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RESEARCH ARTICLE

Influence of habitat fragmentation and habitat amount on soil fungi communities in ancient forests

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Abstract
Context Fungi represent a large part of soil biodiversity as well as an essential role for tree hydromineral nutrition, survival, and carbon cycling. While their local diversity has proven to be shaped by abiotic and biotic factors related to soil, climate and vegetation, their response to landscape fragmentation is still debated.
Objectives In this paper, we focus on ancient forests characterized by the presence of beech at low elevation, a habitat particularly fragmented in South-West France. We aim to assess the effect of past and present forest fragmentation and quantity on soil fungal and functional group diversity. We expect a negative impact of fragmentation and a positive effect of forest quantity on soil fungal diversity, in addition to an influence of local soil factors.
Methods Soils from 41 1ha ancient forest plots across South-West France were sampled along gradients of past and present forest fragmentation and quantity, before their fungal diversity was characterized by metabarcoding of environmental DNA. Hill numbers have been computed and applied to all fungi, trophic guilds and growth forms.
Results We find that past forest fragmentation negatively impacts on soil fungal diversity and in particular for symbiotrophs, while the present forest quantity has a positive impact. In contrast, the species richness of pathotrophic fungi shows a negative correlation with past forest fragmentation. The diversity

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of fungal groups with lower dispersal abilities (e.g., corticioid, microfungi) is negatively impacted by past forest fragmentation, while the diversity of wind-dispersed fungi (e.g., agaricoid) is not influenced by any landscape descriptors.

Conclusions Our results show the complexity of fungal responses to past and present forest fragmentation and demonstrate the long-lasting effect of past fragmentation as well as the positive impact of recent forest quantity in the landscape on fungal diversity associated with ancient forest soils. Our study also highlights the huge diversity of fungi unraveled by eDNA metabarcoding in this habitat and the potential of such techniques to study the landscape ecology of soil communities.

Keywords Landscape ecology · Habitat fragmentation · Fungi · Forest · Metabarcoding · Soil

Introduction

Forest biodiversity suffers from several anthropogenic pressures including deforestation and intensive management that lead to habitat loss and fragmentation (Haddad et al. 2015; Curtis et al. 2018). Habitat fragmentation is defined as the breaking apart of a large continuous area into several smaller patches (Fahrig 2003). Positive and negative effects of habitat fragmentation have been observed on biodiversity, with these contrasting findings contributing to an intense debate in landscape ecology (Fletcher et al. 2018; Fahrig et al. 2019). This debate demonstrates the importance of properly disentangling the influence of habitat loss from the influence of fragmentation per se (Fahrig 2003, 2017). Indeed, in real-word landscapes, those drivers are often intertwined, making it difficult to tease apart their respective effects (Didham et al. 2012). Patch size and isolation have long been used as predictors of fragmentation, until Fahrig (2013) challenged the classical view of patch as a natural unit for species population and communities and formulated the habitat amount hypothesis (HAH). It postulates that the effects of patch size and isolation on species density could both be encompassed in the effects of the amount of habitat present in the landscape. Since then, several studies have tested this hypothesis and the HAH for various groups of organisms including plants, birds, arthropods and mammals (Martin 2018; Watling et al. 2020). It has therefore been suggested that the HAH should be used as a baseline of habitat loss effects, above which fragmentation effects should be contrasted to assess its importance in driving species diversity (Martin 2018).

Fungi represent a major part of terrestrial diversity, are present worldwide and play major roles in ecosystem functioning (Aslani et al. 2022). While their diversity patterns at local scales are partly explained by climate, soil, plant communities and disturbances such as fire (Tedersoo et al. 2014), knowledge about the influence of landscape factors are still scarce (Mony et al. 2020). Grilli et al. (2017) suggest that fragmentation has negative effects on fungal diversity, with important magnitude variations according to the trophic guild. For instance, ectomycorrhizal fungi (symbionts with trees) and saproxylic fungi (dead wood dwelling species) were found to be sensitive to habitat fragmentation while pathogenic fungi were not (Grilli et al. 2017; Boeraeve et al. 2019). The question remains as to whether fungal groups with potentially lower dispersal abilities (such as gasteroid ones, like truffles and corticioid fungi as suggested by Harrington et al. (2021)) would be more sensitive to landscape fragmentation. Indeed, dispersal limitations have predominantly been described for populations at scales larger than the landscape (circa 100 km, Douhan et al. 2011).

In European forest ecosystems, considering the effect of present and past fragmentation is particularly important, since forest fragmentation level is known to have been higher 150 years ago (Hermy and Verheyen 2007; Cateau et al. 2015). Forests remnants presently defined as ancient are still visible although often surrounded by recent forest patches (Dupouey et al. 2002a; Vallauri et al. 2012). Ancient versus recent above-ground forest differences may however be hidden by recent transformation (e.g., logging, plantation). Nevertheless, beech is often present and major differences have been observed in specific lichen (Fritz et al. 2008), beetles (Assmann 1999; Desender et al. 1999), vascular plants (Hermy et al. 1999) and springtails species (Ponge et al. 2006). These organisms share common traits such as a low dispersal capacity and high sensibility to habitat stability. Moreover, ancient forests soils show a lower pH and higher organic matter content (Dupouey et al. 2002b; Berges and Dupouey 2021).

A few studies have evidenced the impact of present/past forest fragmentation on fungal—in particular ectomycorrhizal—communities associated with ancient forests as well as the effect of habitat quantity in the landscape (Hofmeister et al. 2014; Boeraeve et al. 2018a; Mennicken et al. 2020). These pioneering studies highlight the need to further investigate the effect of past and present forest fragmentation on fungal diversity as well as taxonomical and functional groups, in particular symbiotrophic fungi. Recently, the use of environmental DNA metabarcoding (Taberlet et al. 2018) has promoted the study of complex fungal communities and even their functional diversity, through the use of taxonomical databases (Nguyen et al. 2016; Põlme et al. 2020). However, soil metabarcoding remains biased in estimating the response of diversity, because of possible overestimation of rare species and real abundances. The use of Hill numbers (Hill 1973) has been recommended to overcome this bias for plant and fungal eDNA (Calderon-Sanou et al. 2020). It therefore provides an analytical framework to study the response of soil diversity to landscape fragmentation through environmental metabarcoding.

Our study compares the response of soil fungal diversity associated with ancient forests to past and present fragmentation, while controlling for the quantity of surrounding forests in the landscape. We focused our study in the lowlands of South-West France, where fragmentation of ancient forests is particularly high (Vallauri et al. 2012). Moreover, soil variables are important drivers of fungal communities (Tedersoo et al. 2014) and may explain part of the variation in diversity. We therefore hypothesize that: (1) fungal diversity would be negatively correlated with the number of forest patches (*i.e.*, forest fragmentation); (2) fungal and in particular symbiotroph diversity would be positively correlated with the quantity of forest in the landscape and (3) fungi with low dispersal abilities would show a stronger response to fragmentation as compared to wind-dispersed fungi.

