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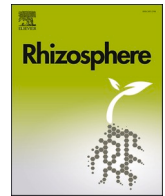
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Effects of *Juniperus communis* encroachment on soil and root fungal communities in subalpine grasslands

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ABSTRACT

Although shrub encroachment has been studied for its effects on fungal communities, the influence of a specific shrub species on belowground fungal communities across grasslands remains unclear. We examined the influence of increasing *Juniperus communis* cover on belowground fungal communities in subalpine grasslands dominated by perennial grasses with different ecologies, *Patzkea paniculata* and *Bromus erectus*. In each grassland, a gradient-based approach was applied to investigate the responses of soil fungal communities to increasing *J. communis* cover. Fungal diversity and composition were assessed using high-throughput sequencing of the internal transcribed spacer (ITS) region. The functional composition of fungal communities was inferred by comparison with ITS reference databases. Although grassland vegetation cover differed, increasing *J. communis* density had no significant effect on the fungal richness of soil or root communities in either grassland. However, *J. communis* density significantly affected fungal beta-diversity and composition. The structuring of root and soil fungal communities along the *J. communis* cover gradient differed significantly between grasslands, leading to both quantitative and qualitative changes in belowground fungal communities including saprophytic, ericoid and pathogenic fungi. Our results suggest that pathways of influence of *J. communis* encroachment on subalpine ecosystem functioning are mediated by changes in the assemblage and composition of fungal communities.

1. Introduction

Fungal communities in soil are one of the most diverse components of soil microorganisms and are known to play a fundamental role in regulating ecosystem functions as decomposers, mutualists, or pathogens of plants and animals (Öpik et al., 2006; Treseder and Lennon, 2015). They contribute strongly to biogeochemical cycles by decomposing organic materials, by mediating the phosphorus and nitrogen cycles and they influence plant productivity and plant diversity (van der Heijden et al., 2008; Hoeksema et al., 2010; Treseder and Lennon, 2015; Maciá-Vicente et al., 2023). So far, several studies have sought to characterize factors that regulate fungal communities in soil, which will

impact ecosystem processes (de Oliveira et al., 2020).

Besides environmental factors, including climate (precipitation and temperature) and soil properties (organic matter, C, N and P contents, pH), it is well established that plants constitute one of the predominant biotic drivers of the diversity and the composition of fungal communities (Chen et al., 2017). Plants can influence fungal communities through their species-specific effects via their functional traits. Plant functional traits related to photosynthesis, carbon chemistry of litter and roots and nutrient acquisition can significantly influence fungal richness and diversity (Hanif et al., 2019). Plants can also influence fungal communities through their exudates, which confer a specific and favorable environment for developing specific fungal communities

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(López-García et al., 2017). Any changes in plant communities appear to feed back as structuring forces driving fungal communities, with a significant impact on ecosystem functioning.

Subalpine grasslands are among ecosystems with rapid changes in plant communities, yet understudied as compared to arctic and alpine communities (Michalet et al., 2024). In these ecosystems, since the 1950s several factors, including abandonment of extensive livestock farming and global climate warming (Tasser et al., 2017; Lavorel, 2019), have led to the modification in plant communities, resulting in the grassland colonization by shrubs (Grau et al., 2019). This process, named shrub encroachment (Archer et al., 2017), resulting in compositional turnover of plant communities characterized by the shift from herbaceous plants to shrubs, has been described in several European mountains and is responsible for altering their functioning (Urbina et al., 2020). Shrub encroachment decreases plant community richness, plant diversity and increases plant productivity (Boscutti et al., 2018). It also alters soil properties such as phosphorus, carbon and nitrogen contents, pH and enzyme activities (Aguirre et al., 2021; Laorden-Camacho et al., 2025).

