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Spatially-explicit effects of small-scale clear-cutting on soil fungal communities in *Pinus sylvestris* stands

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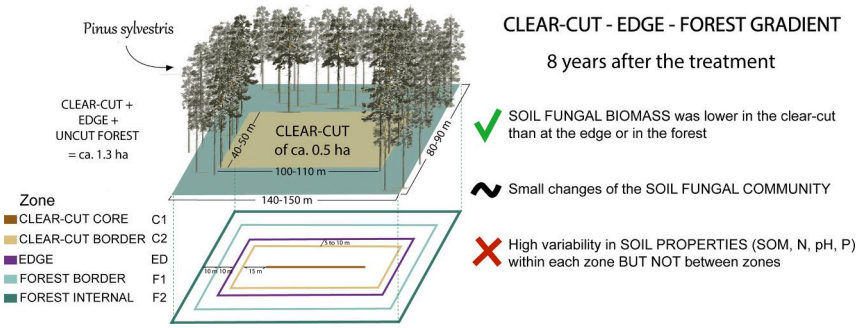
HIGHLIGHTS

- Spatial soil sampling was used to analyze a clear-cut–edge–forest gradient.
- Medium-term changes in soil fungal biomass, composition and diversity were analyzed.
- Small changes in soil fungal composition occurred along the gradient.
- Fungal biomass was lower in the clear-cut zone than at the edge or in the forest.
- No relationship between soil fungi and soil nutrient distribution (SOM, N, P, pH)

GRAPHICAL ABSTRACT



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ABSTRACT

Clear-cutting is a common silvicultural practice. Although temporal changes in the soil fungal community after clear-cutting have been widely investigated, little is known about stand-level variations in the spatial distribution of soil fungi, particularly at the clear-cut edge. We performed spatial soil sampling in three clear-cuts (0.5 ha), edge habitats, and surrounding forests 8 years after clear-cutting to examine the impact of clear-cutting on the soil fungal community (diversity, composition, guilds, and biomass) and soil properties in a managed *Pinus sylvestris* forest in northern Spain. Our analyses showed small differences in the composition of the soil fungal community between edge, forest, and clear-cut zones, with <4 % of the species strictly associated with one or two zones. The richness, diversity, and evenness of the fungal community in the edge zone was not significantly different to that in the forest or clear-cut zones, although the clear-cut core had approximately a third fewer ectomycorrhizal species than the edge or the forest. Saprotrophic fungi were widespread across the clear-cut–forest gradient. Soil fungal biomass varied significantly between zones, ranging from 4 to 5 mg g^{-1} dry soil in the forest and at the forest edge to 1.7 mg g^{-1} dry soil in the clear-cut area. Soil organic matter, pH, nitrogen, and

Abbreviations: ECM, Ectomycorrhizal; N, Nitrogen; P, Phosphorus; SOM, Soil Organic Matter; hell, Hellinger; pa, Presence–absence; ASV, Amplicon Sequence Variant; PMAV, Permutation Multivariate Analysis of Variance.

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phosphorus did not differ significantly between edge, forest, and clear-cutting zones and were not significantly related to the fungal community composition. Overall, our study showed that small-scale clear-cut treatments are optimal to guarantee, in the medium-term, soil fungal communities within harvested areas and at the forest edge that are comparable to soil fungal communities in the forest, even though the amount of fungal biomass in the clear-cut zone is lower than at the forest edge or in the forest.

1. Introduction

Forest clear-cutting is a widely applied silvicultural practice resulting in a noticeable reduction in biomass aboveground and less evident changes to the soil and microbiota belowground (Paillet et al., 2010; Thiffault et al., 2011). The total removal of the forest canopy results in greater soil temperature and moisture amplitudes (Ballard, 2000), which can affect forest understory vegetation (Centenaro et al., 2023), soil nutrient cycling (Chatterjee et al., 2008; James and Harrison, 2016; Thiffault et al., 2011), and soil fungal community composition and diversity (Chatterjee et al., 2008; Jones et al., 2003). Although several studies have used molecular tools to investigate the impact of forest management on soil microbial diversity (e.g., Kohout et al., 2018; Kyaschenko et al., 2017; Rähn et al., 2023; Varenius et al., 2016), these studies focused on the forest interior and the clear-cut area rather than on potential differences in soil properties and fungal communities close to forest edges (Crockatt, 2012). However, microclimatic conditions at the edge of the clear-cut may differ from both the forest interior and the core of the clear-cut area (Crockatt, 2012), leading to differences in soil properties and soil fungal dynamics. To our knowledge, only two studies have used molecular tools to determine how soil fungal communities change with increased distance from the forest edge (Boeraeve et al., 2019; Yang et al., 2022). However, both studies focused on edges of forests surrounded by agricultural land that is regularly harvested and tilled. Furthermore, they focused on deciduous broadleaved species, while little attention has been directed toward unraveling variations in fungal communities and soil properties at the edges of coniferous forests, particularly in Mediterranean ecosystems.

Soil fungal communities are composed of hundreds of different fungal species that can be grouped into guilds (Root, 1967). The two most important guilds are the free-living saprotrophs (e.g., dung saprotrophs, litter saprotrophs, and soil saprotrophs) and the plant root-associated symbiotic fungi (e.g., ectomycorrhizal (ECM) fungi, arbuscular mycorrhizal fungi, and ericoid mycorrhizal fungi). Saprotrophs obtain carbon (C) through organic matter decomposition, whereas symbiotic fungi receive C in the form of photosynthates from the host and root exudates (Smith and Read, 2008). After clear-cutting, ECM fungal communities are left without photosynthesizing host trees whereas saprotrophs have plenty of sources of woody debris to decompose but a lack of new organic plant matter (i.e., no litterfall). Studies analyzing the short (Heinonsalo et al., 2007; Kohout et al., 2018; Parladé et al., 2019; Rähn et al., 2023), medium (Hartmann et al., 2012), and long-term (Chen et al., 2019; Kyaschenko et al., 2017) effects of clear-cutting have reported significant differences in the composition of the fungal community between forest and clear-cut areas. In most cases, a change in the overall soil fungal community was observed, with a slow recovery of the ECM community after the treatment. Furthermore, several studies have reported a decrease in fungal biomass in the short term, i.e., within the first 2 to 5 years after the clear-cut (Bååth, 1980; Forge and Simard, 2000; Grebenc et al., 2009; Holden and Treseder, 2013; Pietikäinen and Fritze, 1995), and in the medium-term, i.e., 8 to 15 years after the treatment (Chatterjee et al., 2008; Hassett and Zak, 2005; Moore-Kucera and Dick, 2008). However, little is known about soil fungal responses at the edge of forests subjected to clear-cuts (Crockatt, 2012). Previous studies, such as Heinonsalo et al. (2007), found higher ECM diversity at the forest edges than in the forest interior, and Yang et al. (2022) found that arbuscular mycorrhizal fungi were significantly more abundant at the forest edge then in the forest interior. Furthermore, some studies have reported a gradient of fungal biomass from the edge of the clear-cut to the forest interior (Malmivaara-Lämsä et al., 2008). Therefore, it is essential to analyze how fungal species respond to such habitat transitions to understand the ecological significance and potential consequences for forest management (Spiecker, 2003).

A forest clear-cut can also affect soil properties, depending on the local climate and annual precipitation. In general, removing forest vegetation exposes the soil to increased temperature variations and leads to an initial increase in soil moisture, that rapidly vanishes with the reestablishment of the vegetation (Keenan and Kimmins., 1993). The available plant residues in clear-cut areas are degraded by saprotrophs and, consequently, the amount of litter and organic matter content decreases (Grelle et al., 2023; Jamroz and Jerzykiewicz, 2022). By contrast, in a forest environment, the deposition of leaves, needles, and dead branches leads to the formation and accumulation of a litter layer. Changes in soil nutrient cycling (e.g., C, N, P) may be related to changes in fungal community composition, abundance, and diversity (Adamo et al., 2021; Högberg et al., 2010; Kjøller et al., 2012; Kyaschenko et al., 2017; Lilleskov et al., 2002). However, soil properties (Stutz and Lang, 2023), as well as microbial communities (Franklin and Mills, 2003), might change within a few meters and, thus, it is important to understand how they change spatially and whether there is greater variability within or between zones (i.e., clear-cut, edge, and forest interior).

