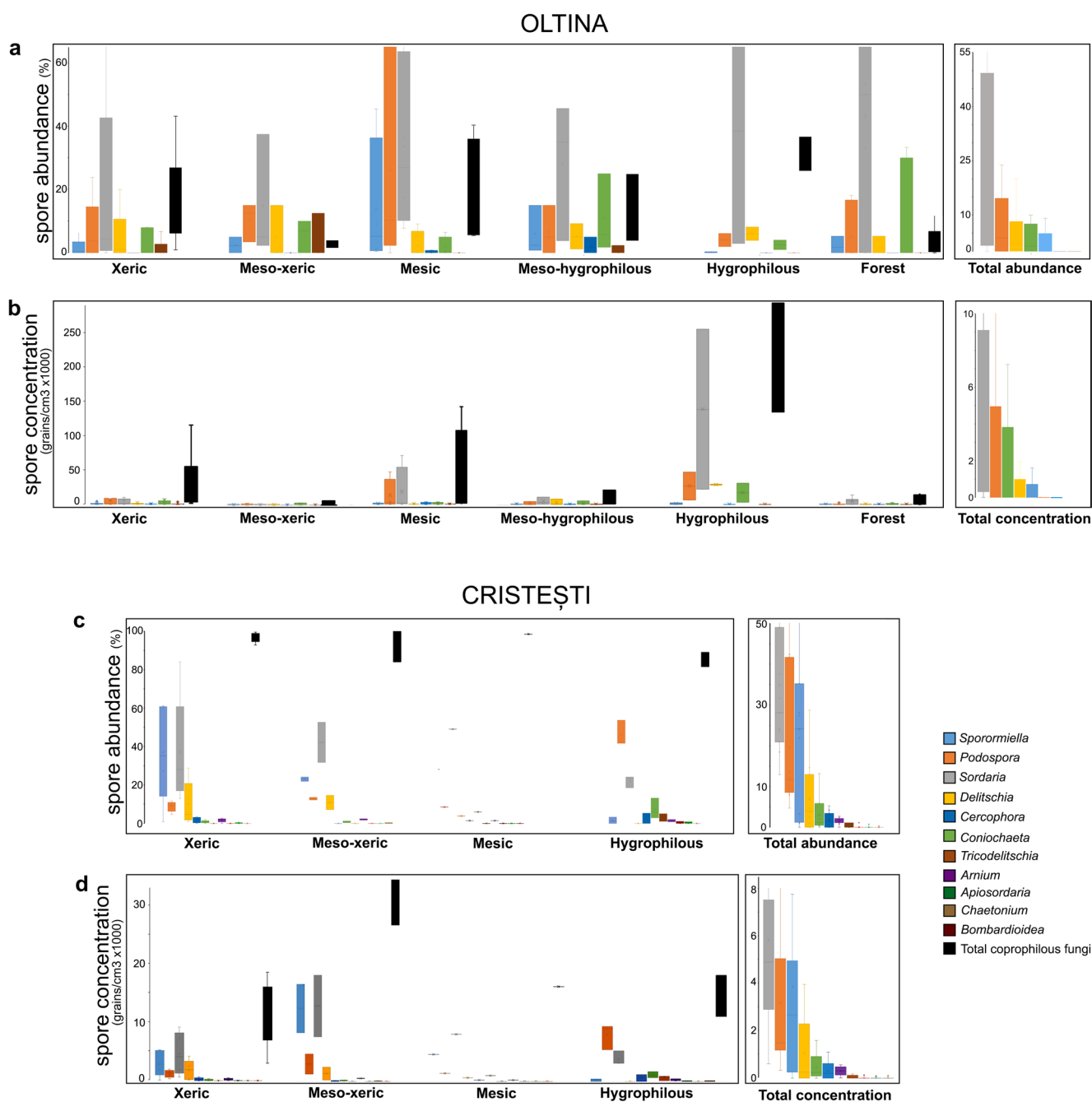


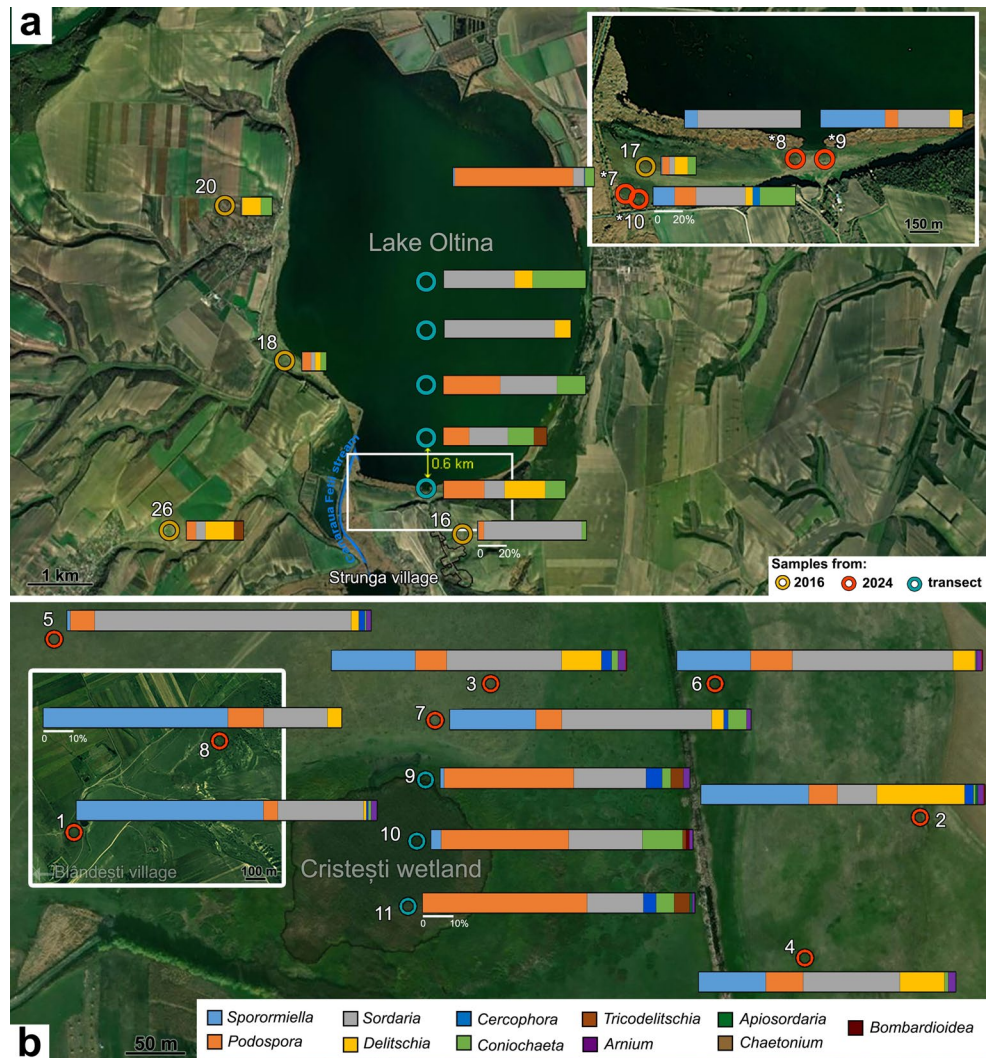
Fig. 5 Box plots showing percentages (**a, c**) and concentration (**b, d**) of coprophilous fungal spore types along a vegetation gradient at Oltina and Cristești

record included *Trichodelitschia*-, *Apiosordaria*-, *Arnium*-, *Chaetomium*-, *Bombardioidea* and *Cercophora*-type. These taxa are exclusively (*Trichodelitschia*, *Bombardioidea*), primarily (*Arnium*, *Delitschia*, *Sordaria*, *Sporormiella*, *Podospora*) or occasionally (*Apiosordaria*, *Cercophora*, *Chaetomium*, *Coniochaeta*) coprophilous ascomycete genera (Shumilovskikh and van Geel 2020). Their persistence in modern and fossil samples is due to thick, pigmented walls, resisting chemical degradation (Baker et al. 2013;

van Asperen et al. 2020, 2021; Shumilovskikh and van Geel 2020; Shumilovskikh et al. 2021).

Sordaria-, *Podospora*-, *Sporormiella*- and *Delitschia*-type were abundant in all grazed habitats, with the *Podospora decipiens*-type predominating on dung. In contrast, the *Coniochaeta*-type, an occasional dung taxon, was most abundant in wetlands and forests (Figs. 2, 3a, b). Schlütz and Shumilovskikh (2017), analysing fungal ascospores of several *Podospora* species, showed that the common

Fig. 6 Composition of coprophilous fungal spore types represented as percentages along Oltina Lake (a) and the Cristești wetland (b) transects