Studies analysing the influence of shrub encroachment on fungal communities have often focused on species diverse grasslands colonised by a community of shrubs. They showed a strong influence of shrubs on fungal communities through their functional composition (Grau et al., 2019; Broadbent et al., 2022; Marchal et al., 2025) and their impacts on soil properties (Collins et al., 2018; Grau et al., 2019; Ding et al., 2020). However, the specific influence of a single shrub species on belowground fungal communities, particularly in relation to the dominant grass species within a grassland, remains poorly understood. Addressing this gap is crucial for disentangling species-specific mechanisms underlying the interactions between shrubs, soil fungi and grassland composition and their consequences on grassland functioning. We expected that the effects of a given shrub species on fungal communities can be strongly influenced by the specific ecologies of herbaceous communities. It has previously been described that the same dominant grassland species can have different impacts on biomass, activity and structure of microorganisms in soils of different fertility (Innes et al., 2004). Similarly, studies conducted by Bezemer et al. (2006) have shown that the effects of plant species or plant communities on microbial communities depend on the nature of soils (sandy or chalky). To our knowledge, no study has investigated the responses of fungal communities to the encroachment of a given shrub species in different types of subalpine grasslands. Such a study could allow us to better understand the dynamics of fungal diversity in a given grassland in response to shrub encroachment linked to global changes.

This study aimed to investigate the influence of encroachment by the evergreen dwarf coniferous species *J. communis* on the belowground fungal communities in two different grassland communities, dominated by two grasses with different functional traits and ecologies *Patzkea paniculata* and *Bromus erectus* (Quétier et al., 2007; Gross et al., 2007). *P. paniculata*, a specialist of subalpine grasslands of the southern Alps, is an extremely conservative species whose exceptionally high dry matter content and low nutrient concentrations result in slow nutrient cycling, along its allelopathic effects on fungal communities (Binet et al., 2013, 2017). In contrast, *B. erectus* is a widespread conservative species with somewhat faster nutrient cycling (Grassein et al., 2010). Focusing on gradients of *J. communis* density within each of these grasslands, we analyzed fungal communities associated with *J. communis* and herbaceous plants by high-throughput sequencing of internal transcribed spacer (ITS1) to characterize their diversity and composition. To gain an insight into the influence of *J. communis* on the belowground fungal communities, we targeted both soil and root-associated fungal communities, known to respond differently to biotic and abiotic factors (Stevens et al., 2020; Fu et al., 2022). We hypothesized that: (i) the effects of *J. communis* cover on belowground fungal communities would differ across grassland types, reflecting the influence of plant species identity on fungal communities (Mouhamadou et al., 2013; Sweeney

et al., 2021), and would be more pronounced in *P. paniculata* grasslands owing to its species specific traits, particularly its allelopathic properties (Viard-Créat et al., 2009); (ii) root and soil fungal communities would have distinct responses to *J. communis* encroachment, driven by differences in host specificity, resource availability, and sensitivity to environmental filtering (Zhang et al., 2022; Fu et al., 2022).

2. Materials and methods

2.1. Field sites

The study was conducted at the Lautaret site in France on the southern slopes of the Guisane valley (45.03°N, 6.24°E), at an average altitude of 1900 m a.s.l. on flysch sediment. The climate is subalpine with a strong continental influence and summer drought. The mean annual precipitation is 956 mm and mean monthly temperatures range between 1.1 °C in January and 19 °C in July (1991–2020) (Météo-France data). Colonization by *J. communis* reflects both land use history, contrasting areas with or without previous cultivation and mowing, and extensification of livestock farming, especially conversion from mowing to summer grazing by cattle in since the 1970s (Devaux, 2016).

2.2. Site vegetation cover

Vegetation cover of sites was determined as described by Marchal et al. (2025). For instance, vegetation cover was assessed on a 10 × 10 m grid with points spaced 1 m apart. At each point, the presence of *J. communis* and/or herbaceous vegetation was recorded to calculate their percentage cover over 100 points. The canopy height was also measured at each point to estimate the average height per plot and the volume of *J. communis* (percentage of coverage × average height × 100).