Overall, to better describe the soil fungal community after a clear-cut, we need to consider potential changes in the fungal spatial distribution and biomass. Moreover, the impact of a clear-cut on the fungal community might gradually change according to the distance from the forest edge. Therefore, understanding the impacts of clear-cutting requires a thorough exploration of the potential variation in the fungal community from the center of the clear-cut to the forested area, passing through (and sampling) the edge of the clear-cut. Furthermore, soil properties within the sampling area may change, which may influence the soil fungal community. Thus, soil properties also need to be considered to better understand changes in soil fungal composition and diversity. The overarching aim of this study was to examine the medium-term impact of small-scale clear-cutting on the soil fungal community (composition, diversity, functional guilds, and biomass) and soil properties along a clear-cut–edge–forest gradient (Fig. 1) in a Mediterranean *Pinus sylvestris* forest (North Spain). Our aims were: (1) to understand whether the soil fungal community at the forest edge was significantly different to communities found in the forest interior and in the clear-cut zone; (2) to unravel potential spatial differences in fungal richness, diversity, and biomass along the clear-cut, edge, and forest gradient; (3) to describe spatial differences in soil properties between clear-cut, edge, and forest interior zones and how this could be related to soil fungal community composition.

2. Materials and methods

2.1. Study site

The study was conducted in a managed monospecific Scots pine (*Pinus sylvestris*) forest known as 'Pinar Grande', located within the province of Soria, in the eastern part of the Castilla y León region, Spain (Fig. 1a). The Pinar Grande forest covers an area of 12,500 ha with an altitudinal gradient ranging from 1100 to 1500 m.a.s.l. and is part of the Sistema Ibérico mountain range. The vegetation includes *Agrostis* sp.,

Brachypodium sp., *Cynosurus cristatus*, *Erica vagans*, *Erica tetralix*, *Lotus* sp., and *Nardus stricta*. The climate is subhumid Mediterranean but affected by continental features. The average annual rainfall is 865 mm, with 69 mm falling in July and August, and 132 mm in September and October. The average annual temperature is 8.8 °C: January is the coldest month with an average temperature of 1.9 °C; July is the warmest month with an average temperature of 17.4 °C with strong temperature excursion between night and day (Ágreda et al., 2015). The summer is hot and dry. The frost period begins in November and ends in April, with frequent frosts in late spring and early fall (Martínez-Peña et al., 2012).

2.2. Clear-cut experiment

Three rectangular areas of 5000 m^2 (ca. 100–110 m × 45–50 m) were clear-cut in September 2012 (Fig. 1b). The term clear-cut has been used to indicate harvested areas ranging from 1 ha to tens of ha (Jones et al., 2003; Prescott et al., 2000). Thus, in our study, given the small size of the harvested stands, we refer to these areas as small clear-cut areas. The 'Pinar Grande' forest, where the three areas were located, comprised a homogeneous stand structure of *P. sylvestris* trees with an average age of 110 years and of similar heights (mean: 25.8 m) and diameters (mean: 43.5 cm) (Fig. 1c). Silvicultural practices are regulated by a management plan. To reduce the density of young forest stands regenerating after clear-cutting, stands are thinned ca.15 years after the original clear-cut and then again, between 50 and 70 years after the original clear-cut. Finally, when the remaining trees are 110 years old (or more) they are clear-cut. Nevertheless, the intensity of the first and second thinning treatment might change due to, for example, the wood price at a given time, recreational needs, decisions of owners, the presence of protected areas, the outbreak of pests, or the presence of firebreak areas. For this reason, the three forest stands used in this study had different tree densities before the final clear-cut owing to differences in the silvicultural management of each area rather than to differences in site fertility: area 1, 287 trees per ha; area 2, 400 trees per ha; and area 3, 687 trees per ha. Indeed, according to Martínez-Peña (2009), the site index of areas 1, 2, and 3 was 2.26, 2.32, and 2.37, respectively. Within the three areas, trees were felled and delimbed with a chainsaw and harvested with a skidder. Subsequently, the branches were chipped on site and the soil was tilled superficially with a harrow pulled by a tractor to encourage natural regeneration from trees surrounding the clear-cut areas (Fig. S1). No artificial regeneration was undertaken, and no other silvicultural treatment was performed in the harvested areas. Stumps were left in the clear-cut areas. An inventory carried out in fall 2015 by Mesa de los Ríos (2017), three years after felling, revealed that regeneration was widespread in the three clear-cut areas: 17,800 *P. sylvestris* seedlings per ha in area 1, 14,700 in area 2, and 18,200 in area 3, ranging in age from 1 to 3 years. When the sampling took place,

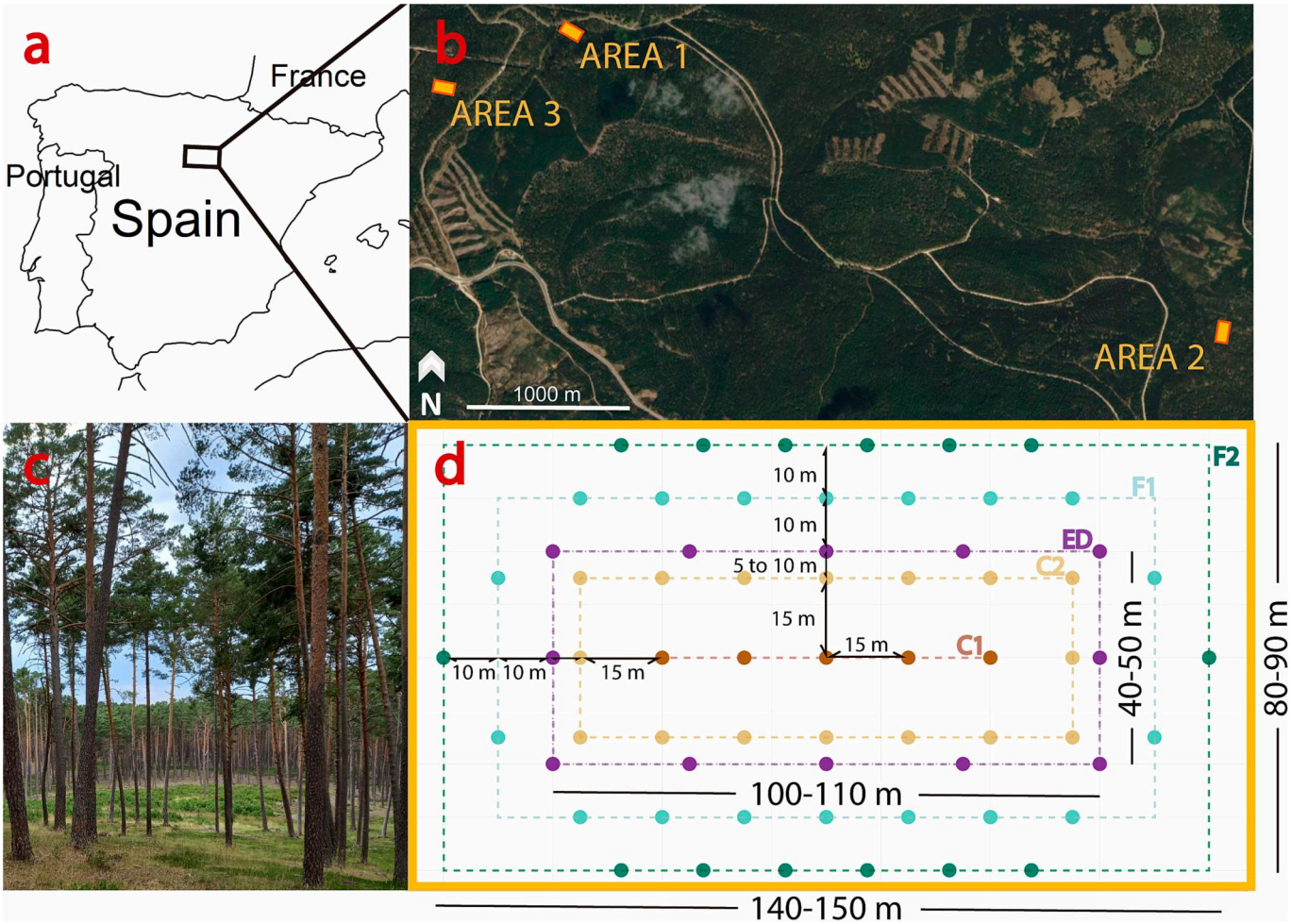

Fig. 1. (a) Map of Spain showing the location of the monospecific Scots pine (*Pinus sylvestris*) forest known as 'Pinar Grande' within the Central Spanish plateau. (b) Location of the three clear-cut areas (Area 1, Area 2, and Area 3) in the Pinar Grande forest. (c) Scots pine forest surrounding the clear-cut of Area 1. (d) Sampling design: for each of the three areas there were five sampling points in the clear-cut core (C1, dark-ocher dots), 16 sampling points 15 m from the clear-cut core and 5–10 m from the edge (C2, light-ocher dots), 12 sampling points at the edge (ED, purple dots), 18 sampling points 10 m from the edge in the forest margin (F1, light-green dots), and 14 sampling points in the forest interior, 20 m from the edge (F2, dark-green dots).

regeneration was homogeneously distributed in the three sampled areas.