Material and methods

Plot selection and measurement of forest fragmentation and quantity

The choice of plots used for the study was based on a regional inventory in South-West France of 257 plots within 187 ancient lowland forest sites (Savoie et al. 2011, 2015; Gouix et al. 2019). From this inventory, 50 plots of a unique habitat (*Fagus sylvatica* and *Quercus petraea* forest) were identified using an a priori analysis to avoid the autocorrelation between the fragmentation (number of patches of ancient forests) and the area of ancient forests in a buffer zone of one km radius (Fig. 1). The buffer zone is a fixed surface around a given patch of habitat (*i.e.,* 314 ha), defined based on a previous study of fungal communities (Mennicken et al. 2020). An ancient forest GIS layer was obtained by digitizing the 1:40,000 min of the Ordnance survey map using QGIS Historical map plug-in (Favre et al. 2013; Herrault et al. 2015; Karasiak et al. 2016). The past fragmentation and ancient forest quantity were retrieved from ancient forest maps, while present forest fragmentation and quantity were obtained from IGN-BD Topo edition 2019 (https://geoservices.ign.fr/bdtopo). The lack of autocorrelation between present fragmentation and forest quantity was also checked (Fig. Suppl. S1). All spatial analysis was performed using ArcGIS 10.8.1 (ESRI 2020). Additionally, we ensured no overlap of buffers to ensure statistical observation independence. From the initial subset of plots, 41 were selected, covering a total of 707,780 ha (Fig. Suppl. S2; N 43.4311°, E 0.5723°). Site position is provided in the associated DRYAD Archive (https://doi.org/https://doi.org/10.5061/dryad.gmsbcc2tg; to be released upon publication).

Soil fungal eDNA sampling

Each ancient forest plot was centered on the 1 ha—inventories from Gouix et al. (2019). Within each plot, 16 sampling points were positioned every 20 m (Fig. S3) following recommendations of Donald et al. (2021) for soil eDNA sampling. For each sample point, five 10 cm depth soil cores were taken (mixing horizon A and part of horizon B) in a radius of 3.5 m and pooled in a composite sample. Rocks, large roots, litter, and organic debris were removed at the collecting step. After sampling each forest patch, the shovel was cleaned with 2% bleach to reduce cross-contamination by eDNA. Within each patch the shovel was dipped in soil near the next sampling point to avoid carrying soil debris from a point to another (Donald et al. 2021). All composite soil samples (c. 100 g/sampling point) were stored on ice for a maximum of

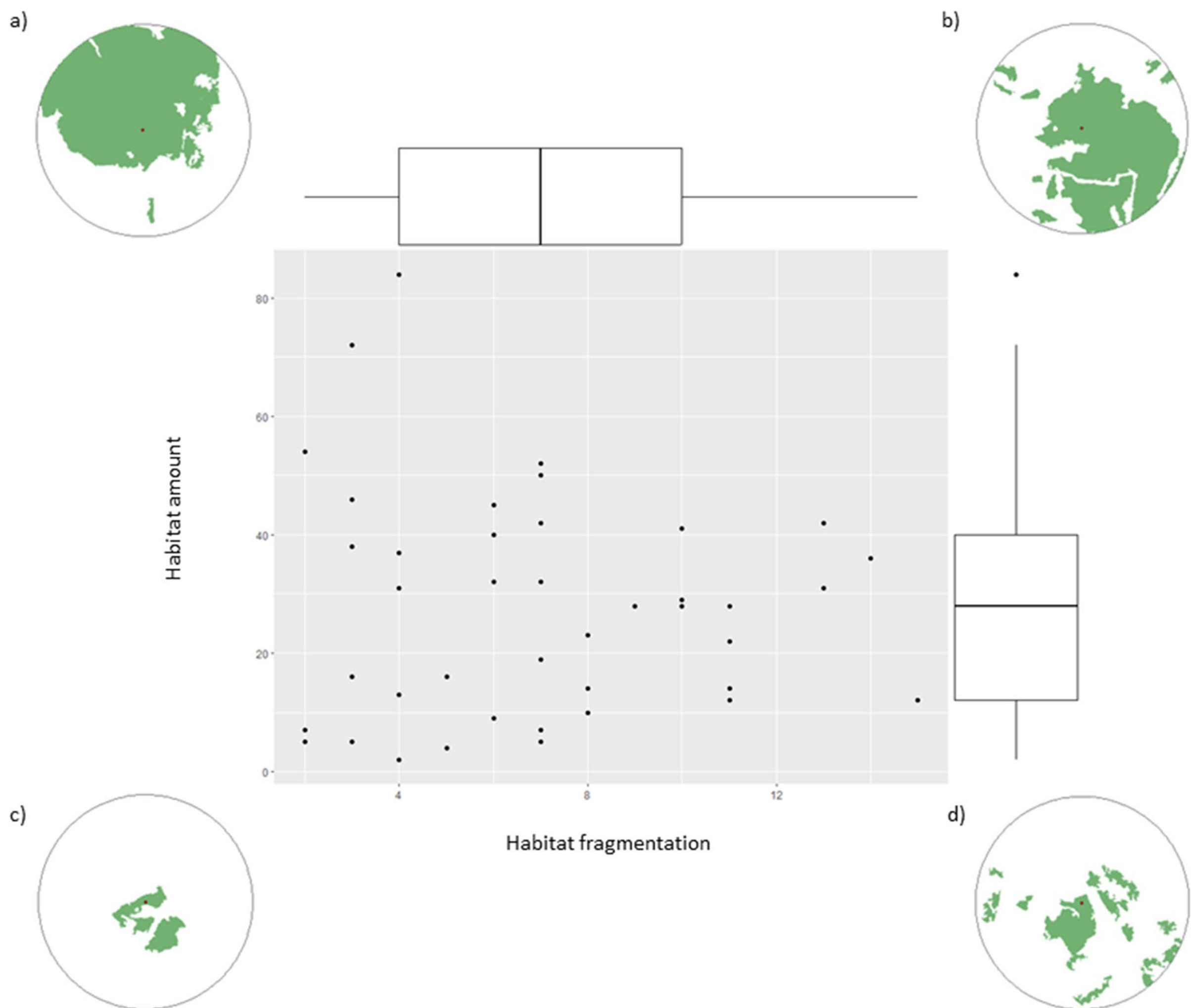

Fig. 1 Distribution of sampled sites according to the number of patches and the proportion of ancient forest in the 1 km buffer. The boxplots show the distribution of values for the number of patches (habitat fragmentation) and the quantity of ancient forest in the buffer (habitat amount). Different landscape structures illustrating extremes in the amount and fragmentation of ancient forest in a buffer zone are presented: **a** high proportion of ancient forest and low fragmentation of ancient forest, **b** high proportion of ancient forests and high fragmentation of ancient forest, **c** low proportion of ancient forest and low fragmentation of ancient forest and **d** low proportion of ancient forest and high fragmentation of ancient forest

12h. The samples were subsequently homogenized, and 15 g from each composite soil sample were transferred to a tea bag mesh surrounded by 45 g of silica gel in a clean Ziploc bag using a clean plastic spoon. The silica gel was replaced every 24 h to ensure a rapid and complete drying at room temperature in the field. The dry samples were then stored until DNA extraction. The leftovers of the composite soil sample were pooled at the plot level and fully air-dried for further chemical analyses. In addition, c. 100 g of separated samples of horizon A and horizon B (10 cm depth) were collected at the center of the plot for chemical analyses.