2.3. Sampling design

To quantify the effects of *J. communis* on fungal communities, we used a classic gradient approach (Ter Braak and Prentice, 1988) considering grassland plots with increasing shrub density. Plots with different *J. communis* cover were located within homogenous landscape units with relatively flat slope (<10°), the same substrate, topography, and management history and all *J. communis* populations were mature. Within each of *P. paniculata*- and *B. erectus*-dominated grasslands we selected six homogenous 10 × 10 m plots with increasing *J. communis* cover, ranging gradually from 100% herbaceous cover to plots up to respectively 47% and 61% *J. communis* (Fig. S1), allowing us to capture a representative sample of colonization within the study landscape. Vegetation cover was quantified as described by Marchal et al. (2025). At each point at 1-m intervals within a 10 × 10 m grid, we recorded the presence of *J. communis* or herbaceous vegetation to calculate the percentage cover of each component.

2.4. Plant functional traits across grasslands

Plant traits were measured according to the methods described in Marchal et al. (2025). Briefly, biomass was estimated in each plot using allometric techniques (Lavorel et al., 2008) and the point method (Levy and Madden, 1993). We collected herbaceous and shrub leaf and stems samples to measure plant traits (leaf dry matter content (LDMC), C, N, P and lignin content) following standard trait measurement protocols (Cornelissen et al., 2003) and calculated community weighted mean (CWM) plant traits, representing the weighted contributions of *J. communis* and herbaceous species, using biomass estimations as described in Marchal et al. (2025).

2.5. Soil and root samplings

Soil and root samples for fungal community analysis were sampled in

the six *P. paniculata* grassland plots (July 2021) and the six *B. erectus* grassland plots (July 2022) at comparable altitudes (1850–1900 m a.s.l.). Sampling was conducted at the peak growing season in both years, allowing for a comparison of the effects of *J. communis* encroachment on fungal communities across two distinct grassland ecosystems. In each plot containing *J. communis*, five soil blocks (30 cm × 25 cm × 20 cm) were randomly collected beneath *J. communis* canopies and five soil blocks beneath adjacent herbaceous vegetation. In addition, five soil blocks were sampled in herbaceous-only plots (0% *J. communis* cover) within both *P. paniculata* and *B. erectus* grasslands, providing reference conditions without shrub-induced shading. A total of 105 soil blocks (55 and 50 from *P. paniculata* and *B. erectus* grasslands, respectively) were sampled and stored at −20 °C until analysis.

2.6. Soil and root DNA extraction, PCR amplification and illumina-based sequencing

The 105 soil blocks from the *P. paniculata* and *B. erectus* grasslands were thawed at room temperature and sieved to 2 mm. A subsample of 150 mg of rhizosphere soil from each soil block was used to extract soil fungal DNA. The root samples from each soil block were carefully collected and thoroughly washed with tap water. Roots were excised in 2–5 mm fragments, ground with liquid nitrogen, and 100 mg were used to extract root-associated fungal DNA. We chose not to sterilize the root surface to capture both internal and closely associated external fungal taxa, enabling a comprehensive analysis of the entire fungal community. The FastDNA SPIN Kit for Soil (MP Biomedicals, Irvine, CA, USA) and the Fast prep DNA Spin kit (MP Biomedicals, France) were used to extract soil and root DNA, respectively. After extraction, the quality and quantity of DNA were investigated with a spectrophotometer (Nanodrop, PeqLab, Erlangen, Germany).

The ITS1 sequences were used as molecular markers for fungal community and these sequences were amplified with the fungal primers ITS5 (White et al., 2000) and 5.8S_fungi (Epp et al., 2012). This primer pair provides broad coverage of fungal diversity. Although these primers amplify a relatively lower proportion of Glomeromycotina sequences compared to other fungal phyla (Ficetola et al., 2010), they are well suited for capturing overall fungal community composition and have been widely used in similar ecological studies (Mouhamadou et al., 2013; Marchal et al., 2021, 2025). These primers were extended by sample specific tags of 8 nucleotide length to allow parallel sequencing of multiple samples. The PCR reactions were performed as reported by Mouhamadou et al. (2013). Three PCR reactions (technical replicates) were performed from DNA extracted from each sample and the three PCR products from each sample were pooled. PCR products were purified with the QIAquick kit in accordance with the manufacturer's instructions (Qiagen, Courtaboeuf, France) and quantified using the Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA, USA). The purified amplicons from roots or soil were pooled separately for sequencing by using equivalent molarities among amplicons. The construction of libraries and sequencing (Illumina MiSeq 150 bp pair-end) were performed by Fasteris (Geneve, Switzerland).