Podospora-type in palynological records (HdV-368) covers the spores of several coprophilous species. Generalist charred and saprophytic fungal spore types such as *Gelasinospora*, *Neurospora* and *Nigrospora*, which can occasionally grow on dung were also found across all grazing intensities. Notably, *Glomus*, a mycorrhizal fungus associated with herbivore trampling and subsequent erosion, was highly abundant in more intensively grazed habitats. This pattern was particularly evident in Oltina region, where stronger biomass removal by herbivores was noted, suggesting a link between high herbivore density, grazing pressure, and increased soil disturbance.

The range of coprophilous fungal spore types identified in our study regions generally aligns with those reported in surface samples from the Pyrenees, France (Cugny et al. 2010; Ejarque et al. 2011), the UK (van Asperen et al. 2020; 2021), Tibet (Basumatary et al. 2020; Wei et al. 2021), and China (Wei et al. 2024). These findings emphasize their cosmopolitan distribution and reinforce the utility of a broader

spectrum of CFS types as indicators of grazing pressure. Nevertheless, the relative abundances of CFS types vary geographically influenced by herbivore type, climate, and habitat characteristics. For example, *Sporormiella* tends to dominate in mountain and alpine environments, while *Sordaria* is more prevalent in lowland agropastoral systems (this study; Cugny et al. 2010; Ejarque et al. 2011; Wei et al. 2024).