2.3. Soil sampling

For each of the three clear-cut areas we designed a systematic grid of ca. 12.5–1.3 ha to cover the clear-cut (0.5 ha), the edge, and the surrounding forest. To properly capture the spatial variation, each of the three areas was divided into five zones: the clear-cut core (C1); the clear-cut margin (C2), which was 15 m from the core; the forest edge (ED), which was 5 to 10 m from C2; the forest margin (F1), which was 10 m from the forest edge; and the forest interior (F2), which was 20 m from the forest edge. The size of the three clear-cut areas slightly differed, ranging from 40 to 50 m wide and 100 to 110 m long (Fig. 1d). Thus, we decided to maintain the same distance between samples in C1 and C2 (15 m) and between soil samples in F1 and F2 (10 m), while adjusting the sampling distance between ED and C2, which ranged from 5 to 10 m (Fig. 1d).

On July 7th, 2020 we collected the soil samples in the three areas. First, we sampled three soil cores (10 cm deep and 5 cm in diameter, ca. 20 cm apart) for each of the 65 points of each area (5 in C1 + 16 in C2 + 12 in ED + 18 in F1 + 14 in F2). Second, the three soil cores collected at each sampling point were pooled in the field to create one pooled soil sample per sampling point. Needles and partially decomposed needles were excluded to reduce the sampling of needle-associated saprotrophs (Clemmensen et al., 2013; Voříšková et al., 2013). We sampled the first 10 cm of organic soil (horizon A) because horizon A was 10 to 20 cm deep in each of the three areas (Martínez-Peña, 2009). The resulting material was used to analyze the soil fungal community. Moreover, an extra soil sample per point per area was collected to determine the soil physicochemical properties. Once in the laboratory, the pH of the soil at the 195 sampling points (three areas × 65 sampling points) was measured using fresh material, whereas for all the other analyses, the samples were lyophilized, sieved (≤2 mm mesh), and ground to a fine powder using a mortar and pestle. The resulting fine powder was stored in a dark, dry location at room temperature before DNA extraction.

2.4. Soil chemical properties

The 195 soil samples were analyzed in the laboratory using the methodology described by Alday et al. (2012). We determined soil characteristics using the following techniques: soil pH using a pH meter in a 1:2.5 soil:deionized water slurry (Allen, 1989), total nitrogen (N) content using the Kjeldahl method (Bremner and Mulvaney, 1982), available phosphorus (P) content using the Olsen method (Olsen and Sommers, 1982); and, finally, total soil organic matter (SOM) using the loss-on-ignition method (Nelson and Sommers, 1996) with the total carbon content derived from the OM values. The soil, in our study site, is siliceous with a sandy loam texture, is markedly acidic (pH 4–5), and has limited water-holding capacity and low fertility levels (Parladé et al., 2019).

2.5. Analyses of free ergosterol and estimation of fungal biomass

Free ergosterol was used as an estimate of fungal biomass (i.e., freshly produced mycelia) (Wallander et al., 2010) at each of the 65 sampling points in each of the three areas by extracting the fungal biomarker ergosterol from approximately 1 g of dried and milled soil. Free ergosterol was extracted as described by Hagenbo et al. (2021) and then converted into fungal biomass using a conversion factor of 3 μg ergosterol mg^{-1} fungal dry matter (Salmanowicz and Nylund, 1988) and multiplied by a correction factor of 1.62 to account for non-extracted ergosterol. Ergosterol content was determined by comparing sample peak areas with those of external standards (Montgomery et al., 2000).

2.6. Soil fungal community analysis

Genomic fungal DNA was extracted from 500 mg of soil using a NucleoSpin® NSP soil kit (Macherey-Nagel, Düren, Germany). DNA concentration was measured spectrophotometrically, and templates diluted to 0.5 ng μL^{-1}. The fungal Internal Transcribed Spacer 2 (ITS2) region was PCR amplified in triplicate using the forward gITS7 primer (Ihrmark et al., 2012) and the reverse ITS4 primer (White et al., 1990). Both primers were fitted with unique 8-bp tags that differed in at least three positions (Clemmensen et al., 2016) to individually identify each sample during a posteriori bioinformatics analysis. We optimized the number of PCR cycles following Castaño et al. (2020), finally using 26–30 cycles in most of the samples. Final concentrations in 50-μl PCR reactions were: 2.5 ng template, 200 μM of each nucleotide, 2.75 mM $MgCl_2$, primers at 200 nM, 0.025 U μL^{-1} polymerase (DreamTaq Green, Thermo Scientific, Waltham, MA, USA) in 1× buffer. PCR cycling conditions were: 5 min at 95 °C, followed by 24–30 cycles of 30 s at 95 °C, 30 s at 56 °C, 30 s at 72 °C, and a final extension step at 72 °C for 7 min. Amplified products were purified using a NucleoMag NGS Clean-up and a Macherey-Nagel size select kit, and quantified using a Qubit fluorometer (Life Technologies, Carlsbad, CA, USA). During the extraction, 10 samples had a low DNA concentration and, therefore, were excluded. The 185 remaining samples were randomly divided into three pools (libraries). Equal amounts (ng) of DNA from each sample were pooled and purified using an EZNA Cycle Pure kit (Omega Bio-Tek, Norcross, GA, USA) to obtain a total of 1 μg of DNA for each library. The three libraries were sequenced by Illumina MiSeq (2 × 300 cycles) at the Centre for Genomic Regulation (CRG) Barcelona, Spain.

2.7. Quality filtering and bioinformatic analysis

MiSeq Illumina reads were provided as demultiplexed (i.e., samples were split into individual per-sample files) paired-end (i.e., forward and reverse) FASTQ files. Sequences (FASTQ files) were first reoriented using PipeCraft 2 (Anslan et al., 2017; pipecraft2-manual.readthedocs.io/en/stable) and then analyzed in R using different functions provided in the DADA2 package (see the DADA2 Pipeline v1.18 (adapted to ITS region) https://benjjneb.github.io/dada2/ITS_workflow.html). Primer strings were removed from sequences and then sequences were filtered, trimmed, dereplicated, and the core sample inference algorithm was applied to the dereplicated data. Consequently, paired-end reads were merged (i.e., matching forward with reverse reads) and an Amplicon Sequence Variant (ASV) table was constructed. Finally, chimeras were removed, and taxonomy was assigned using the most recent UNITE fungal dataset (https://doi.plutof.ut.ee/doi/10.15156/BIO/2483925). After quality control, four out of 185 samples contained no reads and, therefore, were excluded. Subsequently the ASV table was curated with LULU (Frøslev et al., 2017). In all compositional analyses, the ASV abundance table was rarefied to minimize differences in sequencing depth between libraries (McKnight et al., 2019). The sample with the lowest number of reads (i.e., 3202 reads) was used as a reference. The rarefied abundance table contained 5061 ASVs (from now on referred to as the "*overall community*") in 181 samples (area 1, 2712 ASVs in 56 samples; area 2, 2738 ASVs in 63 samples; and area 3, 2719 ASVs in 62 samples). The overall community was used to compute diversity indexes and to perform community analyses. However, to better understand which part of the overall community changed among zones, we split the overall community (5061 ASVs) according to the abundance of ASVs (Qiang et al., 2023) using a two-step process. First, we divided the overall community into two communities: (i) "community A" (4076 ASVs), which comprised only ASVs with <0.1 % of the reads compared with the ASV in the overall community with the maximum number of reads and (ii) "community B" (985 ASVs), which comprised only ASVs with >0.1 % of the reads compared with the ASV in the overall community with the maximum number of reads. Community A represented 80 % of ASVs in the overall community but comprised <6 % of the reads

of the overall community. Conversely, community B represented 20 % of ASVs but comprised 94.7 % of reads. In this way, we were able to reduce the "noise", identifying ASVs with extremely low abundance (i.e., community A) and those with higher abundance (i.e., community B). Second, following the same procedure, we split community B into "community C" (805 ASVs, 82 % of ASVs, 16.1 % of reads of community B) and "community D" (180 ASVs, 18 % of ASVs, 84 % of reads of community B) comprising ASVs with less or >1 % of reads, respectively, compared with the ASV in community B with the maximum number of reads (Fig. 2). By doing so, we identified the core community (i.e., community D), representing the most common/abundant species. All four community matrixes were Hellinger transformed (hell) to account for taxa with a low number of reads (Legendre and Gallagher, 2001).