2.7. Bioinformatic analysis

Read assembly and primary filtering were performed with the OBITools package (Boyer et al., 2016). Only reads with a perfect match to sample-specific tags and allowing up to two mismatches in primer regions were retained. Sequences were filtered by length, keeping reads between 90 and 400 bp, and sequences shorter than 100 bp or containing ambiguous nucleotides were removed using obigrep command (Lentendu et al., 2011). Rare sequences (occurrence <2) were discarded. Identical sequences were dereplicated with obiuniq and annotated with their read counts. The applied thresholds follow commonly accepted practices in DNA metabarcoding studies and are consistent with previous methodological work (Marchal et al., 2021, 2025).

The ecotag function of OBITools was used to taxonomically assign the obtained unique sequences (identical repeated sequences) to the r143 EMBL fungi database using the ITS5 and 5.8S_fungi primers. Highly similar unique sequences were clustered into operational taxonomic units (OTUs) by computing pairwise similarities using Sumatra (OBITools package, <https://metabarcoding.org/sumatra>) and forming 97% similarity clusters (a commonly applied threshold for fungal species-level resolution (Grau et al., 2019) with the Markov Cluster algorithm (MCL) classification process (Van Dongen, 2000). Functional assignments were performed using the FUNGuild pipeline (Nguyen et al., 2016). Only taxa assigned with highly probable and probable confidence rankings were retained for analyses, corresponding respectively to assignment confidence scores ≥0.9 (highly probable) and between 0.75 and 0.9 (probable), as defined by Nguyen et al. (2016).

The MetabaR package (Zinger et al., 2021) was used in R software 4.3.2 to flag and filter out spurious signals, following the validated pipeline described in the package documentation (<https://metabarfactory.github.io/metabarR/> accessed December 05, 2022) which provides diagnostic plots and visualization tools to guide the selection of appropriate flagging thresholds. The extraction, PCR and sequencing control samples allowed us to flag contaminants, nontarget taxa were detected based on a minimum of 0.8 best identity score and tag-jumps were detected and removed using a minimum abundance threshold of 0.01 (33.25% of OTUs, 31% of reads). Finally, PCR outliers were flagged if the compositional dissimilarities in OTUs between PCR replicates from the same sample were not lower than those observed between PCR obtained from different samples, as well as based on a minimum sequencing depth of 0.001 reads (with normalization per technical replicate) per PCR (8.03% of PCRs technical replicates, 9.1% of reads).

We normalized the dataset by sample using the Hellinger standardization method “descostand” function, (package *vegan*). Unique sequences belonging to the same OTUs were aggregated. The final contingency tables display the abundance (normalized read counts) of 3311 OTUs among 103 samples for soils, and 3917 OTUs among 86 samples for roots.

To assess quantitative variations in fungal communities across grasslands, analyses focused on ubiquitous OTUs (i.e., those present across all vegetation types in *P. paniculata* and *B. erectus* grasslands); then the most abundant ones (corresponding to those representing at least 0.5% of the total abundance) were selected, and their relative abundances were subsequently compared in herbaceous plants in the presence/absence of *J. communis*. To evaluate qualitative differences in fungal communities across grasslands, OTUs present in herbaceous plants with and without *J. communis* were compared to identify those that were specifically associated with the presence of *J. communis*.