It has been suggested that influx or concentration data for CFS abundance are more reliable than percentage data, as they reflect the absolute abundance of each taxon independent of pollen input (Etienne et al. 2013; Johnson et al. 2016; Perrotti and van Asperen 2019; Wei et al. 2024). Alternatively, expressing fungal spores relative to their own sum can circumvent the dependency on pollen abundance. We found that in general, trends in percentages calculated to total fungal spores and concentrations followed similar patterns (Fig. 3b, d). Further, comparison of CFS values calculated to total fungal spores, fungal spores plus total

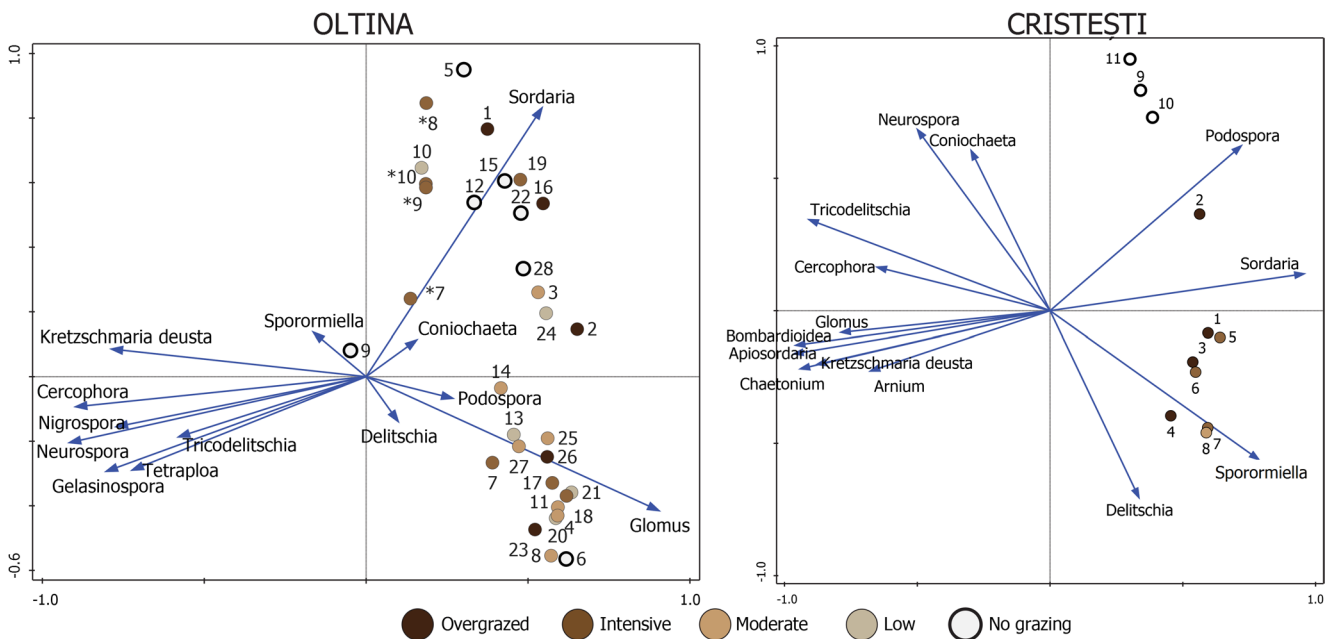


Fig. 7 Principal component analysis (PCA) based on all fungal spores from all samples at Oltina and Cristești

terrestrial pollen sum, and concentrations shows that dissimilarities become particularly evident when high pollen counts lower the relative proportion of CFS in pollen-based percentages (ESM 2 Fig. S1). These findings highlight previous recommendations that independent CFS quantification, such as concentration data or percentages relative to the fungal sum, is preferable.

Coprophilous fungal spore variation with dung type and herbivory abundance/grazing intensity

Our analysis of CFS associated with specific herbivore dung is based on a limited sample set, i.e. cattle dung ($n=2$), sheep dung/moss from corral ($n=3$) and soil samples mixed or from the proximity of cattle or sheep dung ($n=6$). Nevertheless, results from CFS abundance on dung samples show the preference of *Podospora* (*Podospora decipiens*-type) for cattle dung, *Sporormiella* and *Sordaria* for sheep dung, and *Sordaria* as more dung neutral (Fig. 2a, c). Livestock census data show that in Oltina region, sheep and caprine consist 90% of livestock in most villages, while in Cristești they accounted for ca. 70%, with cattle and horses playing a minor role (ESM 1 Table S3). Surface samples from pastures with known livestock composition were dominated by *Sordaria* and *Sporormiella*, closely followed by *Podospora* (Fig. 2b, d). Previous studies provide further context for our findings. Based on a larger modern dung dataset, Richardson (2001) reported that *Podospora* fungi dominates on cattle dung, fungi of *Sporormiella* and *Trichodelitschia* occur slightly more frequent on sheep dung, while those of *Sordaria* are abundant on both but slightly more prevalent on

sheep dung. *Podospora* fungal spores were also found to be abundant on feral cattle dung in the UK (van Asperen 2017), Konik horses' dung in the Netherlands (Baker et al. 2016) and yak and cattle (*Podospora decipiens*-type) in Nepal and Mongolia (Schlütz and Shumilovskikh 2017). In contrast, studies from dry pastures in China found *Sporormiella* and *Delitschia* more abundant on cattle dung, while *Delitschia* and *Sordaria* were dominant on sheep dung.