Molecular biology and sequencing

The DNA extraction was achieved using a Macherey–Nagel NucleoSpin R Soil kit (Allentown, Pennsylvania, USA) following Mennicken et al. (2020) and targeted the soil environmental DNA.

Negative controls (one/series) were included and the extraction resulted in 100 µL of DNA solution, stored at − 20 °C. Amplifications targeted the fungal ITS1 (Fwd: ITS5 GGAAGTAAAAGTCGTAAC AAGG from Epp et al. (2012) and a modified version of Rev:5.8S_Fungi CAAGAGATCCGTTGT TGAAAGTK, Taberlet et al. 2018). For each PCR, a unique set of primers composed of eight nucleotide markers was used to differentiate the different samples during bioinformatic processing (Taberlet et al. 2018). Negative and positive PCR controls were included, and each PCR was done twice with a different pair of tagged—primers. Empty wells were included in each PCR plate to keep unused tag-combination and evaluate possible tag-jumps (Negative sequencing controls). The PCR protocol follows Mennicken et al. (2020). The use of eight-nucleotide tags located in 5´ position of each primer and their unique combination (in Forward and Reverse) for each PCR made it possible to pool all reactions and prepare a unique library. The sequencing was performed by Fasteris company (Life Science Genesupport SA, Plan-les-Ouates, Switzerland), in 2 × 250 bp using the Miseq V3 technology.

Bioinformatics

The raw sequences (fastq for R1 and R2 sequences) were received after quality checks and analyzed with OBItools (Boyer et al. 2016) on the GenoToul cluster (Toulouse, France). The quality filters enabled the removal of sequences unaligned between the R1 and R2 files, sequences shorter than 60 nucleotides and having a qphred score below 50. The bioinformatic analyses was identical to Mennicken et al. (2020), except for the taxonomic identification step requiring an updated reference database. Following recommended practices for the analysis of fungal communities, the Molecular Operational Taxonomic Units (MOTUs) were defined using the "sumaclust" clustering function (Mercier et al. 2013) with a threshold value of 97% (Nilsson et al. 2008). Taxonomic identification of MOTUs was done using the ecotag function (Boyer et al. 2016), comparing our sequences through a global alignment among GenBank reference sequences (Benson et al. 2012). As a result, our sequences were assigned to a putative species as well as a genus, family, and order based on the Genbank taxonomy. The metabaR package (Zinger et al. 2021) was used to detect potential contaminants from our dataset and non-fungi sequences as well as sequences over-represented in our negative controls were removed. Finally, from taxonomic identification, fungal guilds and growth forms were assigned to each MOTUs recognized at the species or genus level using the FUNGUILD database (Nguyen et al. 2016). Raw sequences are all deposited in the DRYAD Archive (https://doi.org/https://doi.org/10.5061/dryad.gmsbcc2tg; to be released upon publication).

Soils analysis

The following parameters were characterized in the LEFE laboratory (Toulouse, France) on the soil composite fine fraction (< 2 mm): particle size distribution (NF ISO 13320), total and organic carbon content (NF ISO 10694), total nitrogen content (NF ISO 13878), pH (H_2O), pH (KCl) (NF ISO 10390) and phosphorus content (Olsen method, EN ISO 10304). Coarse element contents were also quantified (ISO 13320). The cation exchange capacity (CEC) and exchangeable cations contents (Ca, K, Mg, Na, Si) were also measured on the same size fraction (GET, Toulouse, France). The CEC was measured after determining the excess of cobaltihexamine ions in the exchange solution by spectrocolorimetry after 2 h stirring (Aran et al. 2008). The determination of exchangeable cations (for Ca, Na, Mg and K) was carried out by ICP-OES. The samples collected from horizon A and B were only measured for pH (H_2O), pH (KCl), organic carbon and total nitrogen content. All parameters were included in further statistical analysis.

Statistical analysis on fungal diversity

All statistical analysis were performed with RStudio 4.1.0 software (R Core Team 2021). The sample coverage was calculated for each plot (Hsieh et al. 2016) to check if our sample coverage was sufficient and comparable between sites.

To describe the diversity at the plot level, Hill numbers (Hill 1973) were calculated using the *hillR* package (Li 2018) for different q values. The larger the q value, the less contribution rare species have (Alberdi and Gilbert 2019). We used three values for q: q = 0 (specific richness), q = 1 (Shannon's index) and q = 2 (inverse of Simpson's index).

To integrate the effect of soil properties (26 variables) in our models, a principal component analysis was carried out and the three principal axis were extracted using FactoMineR (Le et al. 2008) package. To check for collinearity among the landscape variables, a Spearman coefficient with a Bonferroni correction was used from corrplot and Hmisc packages (Taiyun and Viliam 2021; Harrell 2023).

To test the effects of forest quantity and fragmentation on alpha diversity (q = 0, 1 and 2) of all soil fungi, fungal guilds (symbiotroph, pathotroph, saprotroph) and contrasted growth forms (gasteroid, agaricoid, corticioid, microfungi), linear models were tested through the lme4 package (Bates et al. 2015). All input variables were first standardized, and each model was adapted to comply with linear model conditions (normality and heteroscedasticity of residuals). All models included the first 3 PCA axes related to soil variability as co-variables. To deal with the correlation between ancient and present forest quantity, we built a set of models with or without those variables, to test for the significance of their relationship with each response variable. The first model considered the quantity of ancient forest in the landscape. The second model considered the quantity of present forest. The third model included the quantity of both ancient and present forest. In the last model, neither the quantity of ancient nor present forest were incorporated. The final models therefore do not include correlated variables. For each diversity index-fungal group combination, the best model (among the four candidate models) was selected using the AICcmodavg package (Mazerolle 2020). When the delta AICc between different models was < 2, we computed model-average estimates and the 95% confidence interval for parameters appearing in selected models (Mazerolle 2006). Effect size of each factor for a given model was extracted and represented using effectsize package (Ben-Shachar et al. 2020). Spatial autocorrelation was finally checked for each model using the linear model and the DHARMa package (Hartig 2022).

Results

Taxonomic and functional characterization of soil fungal diversity

In total, 23,456,299 sequences pass the quality filters, resulting in 5134 fungal MOTUs clustered at 97% of identity. The species richness averages 1239 MOTUs per site (845 < > 1897) and the number of sequences per site averages 572,105 reads (211,117 < > 1,011,713). The average sample coverage per site is 0.89 (0.85 < > 0.91) indicating a high sampling coverage of fungal communities. The identification of trophic guilds was possible for 61.5% MOTUs and 81.6% reads (Fig. 2a and b), revealing the dominance of symbiotrophic fungi in reads and MOTUs (Cortinariaceae, Russulaceae, Inocybaceae and Thelephoraceae, Fig. Suppl. S4). Finally, the most abundant growth forms are agaricoid, corticioid, gasteroid and microfungi (37% MOTUs, 60% reads, Fig. 2c and d).