The fungaltraits dataset (https://github.com/traitecoevo/fungaltraits) (Zanne et al., 2020) was used to assign ecological information (primary lifestyle) to ASVs: ECM, ericoid mycorrhiza, arbuscular mycorrhizal, animal endosymbiont, animal parasite, mycoparasite, lichen parasite, litter saprotroph, nectar/tap saprotroph, soil saprotroph, unspecified saprotroph, wood saprotroph, dung saprotroph, plant pathogen, or root endophyte.

2.8. Statistical analysis

2.8.1. Spatial changes in the soil fungal community composition among zones

To understand whether the soil fungal community composition changed between zones, we first checked whether the area × zone interaction (three areas and five zones: C1, C2, ED, F1, F2) was significant when considering the overall community, using a permutation multivariate analysis of variance (PMAV, function adonis2). The area × zone interaction was not statistically significant (i.e., the effect of the gradient was consistent across all areas) and, therefore, we decided to analyze the effect of the clear-cut gradient (using the five zones as a factor) on the fungal community composition when considering the three areas together, running a constrained PMAV (constrained by area). Then, to better understand which part of the overall community produced this effect, we first ran the PMAV on communities A, B, C, and D (see Section 2.7 and Fig. 2 for details of the different communities). Second, we ran a PMAV for saprotrophs and for ECM fungi in community C, excluding the "noise" of community A and focusing only on the community with fewer ASVs that showed significant differences among zones. To visualize fungal compositional differences within areas we used Non-Metric Multidimensional Scaling (NMDS), with ellipses to indicate the 95 % Confident Interval (95 % CI) of each zone.

To visualize the distribution of different taxonomic groups (i.e., phylum, class, order, family, genus, and species) from the clear-cut to the forest, for each zone we considered the relative abundance of each taxonomic group in community C (total), and of ECM and saprotrophic fungi in community C. Due to the high number of samples, we computed the mean relative abundance for each taxonomic unit in each zone for the three areas (5 zones × 3 areas = 15 observations in total). Consequently, to check whether the different zones had significantly different

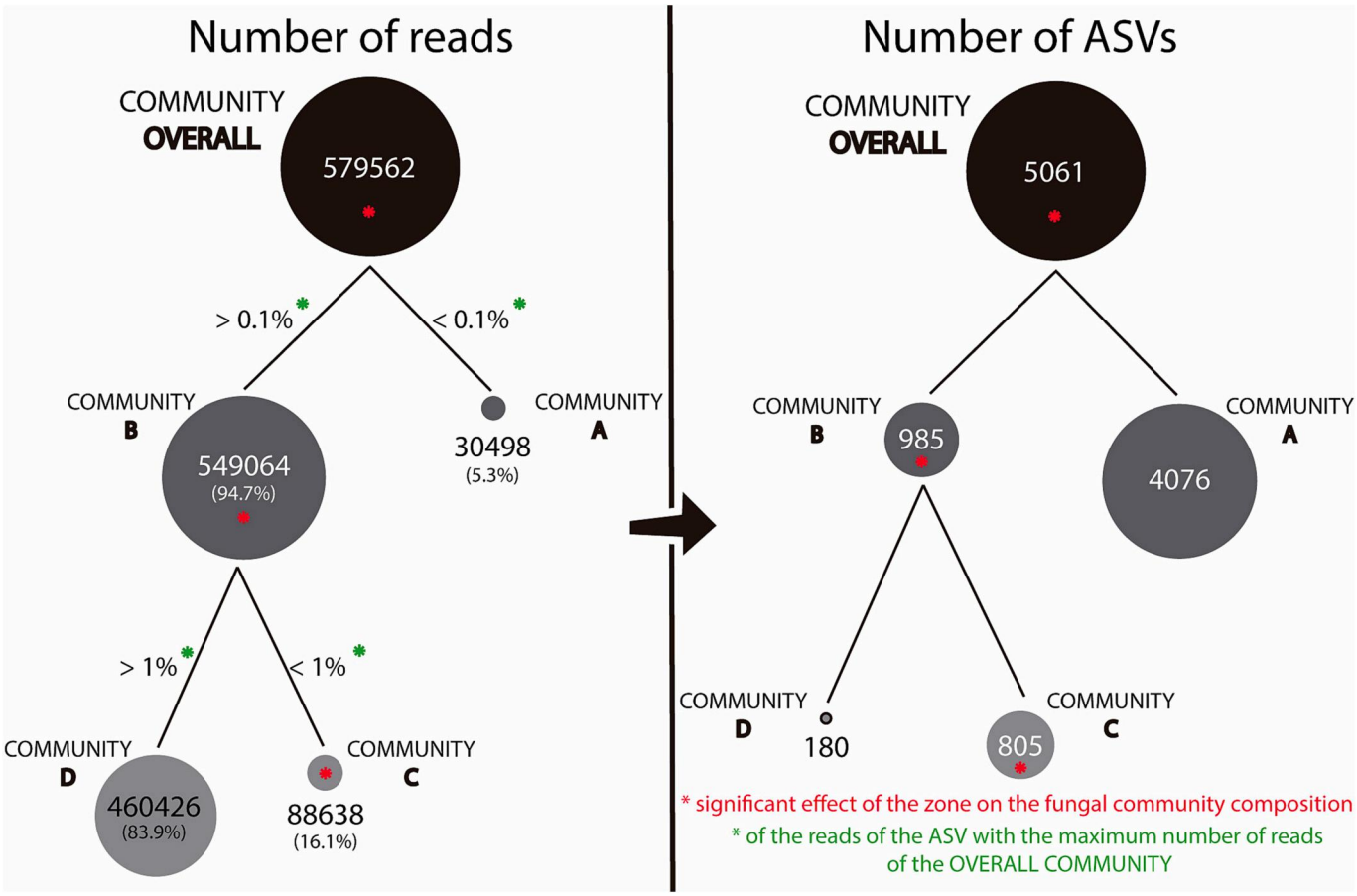

Fig. 2. Filtering steps performed to analyze the soil fungal community. The combined overall community of the three areas (5061 amplicon sequence variants (ASVs) – 579,562 reads) was split into "community A" (4076 ASVs – 30,498 reads, 5.3 % of the overall community) and "community B" (985 ASVs – 549,064 reads – 94.7 % of the overall community) based on ASVs with less or >0.1 % of reads, respectively, compared with the ASV with the maximum number of reads. Community B was then split in "community C" (805 ASVs – 88,638 reads – 16.1 % of community B) and "community D" (180 ASVs – 460,426 reads – 83.9 % of community B) based on ASVs with less or >1 % of the reads, respectively, compared with the ASV with the maximum number of reads. The red asterisk indicates communities that were significantly affected by the factor zone according to the Permutation Multivariate Analysis of Variance (i.e., the overall community, and communities B and C).

abundances, we ran an ANOVA for each phylum, class, order, family, genus, and species.

Finally, to address specific changes in the fungal community between adjacent zones at the species level, we performed an indicator species analysis (with group combinations) using the function "multipatt" (package "indicspecies") on community C.

2.8.2. Spatial changes in fungal biomass and diversity among zones

For each soil sampling point in the three areas, we estimated the species richness (S), Shannon diversity (H), and evenness (J) of the overall community as well as the fungal biomass (ergosterol). To understand whether these variables changed between zones, we first ran a spatial regression model for each area. In this way, we were able to take into account the spatial dependence and the variability of the observations within each area. Afterward, we checked the distribution of residuals of our models (normality and heteroscedasticity assumptions were reasonably met). Then, we used the predicted means of richness, diversity, evenness, and biomass content obtained from spatial models of the three areas to determine whether there were differences among zones using simple linear models, considering fungal richness, diversity, evenness, or biomass as dependent variables and the zone as an explanatory variable (five zones, C1, C2, ED, F1, F2). We performed a Dunnett's test comparing each zone to F2 (control), which was considered to be the zone most representative of the uncut forest. Finally, we attributed a functional guild to all the fungal species in the three areas (overall community) that were classified at genus level, and we determined whether the richness and abundance of ECM fungi, ericoid mycorrhiza, plant pathogens, and saprotrophs changed among zones using the same spatial approach used to analyze diversity indexes and fungal biomass.

2.8.3. Spatial changes in soil properties among zones and relationship with fungal community composition

To understand whether soil properties changed between zones, we used the same spatial approach described in detail in the previous paragraph. Finally, to understand whether soil properties affected soil fungal composition significantly, we used a multivariate permutation procedure (function adonis2) considering the abundance of ECM and saprotrophic fungi (belonging to community C) in relation to pH, SOM, N, and P.