Our study regions have a high but greatly variable livestock density across pastures (range: 1–323; mean: 131, median: 66 animals/ha). Despite this high livestock density, we did not find a clear positive relationship between CFS abundance and livestock density, as high spore concentrations also occurred at low livestock densities (Fig. 4f). Several methodological limitations may explain this weak correlation. First, the livestock census includes animals for an entire village; however, not all these animals are sent to graze on the communal pastures, and the exact number of animals could not be established. Second, sheep and goats, which constitute the largest proportion of livestock, are highly mobile and graze/browse across diverse land-use types not restricted to pastures, which makes it difficult to establish a direct relationship between livestock biomass in each locality and CFS abundance. Finally, CFS concentrations tend to be high in resting places and enclosures further complicating a direct relationship between dung spore abundance and grazing pressure (see ‘The effect of environmental conditions and seasons on coprophilous fungal spore abundance’).

Our examination of total CFS types and selected taxa along a grazing index score, a surrogate for herbivory

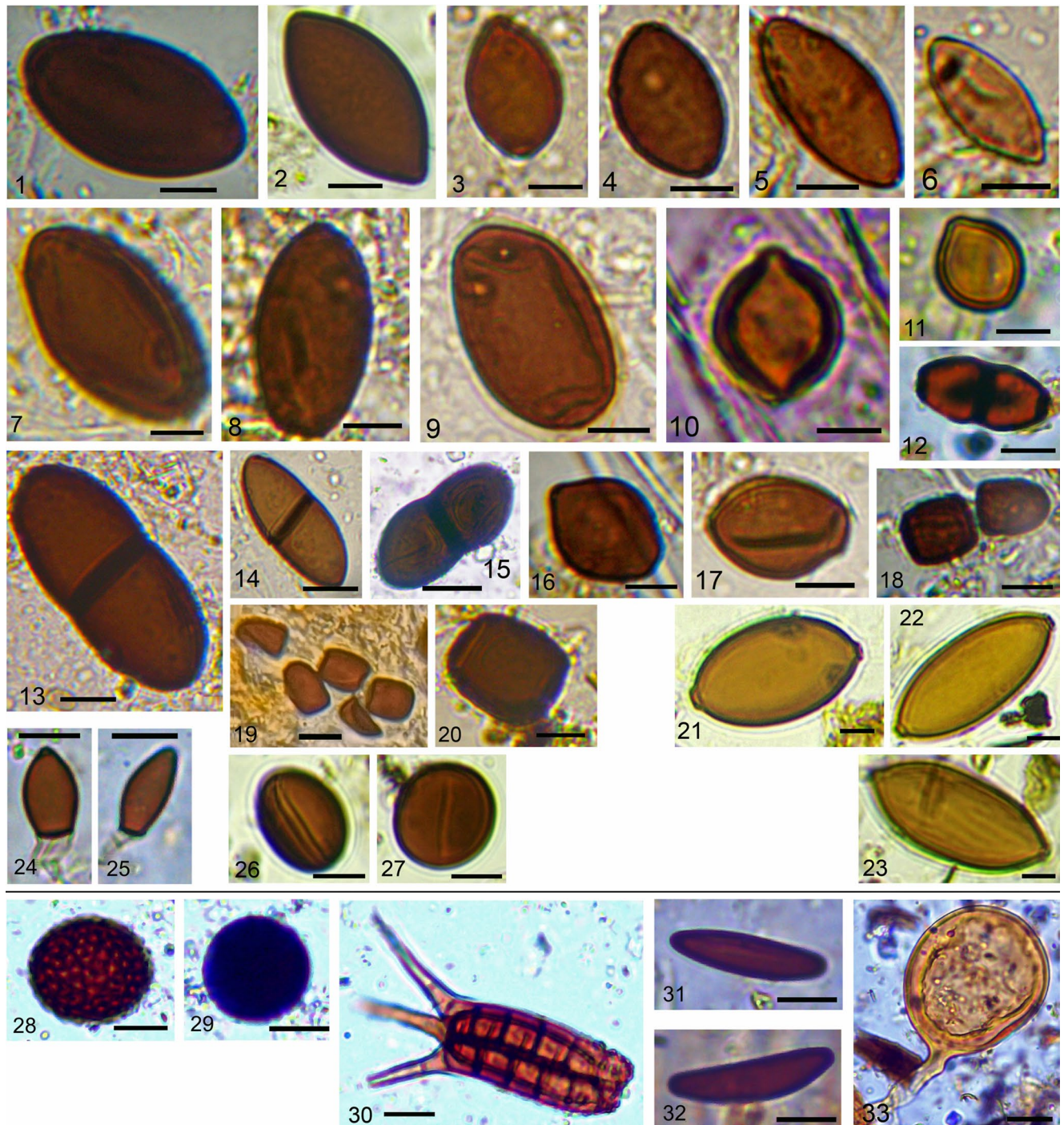


Fig. 8 Selection of fungal spores of obligatory or occasionally coprophilous taxa: 1, 2 *Sordaria*-type (sensu Cugny et al. 2010); 3, 4 *Sordaria* (HdV-55B); 5 sordariaceous ascospores, 6 possibly sordariaceous ascospores; 7–9 *Podospira decipiens*-type; 10 *Chaetomium* thick-walled type; 11 *Chaetomium* thin-walled type; 12 *Trichodelitschia*-type; 13–15 *Delitschia*-type; 16, 17 *Aptosordaria*-

type; 18–20 *Sporormiella*-type; 21–22 *Arnium*-type (HdV-55B-1); 23 *Arnium*-type (HdV-55B-2); 24, 25 *Cercophora*-type; 26, 27 *Coniochaeta* cf. *ligniaria*. Other spores of fungal taxa mentioned in the text: 28 *Gelasinospora*; 29 *Nigrospora*; 30 *Tetraploa*; 31, 32 *Kretzschmaria deusta*; 33 *Glomus*; scale bars = 10 μ m

density, shows that concentrations of total CFS, *Sordaria*-, *Sporormiella*- and *Podospora*-type peak in intensively and overgrazed habitats (Fig. 4a–d). However, *Podospora*-type records additional high values in moderately grazed areas as well as wetlands, thus in ungrazed areas. *Delitschia*-type was most strongly associated with moderately and intensively grazed areas (Fig. 4e). This variation indicates that *Sordaria*- and *Sporormiella*-type are more directly linked to grazing intensity. Consistently, Baker et al. (2016) analysed the abundance of *Sordaria*-, *Sporormiella*- and *Podospora*-type and reported a strong relationship between local herbivore biomass and total fungal spores, *Sordaria*- and *Sporormiella*-type, but a weaker one for less abundant types such as *Podospora*-type.