Site characteristics and selection of co-variables

The landscape is described through its fragmentation (number of patches in the buffer) and the habitat amount (quantity in the buffer). On average, seven ancient forest patches are present in each buffer zone (2 < > 15), and ancient forest covers 28% of the surface (2 < > 84%). Considering the present forest, on average seven patches are present per buffer zone (1 < > 14), and the present forest covers in average 47% of the surface (9 < > 93%).

Soil parameters are synthesized in the form of a PCA (Fig. Suppl. S5a and b) whose three first dimensions explained 59.6% of sites variability. The first axis is driven by pH, C/N and P Olsen; the second one by percentage of carbon and coarse material and the third one by percentage of Si, K, and CEC (Table S1). For all models, no significant spatial correlation in residuals was found.

Responses of the soil fungal diversity

Our results show that forest fragmentation has a significant negative effect on the diversity of the whole community, independently from the weight given to rare species (for q = 0, 1, 2; Fig. 3; Table 1). However, this effect is detected only for past forest fragmentation. The same models show a significant positive effect of present forest quantity in the landscape (for q = 0, 1, 2; Fig. 3; Table 1). The significant and positive effect of PCA axis 1 on the three levels of q evidences the importance of soil pH, P—Olsen and C/N ratio for soil fungal diversity. Present fragmentation is always included in the best model, but never

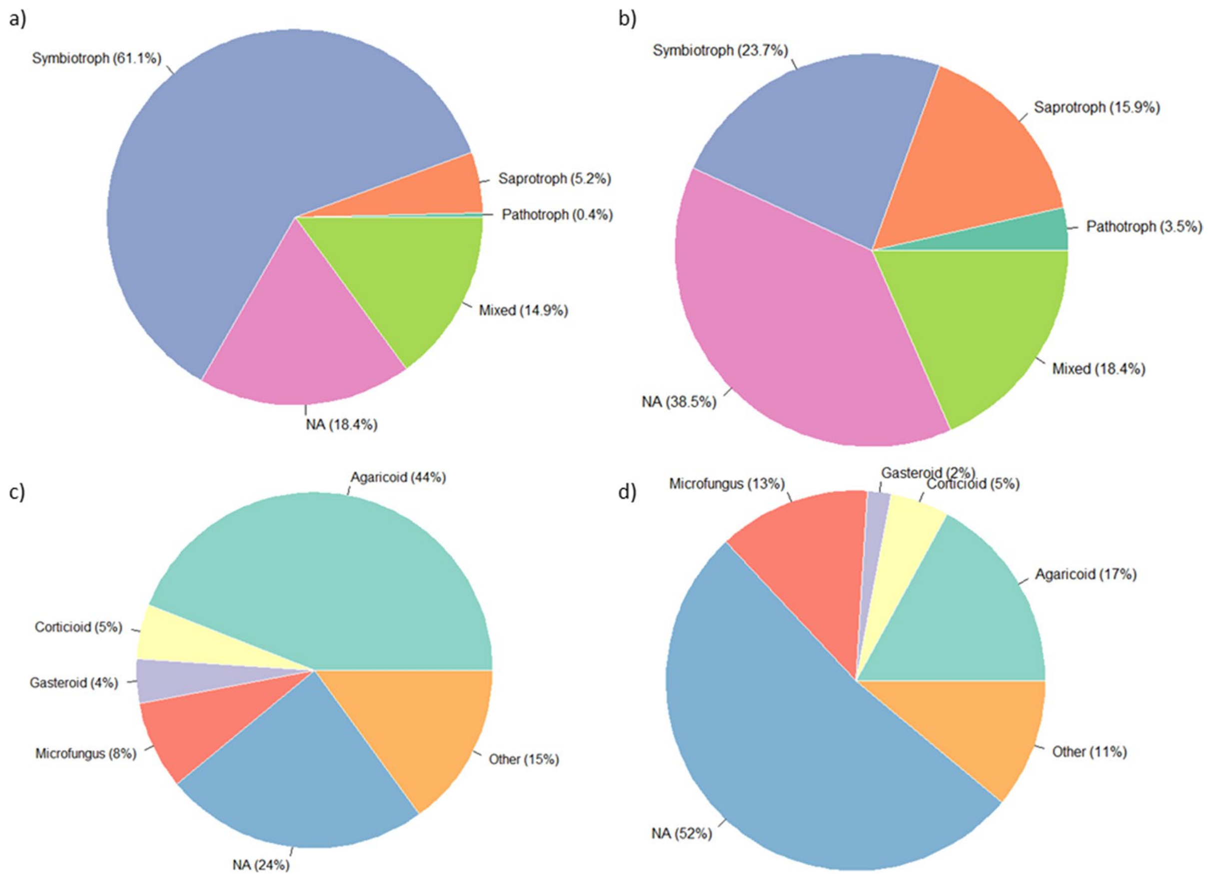

Fig. 2 Pie charts showing **a**, **c** the distribution of MOTUs according to read counts and **b**, **d** to the number of MOTUs. For the trophic guild (**a**, **b**), the "Mixed" class includes different combinations of trophic guilds. The "Not assigned" class contains MOTUs to which no trophic guild could be assigned for lack of taxonomic or functional information. For the growth form (**c**, **d**), the "Other" category includes different combinations of growth mode and other types of growth mode. The "Not assigned" category contains MOTUs to which no growth mode could be assigned due to the lack of taxonomic and functional information

shows a significant effect on soil fungal diversity. Past fragmentation, present forest quantity and PCA axis 1 show a similar effect size, considered as medium for the three q values (Fig. 3; Table 1).

Responses of the different fungal guilds

The diversity of symbiotrophs is also negatively impacted by past fragmentation and positively influenced by forest quantity (Fig. 4a and b; Table 1) and the first axis of the PCA (driven by pH, C/N, P Olsen), *i.e.,* following the fungal diversity pattern except for q = 1. However, the effect size for PCA axis 1 is higher (0.68) as compared to the effect of landscape factors (Fig. 4a and b; Table 1). By contrast, only the species richness of pathotrophs is negatively correlated with ancient forest fragmentation (q = 0 only, Fig. 4c, Table 1) and not significantly shaped by the present forest quantity. Pathotroph species richness (q = 0) is also positively influenced by the soil PCA third dimension (i.e. CEC, Si and K concentration), while the saprotroph species richness (q = 0) is significantly shaped by the first dimension of the soil PCA, as are all fungi and in particular symbiotroph diversity (Table 1). Effect sizes for pathotroph species richness show that forest fragmentation and PCA Axis 3 have similar small to medium effects (average of -0.38 and 0.36 respectively; Fig. 4c). Again, for all trophic modes and orders of q, present fragmentation never shows a significant effect on soil