Statistical analyses were implemented in the R software environment (4.0.5, R Development Core Team 2021) using the following packages: VEGAN v.2.5-6 (Oksanen, 2015) for multivariate analyses, PHYLOSEQ v. 1.40.0 (McMurdie and Holmes, 2013) to rarefy the fungal abundance table, TIDYVERSE v.1.3.0 (Wickham et al., 2019) for plot editing, INDICSPECIES (De Càceres and Legendre, 2009) for indicator species analyses, spaMM v. 4.3.0 (Rousset, 2018) for spatial regression models, DHARMa v. 0.4.6 (Hartig, 2017) for residual diagnostics in mixed regression models.

3. Results

3.1. Spatial changes in the soil fungal community composition among zones

The overall community comprised 5061 ASV fungal species (2712 fungal species in area 1, 2738 in area 2, and 2719 in area 3), of which 46 % were identified to genus level. The overall community was first split into communities A and B; community B was then split into communities C and D (see Section 2.7. and Fig. 2 for explanations of the criteria). Community A comprised 4076 ASVs (1891 fungal species in area 1, 1863 in area 2, and 1848 in area 3), of which 43 % were identified to genus level. Community B comprised 985 ASVs (821 fungal species in area 1, 875 in area 2, and 871 in area 3), of which 72 % were identified to genus level. Community C comprised 805 ASVs (646 fungal species in area 1, 698 in area 2, and 694 in area 3), of which 60 % were identified to genus level. Community D comprised 180 ASV fungal species (175 fungal species in area 1, 177 in area 2, and 177 in area 3), of which 73 % were identified to genus level. Among the five communities, saprotrophs were the most abundant fungal guild throughout the three areas (overall, 31 %; A, 28 %; B, 42.5 %; C, 39 %; D, 58 %), followed by mycorrhizal species (overall, 7 %; A, 5 %; B, 10 %; C, 10 %; D, 11 %), plant pathogens (overall, 4 %; A, 4 %; B, 3 %; C, 3 %; D, 0.7 %), and parasites (overall, 3 %; A, 3 %; B, 3 %; C, 3 %; D, 2.7 %).

The overall community was affected by the zone (hell: $R^2 = 0.03$, $F = 1.18$, $p = 0.028$; presence–absence (pa): $R^2 = 0.03$, $F = 1.2$, $p = 0.007$), even if explaining only 3 % of the variance. Thus, to better understand which part of the fungal community produced this effect, we analyzed communities A and B separately and found that although community A was not significantly affected by zone (hell: $p > 0.05$; pa: $p > 0.05$), community B was significantly affected by zone (hell: $R^2 = 0.03$, $F = 1.21$, $p = 0.039$; pa: $R^2 = 0.03$, $F = 1.29$, $p = 0.008$). When community B was split into community C and D, we found that community C was significantly affected by zone (Fig. 3, hell: $R^2 = 0.03$, $F = 1.22$, $p = 0.002$; pa: $R^2 = 0.03$, $F = 1.32$, $p = 0.001$), whereas community D was not significantly affected by zone (hell: $p > 0.05$; pa: $p = 0.05$). We, therefore, focused our analyses on community C.

Most of the species present in community C were shared along the clear-cut–edge–forest gradient, with <4 % of the species strictly related to one or two adjacent zones, and <1 % of the species significantly associated with a group of three or four adjacent zones. Indeed, out of 805 species, only 21 were associated with a specific zone (12 species were associated with C1, two with C2, three with ED, two with F1, and two with F2), six were associated with two zones, five with three zones, and one with four zones (see Table S1 for the list of species). Furthermore, out of the 27 species that were significantly associated with one or two zones, only four had an indicator value component 'A' equal to 1 (i.e., they occurred at sites belonging to this group only) (*Cladophialophora* sp. (saprotroph), and Helotiales.490 in F1, *Tricholoma saponaceum* (ECM) in F2, and *Pseudeurotium* sp.6 (saprotroph) in C1 + C2), whereas all the others had an 'A' of <1, meaning that they were found mainly in a zone or group of zones but not only in those zones. Out of the 33 species that were associated with 1–4 zones, 23 were classified at genus level, of which 18 species were saprotrophs, three were ECM species, one was a root endophyte, and one was an animal parasite.

We also further analyzed community C to determine whether ECM (79 ASVs) or saprotrophic fungi (315 ASVs) were affected by the zone (Fig. 3). The saprotrophic community was significantly affected by zone (hell: $R^2 = 0.03$, $F = 1.37$, $p = 0.002$; pa: $R^2 = 0.03$, $F = 1.56$, $p = 0.001$) while the ECM fungal community was not significantly affected by zone (hell: $R^2 = 0.03$, $F = 1.01$, $p = 0.186$; pa: $R^2 = 0.02$, $F = 0.97$, $p = 0.487$). However, even though in the ordination analysis the ECM distribution did not appear to differ significantly among zones, when looking at the distribution of the 79 ECM fungal species along the clear-cut–edge–forest gradient (Fig. S2), there were 28 ECM species present in C1 compared with 49 ECM species in C2, 52 in ED, 62 in F1, and 57 in F2. Out of the 305 species in community C that were classified as saprotrophs (Fig. S2), 174 were present in C1 compared with 254 species in C2, 231 in ED, 256 in F1, and 243 in F2.

Interestingly, when analyzing the relative abundance at different taxonomic levels (i.e., phylum, class, order, family, genus, and species) across the clear-cut–edge–forest gradient (Fig. 4), we found that in community C [total], the most abundant phylum was Ascomycota, which significantly decreased in abundance from C1 to F2, followed by Basidiomycota, which significantly increased in abundance from C1 to F2 (Table S2a, Fig. 4). Similarly, among community C saprotrophs, the most abundant phylum was Ascomycota followed by Basidiomycota, with no significant difference among zones (Table S2b, Fig. 4). Conversely, analyses of ECM members of community C revealed that the most abundant phylum was Basidiomycota, which increased in relative abundance toward the forest, followed by the Ascomycota, which decreased toward the forest (Table S2c, Fig. 4).

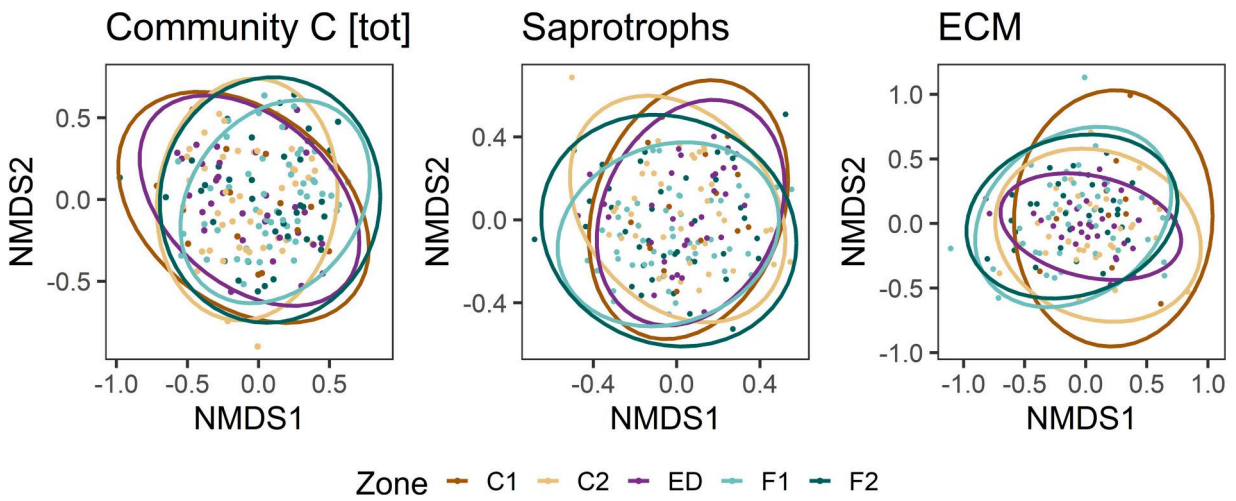

Fig. 3. Non-metric multidimensional screening (NMDS) of the first two axes of community C [total] soil fungal community compositional data (left), and of saprotrophs (middle) and ectomycorrhizal (ECM) fungi (right) belonging to community C. Ellipses showing the confidence interval (CI) 95 % of community differences between clear-cut–edge–forest zones based on the combined data of three areas in Pinar Grande (Spain). Zones: C1, clear-cut core (dark-ocher dots and CI 95 % ellipse); C2, clear-cut border (light-ocher dots and CI 95 % ellipse); ED, edge (purple dots and CI 95 % ellipse); F1, forest margin (light-green dots and CI 95 % ellipse); F2, forest interior (dark-green dots and CI 95 % ellipse).