We also found a strong association between the abundance of CFS and the pollen abundance of the grazing indicator plants *Plantago lanceolata* at both sites, and with Asteraceae Cichorioideae in the Oltina region, and Asteraceae Asteroideae at Cristești (Fig. 2a, c). These findings suggest the benefit of *Plantago lanceolata* and Asteraceae as reliable plant indicator of grazing pressure in Eastern-Central European lowland environments.

The effect of environmental conditions and seasons on coprophilous fungal spore abundance

Our samples collected across vegetation communities with varying moisture conditions and three seasons allowed us to assess the impact of habitat moisture on CFS abundance and diversity. Hygrophilous grasslands with higher moisture conditions supported the greatest CFS diversity and concentrations (Fig. 2b, d). Seasonal differences were also evident: spring samples contained the highest spore concentrations, whereas summer samples had the lowest. Extended water availability from late autumn through winter to moist spring conditions likely reduced dung desiccation of spring samples and promoted spore production and preservation. In contrast, dry conditions in summer 2016 and autumn 2024 probably caused rapid dung desiccation. Our findings agree with studies in temperate regions that reported peak spore production and preservation during moist spring conditions (van Asperen et al. 2016, 2020; Lee et al. 2022) or higher diversity linked to broader climatic variation (Richardson 2001). Dung size also plays a role, with larger droppings drying more slowly than smaller ones under dry conditions (Schlütz and Shumilovskikh 2017).

Other factors may influence variability in dung spore abundance across surface samples, leading to a nonlinear relationship between spore abundance and grazing pressure regimes. For instance, dung tends to accumulate less in actively grazed areas and more in locations where animals drink, chew the cud, and seek shelter (Cugny et al. 2010;

Lee et al. 2022). As a result, the frequent use of hygrophilous grasslands for water access may be another reason for the highest spore abundance, particularly *Podospora*-type, observed in these habitats. Similarly, the high occurrence of *Sporormiella*-type at the sheep corral reflects its role as overnight shelter, whereas that of *Sordaria*-type in woodland margin is likely linked to livestock using these areas for shelter.