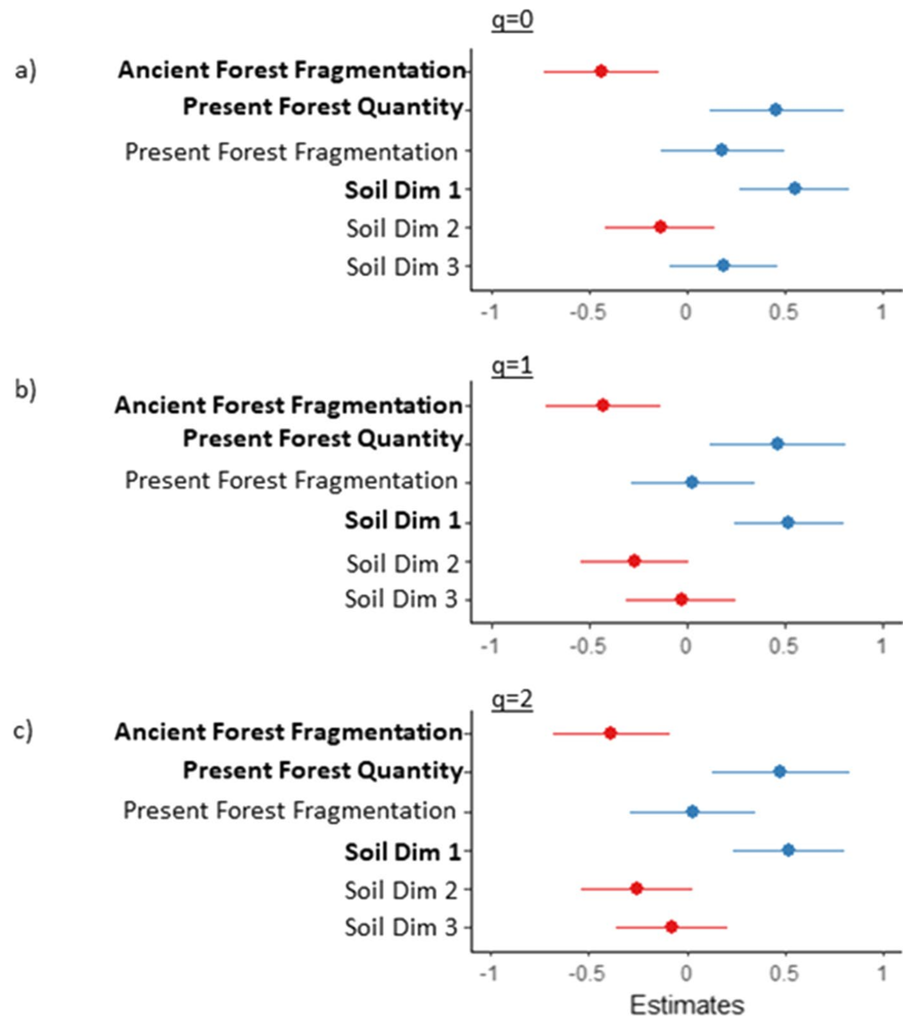

Fig. 3 Size effects of the explanatory variables for the best model (delta AICc = 0) explaining the diversity (q = 0, 1 and 2) of all fungi. Red error bars indicate a negative effect, blue ones a positive effect. Explanatory variables written in bold have a significant effect

fungal diversity but remains included in best models. Ancient and total forest quantity are often removed from best models for pathotrophs and saprotrophs (Table 1).

Responses of the different fungal growth forms

Our results show that forest fragmentation has a negative effect only on the species richness (q = 0) of growth forms associated with a possibly limited dispersal (corticoid, gasteroid, microfungi, Fig. 5, Table 2). Again, only past forest fragmentation has a negative effect on the diversity of these growth forms and present fragmentation was never significant although included in best models. However, past fragmentation is positively correlated with the diversity of most abundant corticioids (q = 1 and 2, Table 2). Agaricoid diversity and corticioid species richness (q = 0, Fig. 5a and b) are also significantly correlated with the present forest quantity (q = 1 only). Only for gasteroids do, both ancient and present forest quantity have a positive effect on species richness (Table 2, Fig. 5c). The first soil PCA axis has a significant effect on all growth forms diversity and q levels (except gasteroid q = 0). In most cases, this effect is positive, except for microfungi (Fig. 5d, Table 2). Additionally, the soil PCA second axis (mostly % of coarse material and C in composite samples and horizons) is negatively correlated with agaricoid and corticioid diversities. Agaricoid diversity is also positively correlated with the soil PCA third axis (driven by CEC, Si and K concentrations). Effect sizes comparison for

Table 1 Results of linear regressions for all fungi and each trophic guild and for the three Hill number (species richness, q=0; Shannon index q=1; inverse of Simpson index, q=2)

	Hill number	R2 adj	Ancient forest fragmentation	Present forest fragmentation	Ancient forest quantity	Present forest quantity	Soil dim 1	Soil dim 2	Soil dim 3
All fungi	q=0	0.33	**(− 82.75); [− 138.61, − 26.88]**	(34.28); [− 25.69, 94.26]		**(86.91); [21.25, 152.58]**	**(104.64); [51.61, 157.68]**	(− 26.28); [− 79.22, 26.66]	(35.32); [− 17.63, 88.26]
	q=1	0.30	**(-11.93); [− 21.2, − 2.66]**	(0.85); [− 9.1, 10.8]		**(14.8); [3.9, 25.68]**	**(16.04); [7.24, 24.84]**	(− 7.84); [− 16.62, 0.94]	(− 2.44); [− 11.22, 6.34]
	q=2	0.32	**(− 7.33); [− 12.33, − 2.33]**	(0.46); [− 4.91, 5.83]		**(7.82); [1.94, 13.7]**	**(8.84); [4.09, 13.6]**	(− 4.55); [− 9.29, 0.19]	(− 0.5); [− 5.24, 4.24]
Pathotrophs	q=0	0.09	**(− 3.77); [− 7.01, − 0.53]**	(− 0.47); [− 3.54, 2.61]			(0.15); [− 2.92, 3.22]	(− 0.17); [− 3.27, 2.94]	**(3.54); [0.37, 6.71]**
	q=1	− 0.07	(− 0.4); [− 2.28, 1.49]	(− 0.98); [− 2.96, 0.99]		(− 1.5); [− 3.72, 0.72]	(− 0.02); [− 1.81, 1.77]	(0.3); [− 1.48, 2.09]	(− 0.32); [− 2.11, 1.48]
	q=2	− 0.10	(− 0.24); [− 1.61, 1.12]	(− 0.5); [− 1.89, 0.88]		(− 0.85); [− 2.49, 0.78]	(− 0.05); [− 1.34, 1.25]	(0.16); [− 1.14, 1.46]	(− 0.37); [− 1.68, 0.94]
Saprotrophs	q=0	0.10	(− 10.43); [− 21.13, 0.26]	(0.83); [− 10.58, 12.25]	(7.17); [− 4.13, 18.46]	(10.04); [− 2.37, 22.46]	**(11.27); [1.11, 21.43]**	(0.71); [− 9.4, 10.82]	(5.73); [− 4.4, 15.86]
	q=1	NA	(− 0.02); [− 0.16, 0.12]	(− 0.05); [− 0.18, 0.08]			(− 0.07); [− 0.20, 0.06]	(0.07); [− 0.07, 0.20]	(0.06); [− 0.07, 0.19]
	q=2	NA	(− 0.05); [− 0.21, 0.12]	(− 0.03); [− 0.18, 0.12]			(− 0.08); [− 0.23, 0.07]	(0.05); [− 0.11, 0.21]	(0.11); [− 0.04, 0.27]
Symbiotrophs	q=0	0.43	**(− 16.30); [− 31.05, − 1.56]**	(14.35); [− 1.48, 30.18]		**(24.28); [6.95, 41.61]**	**(36.95); [23.00, 51.00]**	(− 10.89); [− 24.86, 3.09]	(8.90); [− 5.08, 22.87]
	q=1	0.41	(− 4.43); [− 8.94, 0.08]	(0.6); [− 3.96, 5.15]	(− 4.3); [− 12.06, 3.46]	**(7.71); [0.58, 14.85]**	**(10.41); [6.39, 14.43]**	**(− 5.59); [− 9.61, − 1.58]**	(− 0.71); [− 4.72, 3.31]
	q=2	0.33	**(− 2.97); [− 5.90, − 0.04]**	(0.30); [− 2.85, 3.44]		**(3.89); [0.44, 7.33]**	**(5.81); [3.03, 8.59]**	**(− 3.45); [− 6.22, − 0.67]**	(0.24); [− 2.54, 3.01]