Among community C saprotrophs, the classes Leotiomycetes and Archaeorhizomycetes were particularly abundant and ubiquitous along the clear-cut – forest gradient, while we found significant differences among zones at class level for Tremellomycetes, at order level for Filobasidiales, at family level for Filobasidiaceae, and at genus level for *Naganishia*, increasing in relative abundance toward the edge (Table S2b). At species level, three species increased in relative abundance toward the edge (*Naganishia* sp., Pseudeutotium sp.2 and *Penicillium restrictum*), five toward F1 (*Cladophialophora* sp.11, *Cladophialophora* sp.32, *Mycena epipterygia*, *Penicillium arenicola*, *Pseudoascochyta novae-zelandiae*), and two toward F2 (*Mycena* sp.10, and *Hyaloscypha* sp.10) (Table S2b). Finally, among ECM members of community C we found significant differences among zones at class level for Agaricomycetes (which increased toward the forest) and Pezizomycetes (which increased toward the clear-cut), and at order level for Pezizales (which increased toward the clear-cut). The relative abundance of the family Rhizopogonaceae, as well as of the genera *Rhizopogon* and *Tylospora*, and the species *Cortinarius diasemospermus* and *Tylospora* sp.4 increased toward C2, whereas *Tricholoma saponaceum* was only found in F2 (Table S2c) as confirmed by the indicspecies analysis (Table S1). Nevertheless, the abovementioned classes, orders, families, genera and species (belonging to the saprotrophic and ECM fungal communities) represented a small part (i.e. <2 %) of the relative abundance within their taxonomic group and, therefore, they were not shown in Fig. 4.

3.2. Spatial changes in fungal biomass and fungal diversity among zones

Fungal biomass varied significantly between zones (Fig. 5 and Fig. S3, $F = 7.13$, $p < 0.001$), with significantly higher biomass values present in samples from the forest interior (F2, mean: 4.5 ± 0.64 mg g^{-1} dry soil), forest border, (F1, mean: 5.1 ± 0.56 mg g^{-1} dry soil) and edge (ED, mean: 4.2 ± 0.68 mg g^{-1} dry soil) compared with both the clear-cut core area (C1, mean: 1.7 ± 0.59 mg g^{-1} dry soil, $p < 0.05$) and the clear-cut border at 10 m from the edge (C2, mean: 1.63 ± 1.02 mg g^{-1} dry soil, $p < 0.05$).

Furthermore, the richness, diversity, and evenness of the overall community and of individual guilds (i.e., saprotrophs, ECM, and pathogens) throughout the three plots were not affected by zone ($p > 0.05$).

3.3. Spatial changes in soil properties among zones and relationship with fungal community composition

SOM, pH, N, and P did not differ significantly between zones (p values > 0.1, Table S3, Fig. 5). The mean value of SOM in C1, C2, and ED was 8.38 %, 7.81 %, and 8.66 %, respectively, with values ranging between 4 and 17 %, and 9.39 % and 9.98 % in F1 and F2, respectively, with values ranging from 4 to 23 % (Fig. 5). pH values ranged between 3.40 and 5.95 (mean 4.7), N values between 0.11 and 0.79 % (mean 0.21 %), and P ranged between 0 and 29 mg kg^{-1} with a mean value of 3.74 mg kg^{-1}. Furthermore, spatial changes in soil properties were not significantly related to the saprotrophic (p values > 0.1 for SOM, N and P; p value > 0.09 for pH) or ECM communities (p values > 0.1 for SOM and N; p values >0.06 for pH and P) (Table S4).

4. Discussion

Forest fragmentation and the tendency to create smaller clear-cut areas naturally increases edge areas and, therefore, it is important to consider them when studying soil fungal changes because they might provide niches for some species (Smith et al., 2018; Heinonsalo et al., 2007). Furthermore, previous studies have mainly focused on forest edge adjoining agricultural lands (Boeraeve et al., 2019; Yang et al., 2022), while we put the focus on forest edges adjoining harvested forest patches. Our analyses, eight years after the clear-cut, revealed small changes in the fungal community composition along the clear-cut–edge–forest gradient (<3 % of the compositional variance explained by zones), and highlighted a significant decrease in soil fungal biomass in both the clear-cut border and the core compared with edge and forest zones. Moreover, the spatial distribution of nutrients was not homogeneous, with spatial variability of all measured variables. Nevertheless, the variability of SOM, N, pH, and P within each zone was higher than the variability between zones and no significant relationship between fungal community composition and any of the four soil variables analyzed was found. The composition of the soil fungal communities in the five zones was highly similar, suggesting that there was a good colonization rate along the clear-cut–edge–forest gradient, with mycorrhizal fungi present not only in the forest but also at the edge and in the clear-cut area, which started to regenerate naturally a few years after the clear-cut treatment. Nevertheless, a few species were found to be significant indicators of the forest, clear-cut, or edge zone

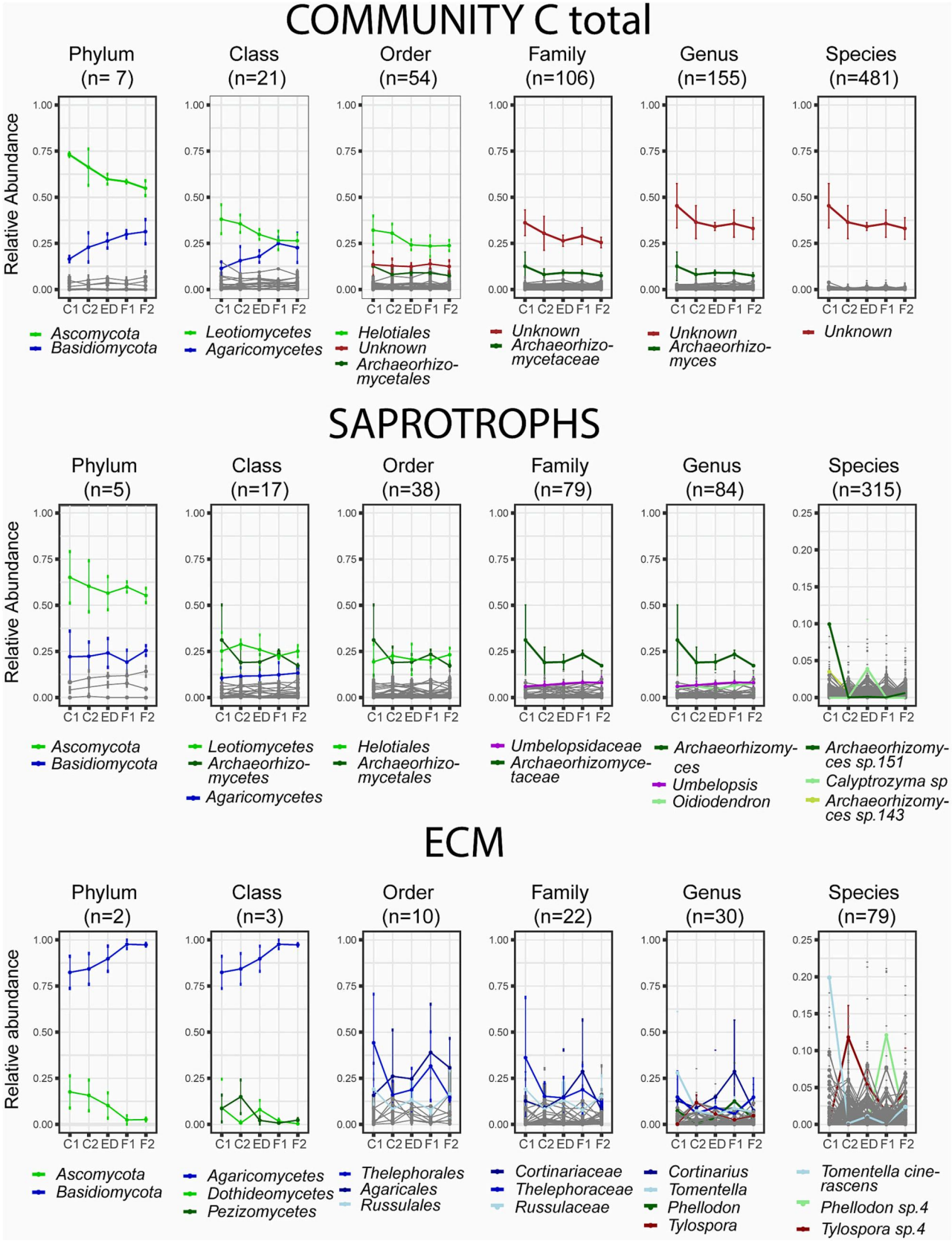

Fig. 4. Relative abundance of community C [total] taxonomic units (i.e., phylum, class, order, family, genus, and species), and of ectomycorrhizal (ECM) fungi and saprotrophic fungi that belong to community C. The names reported below each panel correspond to the most abundant groups. For the full list of community C taxonomic units, see Tables S2a, S2b, S2c.