Transport and deposition of coprophilous fungal spore into the wetland and lake

While CFS typically have short dispersal distances, (i.e. a few meters) they can be transported further by wind and water, e.g. streams and runoff (Baker et al. 2016; Lee et al. 2022). Surface samples from the Cristești wetland (~300 m diameter) show a homogeneous dung spore composition across three coring points, with *Podospora*- and *Sordaria*-type dominant and *Sporormiella*-type present in low amounts at the margins and mid-point (Figs. 1b, 6b). This contrasts with the composition on adjacent pasture, where *Sordaria*- and *Sporormiella*-type dominate. The difference in CFS composition between pasture and wetland raises the question of whether it results from transportation mechanisms reflecting a broader-scale herbivore signal or ecological factors. Livestock species exhibit species-specific behavioural adaptations. For example, sheep are more vulnerable to gastrointestinal parasite infections, which thrive in moist environments, than cattle (May et al. 2022). As a result, sheep tend to avoid wetland areas, whereas cattle more frequently graze in wetter environments. Consequently, the high abundance of predominantly cattle-associated CFS *Podospora*-type in the Cristești wetland may be linked to cattle entering this habitat. Fungi producing *Podospora*-type are also often associated with wild herbivores, including rabbits, deer, and birds (Krug et al. 2004; Richardson 2001; Baker et al. 2016), suggesting that wildlife seeking shelter in wetlands may further contribute to these compositional differences.

At Lake Oltina, the 3 km surface sample transect reveals spatial heterogeneity in CFS composition (Fig. 6a). *Sordaria* and *Delitschia* were the most abundant spores across all coring points, with the highest proportions recorded in the central lake area (1.5–3 km). *Podospora* peaked at intermediate and lake-edge points (0–1.5 km). Surface samples near the lake showed high values for *Delitschia*, *Podospora*, *Sordaria*, *Coniochaeta* along with low amounts of *Sporormiella*. This fluctuation in CFS composition and abundance among samples along coring points within the lake and surrounding pastures suggest that they reflect grazing activity across a broader landscape (Baker et al. 2016). Variations in spore assemblages within the lake may also be influenced by stream transport, where stronger hydrodynamic energy

moves spores toward the lake centre (Etienne et al. 2013). The similarity between the CFS composition in central lake surface samples and soil samples from the southern shore, where the Canaraua Fetii stream enters, suggests that water transport contributes to CFS deposition in the lake centre (Fig. 6a).

Insights into fossil coprophilous fungal spore assemblages

Our literature review of more recent European palaeoecological records with CFS quantification reveals several key findings. First, like our surface samples from agropastoral systems, *Sordaria*-, *Podospora*- and *Sporormiella*-type emerge as the dominant dung spore types, with *Cercophora*-type following closely (Table 2). *Podospora* and *Sordaria*, the most abundant taxon in modern surface samples, also dominates the fossil record. A notable feature of fossil datasets is the quantification of *Sporormiella*-type as the sole dung-associated taxon. Initially used to reconstruct megafaunal grazing (Gill et al. 2009), *Sporormiella*-type has become a key indicator of herbivore activity. However, given the complex ecology of dung fungi, reliance on a single taxon may oversimplify past grazing reconstructions. In contrast, *Delitschia*-type, which is abundant in surface samples, was rarely reported in fossil records, suggesting it is underused in palaeo-grazing studies. Several other primarily and occasionally CFS types, including *Arnium*, *Apiosordaria*, *Bombardioidea*, *Chaetomium*, *Coniochaeta*, *Saccobolus*, *Trichodelitschia* and *Tripterospora* are infrequently or sporadically recorded in our surface samples, but generally contemporaneous with high values of common CFS taxa. Among these taxa, *Coniochaeta*-type was consistently found in our surface samples from grazed areas, suggesting that this fungal spore can serve, in association with primary CFS, as a grazing indicator. Fungal spore analysis from geoarchaeological context, also reported a wide range of coprophilous and saprophytic fungal spores indicative of pastoral farming, i.e. *Arnium*-type, *Coniochaeta* cf. *lignaria*, *Chaetomium*, *Delitschia*, *Podospora* group, *Podospora decipiens*-type, *Podospora curvispora*, *Saccobolus minimus*-type, *Sordaria*-type, *Sporormiella*-type, *Apiosordaria*, *Gelasinospora* (Shumilovskikh et al. 2016). Furthermore, while *Glomus* is frequently noted in fossil records, its co-occurrence with dung-associated fungi is rarely discussed in relation to herbivore activity. This oversight may obscure potential links between high herbivore density, increased biomass removal, and soil erosion.

This non-exhaustive review highlights that, although recent studies have increased the number of identified CFS, comprehensive taxonomic reporting remains inconsistent. Coprophilous fungal spores tend to be more systematically documented

in studies focusing on past human activities in archaeological contexts (Marinova et al. 2006; Shumilovskikh et al. 2016) or in research conducted by specialists with strong focus on non-pollen palynomorphs (Shumilovskikh et al. 2016, 2021, 2022). While our surface samples identified a relatively low number of dung-associated types (11), ongoing research and updated databases continue to refine and expand the list of recognised types (*Xylariales*, *Alternaria*, *Curvularia*, *Podosordaria*, *Pleospora*, *Saccobolus*, *Tripterospora*, *Podospora curvispora*), that can be determined in the fossil records (see Shumilovskikh et al. 2016; van Asperen et al. 2021).