Explanatory variables written in bold have a significant effect

For each explanatory variables, estimates are in brackets and the 95% confidence interval in square brackets

corticioids shows that effects of soil PCA axis 1 and forest quantity are both medium to large, while past fragmentation and other soil PCA axis having smaller effects (Table 2, Fig. 5b). For gasteroids, whose species richness is influenced by the two first PCA axis, and both present and past forest quantity, all effect size are relatively similar and considered as medium (Table 2, Fig. 5).

Discussion

Our study on fungal diversity from ancient forests soils of South-West France, joins recent works exploring the landscape ecology of microbial communities. Our results illustrate the mixed effect of both present forest quantity and past landscape fragmentation on soil fungi, and the need to consider several landscape

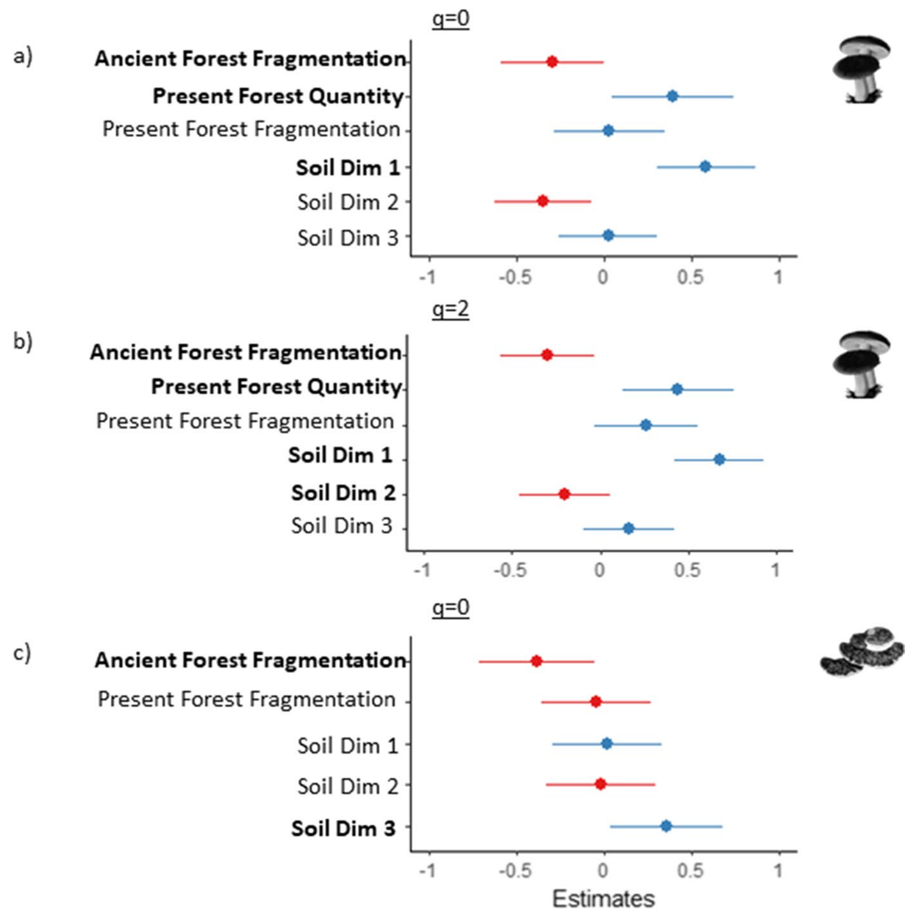

Fig. 4 Size effects of the explanatory variables for the best model (delta AICc = 0) explaining the diversity of symbiotrophic (q = 0 and 2, **a** and **b** respectively) and pathotrophic fungi (q = 0, **c**). Red error bars indicate a negative effect, blue ones a positive effect. Explanatory variables written in bold have a significant effect

descriptors when studying hyper-diverse communities such as fungal ones as suggested by Su et al. (2022).

Our results evidence the negative relationship between past forest fragmentation and the local diversity of fungal communities associated with ancient forest soils, whatever the Hill numbers used to describe the fungal diversity. We therefore validate our first hypothesis, but only for past and not present forest fragmentation. This pattern could reflect an extinction debt for fungal communities in ancient forests (Jackson and Sax 2010). Dispersal limitations could explain this result, which is further supported by the negative relationship between past forest fragmentation and microfungi and corticioid diversity, and the lack of effect for the wind-dispersed agaricoid fungi. Several studies point out the dispersal limitations of either ectomycorrhizal or dead wood associated fungi in fragmented habitats (Peay et al. 2012; Abrego and Salcedo 2014; Boeraeve et al. 2018b) but did not necessarily highlight this legacy effect. Considering the current dynamic of forests throughout Europe, ancient forests would appear to be less and less fragmented (Palmero-Iniesta et al. 2020). However, our results suggest a long-lasting legacy effects of forest fragmentation on soil fungal communities, opening major questions on the conservation of these unique habitats, as well as their positive impact on surrounded forest biodiversity.