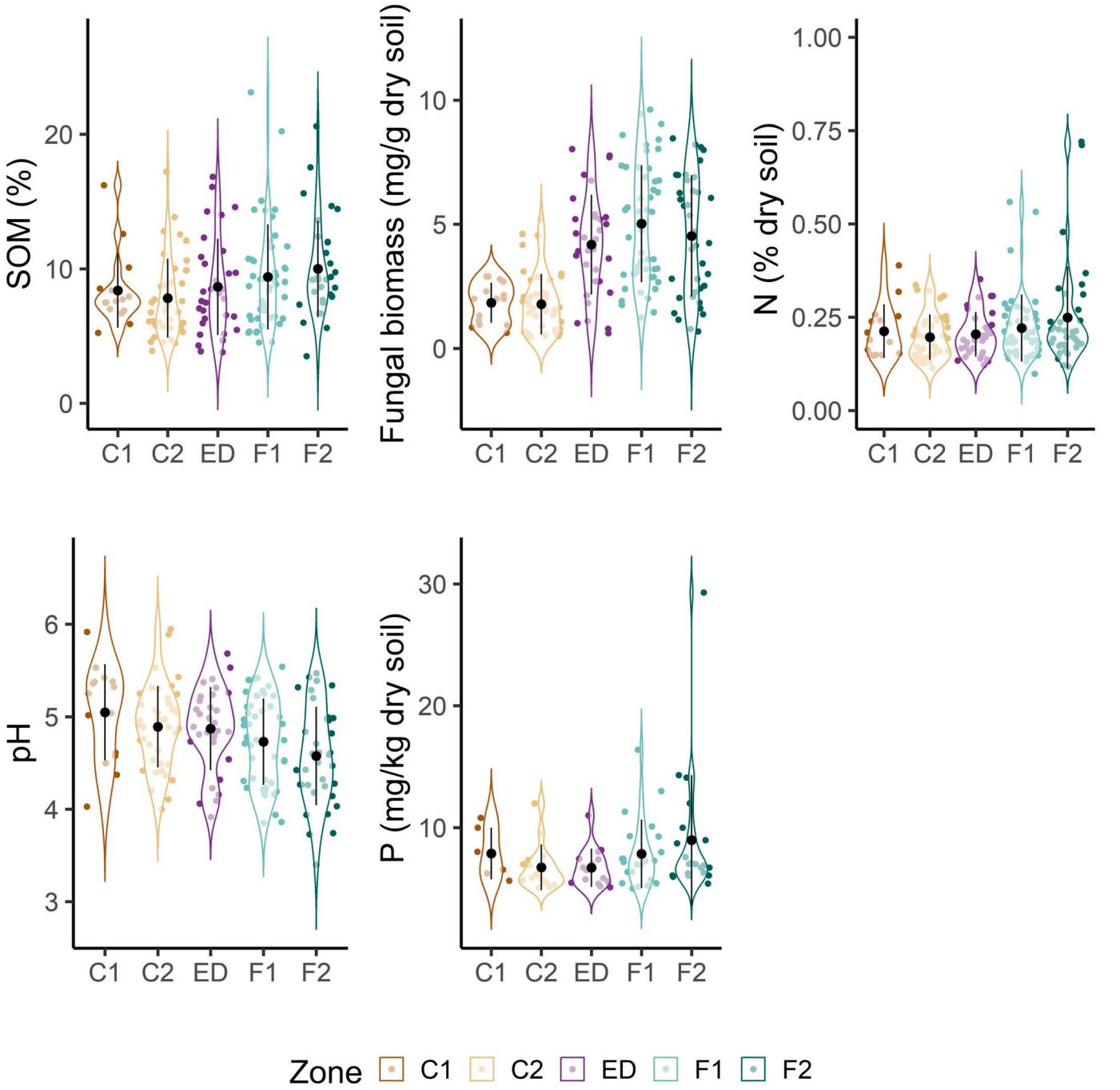

Fig. 5. Physicochemical properties of soil in the five zones (C1, clear-cut core; C2, clear-cut border; ED, edge; F1, forest margin; F2, forest interior): fungal biomass (mg g^{-1} dry soil), soil organic matter (SOM, %), pH, phosphorous (mg kg^{-1}) and nitrogen (%). Data for the three study areas were combined. Black dots and black vertical bars indicate mean values and standard deviation, respectively.

communities.

4.1. Spatial changes in the soil fungal community composition among zones

Fungal richness, diversity, and evenness analyses of the overall community did not reveal significant differences among zones. Conversely, PMAV revealed small (the variance explained by zones was ca. 3 %) but significant differences in fungal community composition between zones, identifying in community C the part of the community that changed (in terms of presence and abundance) along the clear-cut–edge–forest gradient because of the new site conditions. Nevertheless, in the community C total, only 3 genera (out of 155) and 14 species (out of 481) presented significant differences among zones. Moreover, the relative abundance of these genera and species was lower than 0.1 %, highlighting that 8 years after the clear-cut most of the fungal species are shared throughout the clear-cut – edge – forest gradient. On the other hand, community D, did not present significant differences, indicating that the most abundant saprotrophic and ECM species were widespread among zones.

Analysis of community C ECM fungi revealed that Ascomycota increased and Basidiomycota decreased in harvested plots. Hartmann et al. (2012) reported a similar finding when comparing the fungal communities of harvested Douglas fir and spruce forests 10–15 years

after they were clear-cut, with those of natural forests. However, in those experiments, the change in fungal community was related to the removal of the forest floor in harvested plots. In our study, the forest floor was tilled and the chipped branches were left on the ground. This suggests that tilling the soil and covering it with a layer of wood pieces may have contributed to the change in the fungal community composition. Analysis of ECM fungi at species level revealed that although most of the 79 ECM species were widespread across the forest, edge, and even the clear-cut border, a third of the ECM species were not present in the center of the clear-cut, including *Tomentella* sp., *Tomentella* sp.1, *Tomentella* sp.5, *Suillus variegatus*, *Sebacina* sp., *Sebacina* sp.1, *Russula torulosa*, *Russula amethystine*, *Rhizopogon luteolus*, *Laccaria bicolor*, *Cortinarius diasemospermus*, and *Amanita porphyria*. This might be because colonization of the clear-cut core by these species was hampered by the lack of standing trees in the clear-cut area. Directly after clear-cutting, root systems left in the harvested area fragment and there is a decrease in the direct supply of carbon to ECM fungi via symbiotic roots (Chatterjee et al., 2008; Jones et al., 2003). Thus, in the center of the clear-cut, some members of the ECM community may have been negatively affected by the clear-cut and then started to colonize this area again when the area began to regenerate a few years after the clear-cut treatment. Nevertheless, the relative abundance of these species was <0.1 % and, hence, these differences in species presence or absence had little impact on the ordination analysis. Interestingly, Martínez-Peña et al. (2012) monitored edible sporocarps for 9 years in the same forest used in this study. Among the 30 edible ECM fungi recorded in their study, *Boletus edulis*, *Suillus luteus* and *Lactarius deliciosus* produced >60 % of the fruiting bodies collected (60.7 kg). Interestingly, the molecular approach used in our study revealed that the mycelium of these three fungal species were present in <3 %, 20 %, and 11 % of the 181 soil samples (in the overall fungal community), respectively. Moreover, in this study, the ECM fungal species *Cenococcum geophilum* and *Rhizopogon verii* were present in >65 % of the soil samples, however, they were not found by Martínez-Peña et al. (2012). Among the 10 edible saprotrophic fungi recorded in Martínez-Peña et al. (2012) *Rhodocollybia butyracea*, *Lycoperdon perlatum*, and *Macrolepiota procera* produced >98 % of the fruiting bodies collected (3.3 kg), however only *Lycopendrun* sp. was detected in 5 % of the 181 soil samples analyzed in this study. Furthermore, in this study, the saprotrophic fungal species *Oidiodendron chlamydosporicum*, and *Umbelopsis dimorpha* were present in >98 % of the soil samples, however, they were not found by Martínez-Peña et al. (2012). In this regard, the study of Martínez-Peña et al. (2012) was focused on edible fungi that produced visible fruiting bodies, in contrast, our study was focused on the soil fungal community present belowground, thus including also species that might not produce fruiting bodies every year or that do not produce easily visible fruiting bodies (e.g., *Cenococcum*, *Umbelopsis*, *Sebacina*, *Terfezia*).