Conclusions and implications for palaeoecological reconstruction

This study represents the first exploration of fungal spore diversity and their environmental drivers in the European lowland, predominantly agropastoral landscapes. We identified *Sordaria*-, *Podospora*-, *Sporormiella*- and *Delitschia*-type as the most robust indicators of grazing pressure and/or herbivore density, with the abundance of *Sordaria* and *Sporormiella* peaking at higher grazing intensity. The co-occurrence of *Arnium*-, *Apiosordaria*-, *Cercophora*-, *Chaetomium*-, *Coniochaeta*- and *Gelasinospora*-type suggests that these taxa also reliably indicate grazed environments. *Glomus* emerged as a strong marker of heavily grazed landscapes. However, a quantitative reconstruction of herbivore density and type remains challenging due to animal mobility, spore dispersal, and sediment incorporation. CFS abundance was also high in moist habitats, spring samples, and areas where animals drink and rest. In terms of CFS quantification, concentrations or percentages relative to the fungal sum is preferable. Our findings also emphasise the complementary value of plant indicators such as *Plantago lanceolata* and Asteraceae alongside CFS in strengthening grazing regime reconstructions in Eastern-Central European lowlands.

Our non-exhausted review of previous palaeoecological studies points that the full potential of dung spores remains underexplored. To improve reconstructions of past herbivore activity and changes in grazing pressure regime, we recommend that future palaeoecological studies: (i) incorporate a broader diversity of fungal spore types; (ii) consult updates of fungal spore databases and identification protocols; (iii) need to standardize identification methodologies to enhance comparability across studies; and (iv) integrate other proxies such as faecal biomarkers and high-throughput sequencing to refine taxonomic resolution and improve detection sensitivity.

Table 2 The common coprophilous fungal spores found in fossil records from Europe: +++ abundant; ++ common; + rare; – absent

Sites (m a.s.l.)	<i>Sporormiella</i>	<i>Sordaria</i>	<i>Podospira</i>	<i>Cercophora</i>	<i>Delitschia</i>	<i>Armiium</i>	Others: predomi- nantly coproph. fungi	Others: occasion- ally coproph. fungi	Others: ero- sion indicating fungi	References
Lake Olina (50) RO	+	+++	++	+		–	–	–	–	Feurdean et al. (2021)
Mangalia (0) RO	+	+	+	+	+	+++	–	<i>Coniochaeta</i> (+++)	<i>Glomus</i> (+++)	Feurdean et al. (2025)
Sultana (13) RO	+	+	++	++	+	+++	–	<i>Coniochaeta</i> (+)	<i>Kretzschmaria deusta</i> (+)	Hanganu et al. (2025)
Vinderelu (1,684) RO	++	++	+	+	–	–	–	<i>Gelasinospora</i> (+)	<i>Glomus</i> (+++) <i>K. deusta</i> (+) <i>Glomus</i> (+)	Florescu et al. (2024)
Gropil (1,920) RO	+	+	++	+	–	+	<i>Chaetomium</i> (+)	<i>Chaetomium</i> (+)	<i>K. deusta</i> (+)	Florescu et al. (2024)
Sudza (134) RUS	+	+	+	+			Bombardioidea (+)	<i>Coniochaeta</i> (+)	<i>Glomus</i> (+)	Shumilovskikh et al. (2019)
Omelchenki (88) UA	–	++	++	–	–	+++	–	<i>Gelasinospora</i> (+) <i>Neurospora</i> (+) <i>Apiosordaria</i> (+)	<i>Glomus</i> (+++)	Lukanina et al. (2023)
Kardashynskyi (4) UA	+	–	–	–	–	–	–	–	–	Granz et al. (2024)
Varna (0) BG	–	++	++	++	–	–	<i>Chaetomium</i> (++)	–	<i>Glomus</i> (+++)	Filipova-Marinova et al. (2016)
Durankulak (1) BG	–	++	+	++	–	–	<i>Chaetomium</i> (+)	–	<i>Glomus</i> (+++)	Marinova and Atanasova (2006)
Marathon (1) GR	–	+	–	–	–	–	–	–	–	Kouli et al. (2009)
Elaia (52) GR	++	++	++	+	++	++	<i>Saccobolus</i> (+) <i>Chaetomium</i> (+)	<i>Apiosordaria</i> (+) <i>Coniochaeta</i> (+++)	<i>Glomus</i> (+++)	Shumilovskikh et al. (2016)
Dikili Tash (71) GR	+	++	+	+	–	–	<i>Chaetomium</i> (+)	–	<i>Glomus</i> (+++) <i>K. deusta</i> (+)	Glaes et al. (2017)
Limni Volvi (30) GR	++	–	–	–	–	–	–	–	–	Ganz et al. (2025)
Limni Zazari (606) GR	++	–	–	–	–	–	–	–	–	van Vugt et al. (2025)
Limni Vegoritis (519) GR	+++	–	–	–	–	–	–	–	–	Morales-Molino et al. (2024)
Kokad (112) H	+	+	–	–	–	–	–	–	–	Magyari et al. (2022)
Bruszezwo region PL	+	+++	+	++		+	–	–	–	Kolaczek et al. (2025)
Paríž Creek (123) SK	+		+							Jamrichová et al. (2014)
Santovka-PB (150) SK	+	–	–	–	–	–	–	–	<i>Glomus</i> (+++)	Šolcová et al. (2020)
Biele skaly (1,200) SK	+	+++	+	+	–	+	–	<i>Coniochaeta</i> (+)	<i>K. deusta</i> (+)	Wieżik et al. (2023)
Sklářské Valley (800) CZ	+	–	+	–	–	–	–	–	<i>Glomus</i> (+)	Kozáková et al. (2021)
Eustach (387) CZ	++	++	+	+	–	–	<i>Trichodelitschia</i> (+) <i>Chaetomium</i> (+)	<i>Apiosordaria</i> (+) <i>Coniochaeta</i> (+)	<i>K. deusta</i> (+++)	Bobek et al. (2018)
Dářko (650) CZ	+	+	+	+	–	–	<i>Chaetomium</i> (+)	<i>Coniochaeta</i> (+)	<i>K. deusta</i> (++)	Roleček et al. (2020)
Pekna (730) CZ	++	++	+	+	–	–	–	<i>Gelasinospora</i> (+)	<i>Tripterospira</i> (+), <i>K. deusta</i> (++)	Kraklow et al. (2024)