Following our second hypothesis and the HAH hypothesis, the present quantity of forest in the landscape has a positive effect on soil fungal diversity. This result highlights the importance of considering the negative impact of habitat loss, not only of fragmentation, on fungal communities. Moreover, in our study zone, the forest cover doubled on average compared to the records of the nineteenth century, potentially creating new habitats for fungal diversity. However, only the present and not ancient forest quantity has a positive effect on soil fungi diversity, contrasting with Mennicken et al. (2020). The landscape

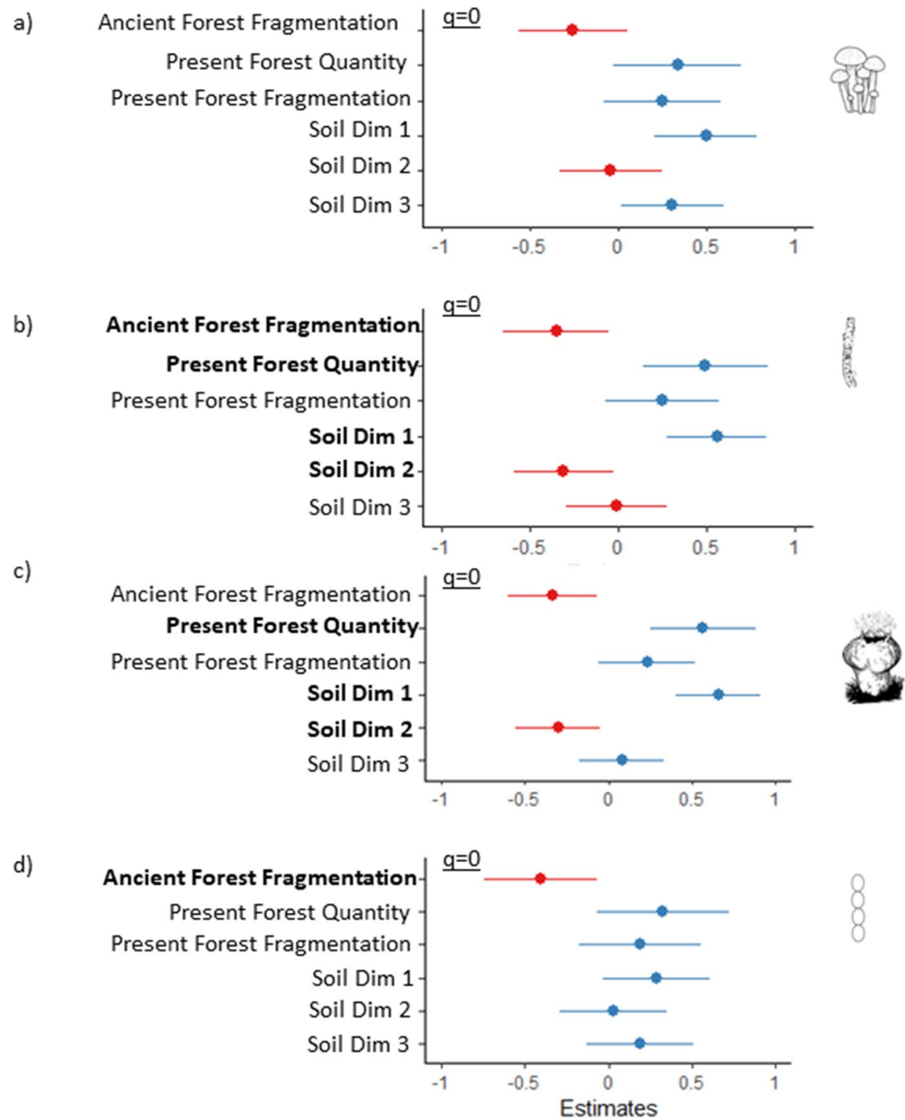

Fig. 5 Size effects of the explanatory variables for the best model (delta AICc = 0) explaining the species richness (q = 0) of agaricoid (**a**), corticioid (**b**), gasteroid fungi (**c**) and microfungi (**d**). Red error bars indicate a negative effect, blue ones a positive effect. Explanatory variables written in bold have a significant effect

context is clearly different from the Cevennes National Park where this previous study took place. Indeed, quantities of ancient and present forest were particularly contrasted (Mennicken et al. 2020), and present forest fragmentation was reduced but was not studied per se. Future meta-analyses would be particularly useful to integrate these different results and contribute to a larger synthesis on the effect of forest quantity for soil fungal communities. More generally, including landscape descriptors should be considered in future studies on soil fungal communities, as they could explain part of diversity changes, exactly as soil characteristics do. On this aspect our results confirm the importance of soil factors such as pH, C/N ratio and P Olsen, as observed in large-scale analysis on fungal diversity (Tedersoo et al. 2014).

Through the analysis of eDNA metabarcoding, our results also show the variable response across trophic guilds. We evidence a negative relationship between forest fragmentation and symbiotroph diversity for at least two Hill numbers. This guild was the most abundant in MOTUs and reads, as in most temperate forest soils, including ancient forest ones (Spake et al. 2016; Mennicken et al. 2020). The dominant families (Fig. Suppl. S3) belonged to symbiotroph fungi and in particular ectomycorrhizal ones, which is also highlighted by studies based on fruiting bodies in ancient forests (Hannoire and Corriol 2019). Our results show

Table 2 Results of linear regressions for the diversity of fungal growth forms and for the three Hill number (species richness, q = 0; Shannon index q = 1; inverse of Simpson index, q = 2)

	Hill number	R2 adj	Ancient forest fragmentation	Present forest fragmentation	Ancient forest quantity	Present forest quantity	Soil dim 1	Soil dim 2	Soil dim 3
Agaricoid	q = 0	0.23	(− 7.43); [− 18.26, 3.4]	(6.5); [− 5.26, 18.26]	(8.45); [− 2.81, 19.71]	(11.86); [− 0.44, 24.17]	**(16.42); [6.21, 26.63]**	(− 0.52); [− 10.66, 9.61]	**(11.25); [1.15, 21.35]**
	q = 1	0.16	(− 3.12); [− 6.63, 0.4]	(0.89); [− 2.67, 4.46]	(− 3.23); [− 9.31, 2.85]	(5.21); [− 0.27, 10.69]	**(4.78); [1.63, 7.92]**	(− 2.52); [− 5.66, 0.62]	(− 0.23); [− 3.37, 2.91]
	q = 2	0.12	(− 1.48); [− 3.92, 0.95]	(− 0.26); [− 2.95, 2.42]		(2.5); [− 0.29, 5.3]	**(3.05); [0.74, 5.37]**	(− 1.61); [− 3.92, 0.69]	(0.21); [− 2.08, 2.5]
Corticioid	q = 0	0.44	**(− 4.54); [− 8.20, − 0.89]**	(3.18); [− 0.75, 7.10]		**(7.76); [3.47, 12.06]**	**(9.01); [5.54, 12.48]**	**(− 4.12); [− 7.58, − 0.66]**	(1.11); [− 2.35, 4.58]
	q = 1	NA	**(0.14); [0.05, 0.23]**	(0.05); [− 0.05, 0.15]	(0.08); [− 0.02, 0.19]	(0.1); [− 0.02, 0.22]	**(0.25); [0.16, 0.34]**	(0.05); [− 0.04, 0.14]	(− 0.02); [− 0.11, 0.07]
	q = 2	NA	**(0.23); [0.12, 0.35]**	(0.04); [− 0.08, 0.16]	(0.08); [− 0.06, 0.22]		**(0.25); [0.13, 0.37]**	(0.08); [− 0.03, 0.2]	(− 0.02); [− 0.14, 0.09]
Gasteroid	q = 0	0.29	(− 1.64); [− 3.33, 0.05]	(1.21); [− 0.46, 2.89]	**(2.2); [0.54, 3.86]**	**(2.66); [0.84, 4.47]**	**(2.98); [1.51, 4.46]**	**(− 1.6); [− 3.08, − 0.13]**	(− 0.03); [− 1.5, 1.44]
	q = 1	0.35	(0.19); [− 0.79, 1.17]	(− 0.03); [− 1.07, 1.01]	(0.81); [− 0.23, 1.84]	(0.86); [− 0.3, 2.02]	**(2.28); [1.34, 3.22]**	(− 0.65); [− 1.58, 0.29]	(0.31); [− 0.63, 1.25]
	q = 2	0.30	(0.01); [− 0.8, 0.82]	(− 0.15); [− 1.01, 0.71]	(0.73); [− 0.12, 1.59]	(0.69); [− 0.27, 1.66]	**(1.75); [0.97, 2.53]**	(− 0.58); [− 1.35, 0.2]	(0.28); [− 0.5, 1.05]
Microfungus	q = 0	0.11	**(− 10.59); [− 20.58, − 0.6]**	(3.72); [− 6.96, 14.4]	(8.46); [− 1.96, 18.88]	(9.92); [− 1.64, 21.48]	(7.75); [− 1.73, 17.24]	(1.71); [− 7.7, 11.12]	(6.3); [− 3.12, 15.73]
	q = 1	0.23	(− 1.15); [− 3.01, 0.72]	(− 1.57); [− 3.35, 0.20]			**(− 2.65); [4.42, − 0.88]**	(0.01); [− 1.77, 1.80]	(0.88); [− 0.94, 2.71]
	q = 2	0.30	(− 0.47); [− 1.34, 0.41]	(− 0.65); [− 1.49, 0.18]			**(− 1.62); [2.45, − 0.79]**	(0.06); [− 0.78, 0.91]	(0.22); [− 0.64, 1.08]