Similarly, Collado et al. (2020) did not find a significant correlation between the amount of sporocarp biomass and the amount of mycelium found in the soil. In this study, three years after harvesting, regeneration was already established in the clear-cut (Mesa de los Ríos, 2017), which can explain the presence of ECM fungi in that zone. In the eight years between clear-cutting (i.e., 2012) and soil sampling (i.e., 2020), pine seedlings had time to regenerate naturally and spread their root systems through the soil, recreating favorable conditions for the survival of ECM fungi.

Similar to the findings of Jonsson et al. (1999), we did not find any age-associated differences in ECM fungal communities between the forest (>100 year old *P. sylvestris*) and the clear-cut (2 to 7 year old *P. sylvestris*). This is interesting because Jonsson et al. (1999) studied a self-regenerating *P. sylvestris* boreal forest under natural conditions, whereas we focused on self-regenerating Mediterranean *P. sylvestris* forest stands subjected to clear-cut. This suggests that the dimensions of the clear-cut where adequate to allow the widespread establishment of seedlings and of a soil fungal community largely corresponding to that found in the soil of the surrounding trees.

When looking at the distribution of the 315 saprotrophic species across the zones, we expected to find clear differences in the fungal communities given that the complete removal of the tree cover and the site preparation could have affected the saprotroph community composition (Kyaschenko et al., 2017; Parladé et al., 2019). Immediately after the clear-cut, the branches of the harvested trees were chipped on site (in each of the three areas) and the soil was tilled superficially with a harrow pulled by a tractor. This process, apart from softening and homogenizing the soil surface and encouraging natural regeneration from the surrounding trees (Karlsson et al., 2002), could also have triggered the fungal community, in particular the saprotrophs, due to the imminent availability of dead wood material that could be decomposed (Kyaschenko et al., 2017). Nevertheless, we sampled 8 years after the clear-cut event, leaving enough time for the saprotrophs to colonize the different zones. Interestingly, the high abundance of the class Archaeorhizomycetes throughout the zones found in the present study was reported in previous studies analyzing coniferous forest soils (Rosling et al., 2011).

4.2. Spatial changes in fungal biomass and fungal diversity among zones

The silvicultural treatment had a significant impact on the soil fungal biomass, with mean fungal biomass values of <2 mg g^{-1} dry soil in the clear-cut core and border, whereas values were on average twice as high at the edge and at 10 and 20 m from the edge to the uncut forest. Although we sampled only the organic layer (0–10 cm), our findings are in line with those of Awad et al. (2019) who found that the mean total fungal biomass in mineral topsoils (0–12 cm depth) of several conifer forests located throughout Germany was 5.2 ± 0.8 mg g^{-1} (soil dry weight). Interestingly, Malmivaara-Lämsä et al. (2008) observed an increase in fungal biomass from the edge to the first 20 m of forest. However, in our study, fungal biomass values from the edge to the inner forest did not differ significantly, with values peaking at 10 m into the forest and then slightly decreasing at 20 m into the forest. Some studies have reported a steep decrease in fungal biomass within the first 3 years after the clear-cut (Bååth, 1980; Forge and Simard, 2000; Pietikäinen and Fritze, 1995), whereas Hannam et al. (2006) found that 4.5 years after harvesting, there were no significant differences in fungal biomass among harvesting treatments. The few studies that have focused on the medium-term effect of a clear-cut on fungal activity are in line with our findings, showing the persistence of lower fungal biomass levels in harvested forests (8 to 15 years after the treatment) compared with uncut ones (Chatterjee et al., 2008; Hassett and Zak, 2005; Moore-Kucera and Dick, 2008). In our study areas, the fungal biomass at the edge did not present significant difference with F2, suggesting that it was not significantly reduced by the clear-cut. In the clear-cut, the recovery of the fungal biomass should be facilitated by the presence of a new cohort of trees growing in the area, providing an increasing carbon input over time. Moreover, the spatial systematic sampling used in this study allowed us to see how the clear-cut spatially affected fungal biomass values, which were low and homogeneous within the clear-cut, slightly higher along the edge, and even higher within the forest, with increased spatial variability. Similarly, the distribution of the soil property values analyzed in this study, in particular, SOM, N, and P (Fig. 5) revealed a higher level of variability in F1 and F2 compared with samples collected from the edge or the clear-cut. Some studies at small spatial scales (15–20 cm to 1–2 m) have shown that the distribution of soil microbes and fungi in forest soils seems to reflect the zone of influence and positioning of single trees, with patch sizes of similar microbial communities from one to several meters (Pennanen et al., 1999; Saetre and Bååth, 2000).

4.3. Spatial changes in soil properties among zones and relationship with fungal community composition

There were no significant spatial changes in SOM, N, and pH, or in P

distribution among the zones. Forest harvesting alters the organic matter cycle by changing litter inputs and the decomposition regime and has been thoroughly studied (Bååth, 1980; Dai et al., 2001; Jamroz and Jerzykiewicz, 2022). Furthermore, the majority of organic N present in the soil is stored in soil organic residues (Blume et al., 2015). For this reason, we expected that variations in SOM might lead to changes in N availability. In our study areas, branches of harvested trees were chipped and mixed with the soil immediately after the clear-cut, providing a large amount of organic residue and compensating for the lack of litterfall in the harvested areas. In parallel, the establishment of a new cohort of trees regenerating in the clear-cut recreated the equilibrium, absorbing nutrients from the soil and providing new plant residues and, therefore, new organic matter that could store organic N. Similarly, Martínez-Mena et al. (2002) found that nine years after tree and shrub removal in a Mediterranean forest, the total nitrogen content of the soil did not differ significantly from that of undisturbed forest.

Strong variations in soil pH (1.5 to 5 units) have been found to have a significant effect on fungal diversity (Glassman et al., 2017; Rousk et al., 2010; Tedersoo, 2020). In our study areas, which were characterized by a markedly acidic siliceous soil (pH 4–5) with a limited water-holding capacity, the difference in pH between the forest and the clear-cut was <1 unit. This is probably why pH was not relevant in explaining fungal community dynamics. Similarly, the available P content of the soil was not affected by the clear-cut, probably because P is strongly bound to mineral particles that decrease its mobility and susceptibility to leaching (Keenan and Kimmins., 1993). Similar to our findings, Martínez-Peña et al. (2012) who collected mushroom fruit-bodies for nine years in the same forest used in this study, found no significant correlation between soil pH and P and the overall ECM community.

5. Conclusion

Small-scale clear-cuts led to small medium-term changes in the composition of the soil fungal community along the clear-cut–edge–forest gradient of *P. sylvestris* stands, with homogeneous soil properties along the gradient. However, 8 years after the clear-cut treatment, soil fungal biomass in the clear-cut area was still significantly lower than at the edge or in the forest. Analyzing these findings from a management perspective revealed that a small-scale clear-cut treatment did not impair, in the medium-term, the presence of a fungal community within the harvested areas and soil properties comparable to that of the forest, while provoking a decrease in soil fungal biomass, which was probably due to the abrupt change in carbon inputs after tree harvesting. Nevertheless, further research is needed to better understand how the size of the clear-cut affects not only fungal but also bacterial and archaeal communities at the edges of different forest types.

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CRediT authorship contribution statement

Giada Centenaro: Data curation, Formal analysis, Writing – original draft preparation. **Sergio de-Miguel:** Data sampling, Conceptualization, Formal analysis, Funding acquisition, Methodology, Supervision, Writing – review and editing. **Svetlana Dashevskaya:** Writing – review and editing. **José Antonio Bonet:** Data sampling, Conceptualization, Formal analysis, Funding acquisition, Methodology, Writing – review and editing. **Fernando Martínez Peña:** Data sampling, Conceptualization, Writing – review and editing. **Rubén Escribano Gil de Gómez:** Writing – review and editing. **Angel Ponce:** Data sampling, Writing – review and editing. **Josu G. Alday:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Supervision, Writing – review and editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Giada Centenaro reports a relationship with University of Lleida that includes: employment. Sergio de Miguel Manana reports a relationship with University of Lleida that includes: employment. Svetlana Dashevskaya reports a relationship with University of Lleida that includes: employment. Jose Antonio Bonet reports a relationship with Cataluna Forest Technology Centre that includes: employment. Fernando Martinez Pena reports a relationship with Centro de Investigación y Tecnología Agroalimentaria de Aragón that includes: employment. Angel Ponce reports a relationship with University of Lleida that includes: employment. Josu G. Alday reports a relationship with University of Lleida that includes: employment.

Data availability

Data will be made available on request.

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