Table 2 (continued)

Sites (m a.s.l.)	<i>Sporormiella</i>	<i>Sordaria</i>	<i>Podospora</i>	<i>Cercophora</i>	<i>Delitschia</i>	<i>Arniium</i>	Others: predominantly coproph. fungi	Others: occasionally coproph. fungi	Others: erosion indicating fungi	References
Blatensk' a slat' (1,275) CZ	++	+	+	–	–	–	–	–	–	Kraklow et al. (2024)
Puścizna Mala (613) PL	+	+	–	+	–	–	–	–	–	Kolaczek et al. (2018)
Puścizna Krauszowska (655) PL	+	+	–	+	–	–	–	–	–	Kolaczek et al. (2018)
Lake Bohinj (500) SLO	+	–	–	–	–	–	–	–	<i>K. deusta</i> (+)	Andrič et al. (2020)
Ombrore (2) I	+	–	+	+	–	–	–	–	<i>Glomus</i> (+)	Biserni and van Geel (2005)
Lac de Moras (304) F	+++	+++	++	++	–	–	<i>Trichodelitschia</i> (+) <i>Chaetomium</i> (+)	<i>Coniochaeta</i> (+++)	<i>K. deusta</i> (++)	Doyen and Etienne (2017)
Col des Glières (1,400) F	+++	–	++	+++	–	–	–	–	–	Julien et al. (2025)
Beaurieux Les Grèves (150) F	++	+++	++	+++	–	–	<i>Chaetomium</i> (+++)	<i>Coniochaeta</i> (+++)	<i>Glomus</i> (+++)	Innes et al. (2019)
Fénay (340) F	++	++	+	++	–	–	–	<i>Apiosordaria</i> (+)	<i>K. deusta</i> (+)	Laine et al. (2010)
Lake Montcortès (1,027) F	+++	++	+	+	++	+	<i>Chaetomium</i> (+++)	<i>Coniochaeta</i> (+++)	<i>Glomus</i> (++)	Montoya et al. (2018)
Northern Vosges Mountains (400) F	++	+++	+	+++	–	–	–	–	–	Gouriveau et al. (2020)
Sourzay (1,130) F	+++	++	++	++	+	–	<i>Trichodelitschia</i> (++) <i>Chaetomium</i> (+)	<i>Apiosordaria</i> (++) <i>Coniochaeta</i> (+) <i>Cercophora</i> (++) <i>Coniochaeta</i> (+++)	<i>Glomus</i> (++) <i>K. deusta</i> (+)	Cugny et al. (2010)
Lake Banyoles (170) E	–	+	+	++	–	–	–	<i>Coniochaeta</i> (+++)	<i>Glomus</i> (+++) <i>K. deusta</i> (+)	Revelles and van Geel (2016)
La Draga (170) E	–	+	+	++	+	–	<i>Coniochaeta</i> (+++) <i>Chaetomium</i> (+++) <i>Apiosordaria</i> (+)	<i>Coniochaeta</i> (+++) <i>Apiosordaria</i> (+)	<i>Glomus</i> (+++) <i>K. deusta</i> (++)	Revelles et al. (2016)
Estanya lakes (900) E	+++	–	–	–	–	–	–	<i>Coniochaeta</i> (++)	<i>Glomus</i> (++)	Riera et al. (2006)
Uitgeest (1) NL	+++	++	++	+	–	++	<i>Chaetomium</i> (+)	<i>Coniochaeta</i> (+)	<i>Glomus</i> (+)	Van Geel et al. (2003)

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Data availability All datasets used in this study are presented in the Supplementary Material.

Declarations

Competing interests The authors have no competing interests to declare that are relevant to the content of this article.

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