Explanatory variables written in bold have a significant effect

For each explanatory variables, estimates are in brackets and the 95% confidence interval in square brackets

that their diversity—as well as all fungal ones—is also positively influenced by the present forest quantity, and that both landscape and soil factors have a similar range of effects. Compared to studies testing the effect of the forest extent, that focused on the patch size (Peay et al. 2012), our results invite further consideration of the HAH in studies on symbiotrophs, and in particular for ectomycorrhizal fungi. For other trophic guilds, thar are less abundant in our dataset, results are more heterogeneous. Only the species richness of pathotrophs is strongly negatively influenced by the past forest fragmentation, which confirms the negative impact of fragmentation for pathogenic dead wood associated species (Abrego and Salcedo 2014). Inventories on ancient and old-growth beech forests have already highlighted the importance of ancient forests for the conservation of dead-wood associated fungi (Parmasto 2001; Dvorak et al. 2017). Using

soil eDNA may underestimate their diversity (Frøslev et al. 2019) but has at least confirmed the importance of ancient forests, especially the less fragmented ones, for the conservation of this trophic guild.

Using taxonomical and functional databases also allows for the investigation of if wind dispersed fungi were less impacted by landscape factors as compared with fungi with more limited dispersal abilities. Our results clearly show that agaricoid fungi—that are often wind dispersed- are not affected by forest fragmentation nor quantity forest in the landscape. As with many fungi, their diversity is rather influenced by pH, C/N ratio and P Olsen in soils. On the other hand, fungi with low dispersal abilities show different response to landscape gradients. Corticioid and microfungi species richness is negatively impacted by past fragmentation, while gasteroid fungi diversity is both positively influenced by past and present forest quantity. Gasteroid species can be dispersed by large mammals (*i.e.,* wild boar, particularly abundant in South-West France) or even small mammals (Borgmann-Winter et al. 2023), that are frequent in forest habitats including ancient forests. The lack of fragmentation effect suggests that mammal dispersal occurs at least at the landscape scale. Recent reviews on fungal dispersal by mammals also confirm this trend (Vašutová et al. 2019), and dispersal limitations may rather apply for corticoid and microfungi in our case. Indeed, corticioid fungi dispersal is generally overlooked but may occur even at shorter distance (e.g., insect dispersal, Harrington et al. 2021). Many species show a high degree of endemism (Ghobad-Nejhad et al. 2012) and ancient forest would be a good habitat for their diversity. Moreover, corticioid diversity was positively influenced by the fragmentation, for higher orders of q, suggesting that corticoid fungi would be more diverse in the more fragmented ancient forests, contrary to the pattern observed for all fungi and major trophic guilds. A mapping of the dead wood abundance in ancient forest landscapes including small diameter one (Juutilainen 2016) would however be required to further document this pattern.

Finally, from this soil eDNA metabarcoding experiment, we detect 5134 MOTUs, relatively more than in ancient forests of Cevennes National Park (Mennicken et al. 2020). This high number shows how apparently managed and fragmented forests contribute to maintain a diverse soil fungal diversity at a regional scale. Metabarcoding often overestimates the number of species compared to fungal fruiting bodies inventories, but interestingly several studies on ancient forests have used comparable methodologies. In ancient forest of the Alps, at higher elevation, 842 MOTUs were detected over 62 sites, and an average of 88 MOTUs were detected per site (Mollier 2023); *i.e.,* circa 10 times less alpha diversity as observed in our study. The spatial design is different as 1 ha was sampled instead of a 15 m radius plot—leading to a larger plot size in our study. Sampling a larger area—1 ha—could explain the higher diversity estimates, especially considering the local heterogeneity of ancient forests. Similarly, the high number of MOTUs at a regional scale could be related to a high heterogeneity of fungal communities associated with ancient forest soils. Such a heterogeneity could be an important feature of ancient forests, already suggested on other old-growth pine forests for example (Wulf and Kolk 2014) and shall be characterized to potentially understand its importance on fungal diversity.

Conclusions

Our study provides a comprehensive characterization of soil communities based on eDNA in ancient forests and highlights the negative effect of past fragmentation on all soil fungi and especially on symbiotroph diversity. Additionally, the habitat amount has a positive effect for most fungi, in relation with the quantity of present forest in the landscape. We also evidence that the diversity of fungal growth forms associated with a long-distance dispersal is not impacted by past or present fragmentation. By studying ancient forests over South-West France, we illustrate an important legacy effect, inherited from the past forest fragmentation. Finally, this study strengthens the need to consider landscape ecology and its impacts on diversity, including on soil fungi. In a context of global change, where habitat fragmentation and land-use changes are threatening biodiversity, our study highlights the importance of reasoning at the landscape scale and considering forest history, for the conservation of soil fungi and their functional diversity, at least in ancient forests.

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Author contributions All authors contributed to the design and development of the study. Data collection was carried out by AR, MR, OF, SM, JMS and LG. DNA extraction in the laboratory was carried out by AR and CRC with the help of SM. The various bioinformatics steps were carried out by MR and AR. Physico-chemical analyses were carried out under the supervision of LG and PO. Statistical analyses were carried out by AR and LG. The final manuscript was written by AR, MR and AB. All the authors read and approved the manuscript.

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Data availability The raw sequences produced in this study are deposited on DRYAD repository (https://doi.org/https://doi.org/10.5061/dryad.gmsbcc2tg; to be released upon publication), together with the R code, the description of sites (including soil characteristics) and of fungal MOTUs taxonomy.

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

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