2.8. Statistical analysis

The normality and homoscedasticity of the data were tested using the Shapiro–Wilk test and Levene's test, respectively. Means of both OTU richness, abundance of the most abundant ubiquitous fungal OTU and abundance of fungal phyla were compared with ANOVAs followed by Tukey's honest significant differences test or the Kruskal–Wallis rank test, followed by Dunn's test for pairwise multiple comparisons of the ranked data with Bonferroni correction for nonparametric data. Wilcoxon test was used to compare plant functional traits across the two studied grasslands. The non-metric multidimensional scaling (NMDS), with Bray–Curtis dissimilarity (Bray and Curtis, 1957) and based on OTU abundance, was used to evaluate the turnover in fungal communities (i.e., beta-diversity) to identify discontinuities in OTU composition. $P < .05$ was considered statistically significant. All statistical analyses were performed using the R package *vegan*.

3. Results

3.1. Variations in plant and abiotic parameters across grasslands

J. communis biomass and community weighted mean plant functional traits in *P. paniculata* and in *B. erectus* grasslands were measured (Table S1). No significant differences in these parameters were observed between both grasslands (Table S2). Similarly, temperature and precipitation data from the study sites over the two sampling years were compared and revealed no significant differences for either parameter (Table S3).

3.2. Richness of root and soil fungal communities

Fungal community richness in both roots and soil differed significantly between grassland types. Root fungal richness was higher in *B. erectus* grasslands, whereas soil fungal richness was higher in *P. paniculata* grasslands (Fig. 1). However, comparison between herbaceous communities and those encroached by *J. communis* revealed no significant differences in root or soil fungal richness within either *P. paniculata* or *B. erectus* grasslands (Fig. 2).

Within each grassland type, root fungal richness associated with herbaceous plants did not vary with *J. communis* cover (Fig. S2 A and B). However, root fungal communities associated with *J. communis* showed variations with increasing cover. In *P. paniculata* grasslands, root fungal richness increased between the lowest (15% and 20%) and the highest *J. communis* covers (27% and 47%) (Fig. S2 C) while in *B. erectus* grasslands, no significant differences in root fungal richness were observed between the lowest and highest levels of *J. communis* cover (Fig. S2 D).

Soil fungal richness associated with herbaceous plants also remained unchanged across the *J. communis* cover gradient within each grassland type (Fig. S2 E and F). In contrast, soil fungal communities associated with *J. communis* varied along the gradient of increasing cover. In

P. paniculata grasslands soil fungal richness increased from the lowest *J. communis* cover (15%) to the higher cover levels (20%, 27%, and 47%) (Fig. S2 G), whereas in *B. erectus* grasslands, soil fungal richness did not vary significantly along the *J. communis* cover gradient (Fig. S2 H).

3.3. Beta-diversity of root and soil fungal communities

Root fungal communities clustered into two distinct groups according to grassland type (Fig. 3A). Each group was divided into two subgroups, separating herbaceous plots and from those colonized by *J. communis* (Fig. 3A).

Within each grassland, fungal communities associated with herbaceous plants were examined to better characterize *J. communis* encroachment effects. In *P. paniculata* grassland (Fig. 3B), herbaceous root fungal communities were subdivided into two groups according to *J. communis* cover, contrasting 0% cover and all other *J. communis* cover values. In *B. erectus* grassland (Fig. 3C), three non-overlapping root fungal groups were identified, with changes between 0% and 17% *J. communis* cover and between 44% and 61% *J. communis* cover.

Concerning soil fungal communities, two distinct non-overlapping groups of fungal communities were also observed according to grassland type, with each group divided into two subgroups according to plant composition (*J. communis* encroached or herbaceous) (Fig. 4A). Within each grassland, soil fungal communities associated with herbaceous plants changed along encroachment gradients, resulting in different types of grouping. Two groups of fungal communities (0–15 % and all other *J. communis* cover) can be distinguished in the *P. paniculata* grassland according to the increasing *J. communis* cover (Fig. 4B), while there was no separation of fungal communities in the *B. erectus* grassland whatever *J. communis* cover (Fig. 4C).

3.4. Composition of fungal communities at phylum level

Concerning root fungal communities, Basidiomycota, Ascomycota

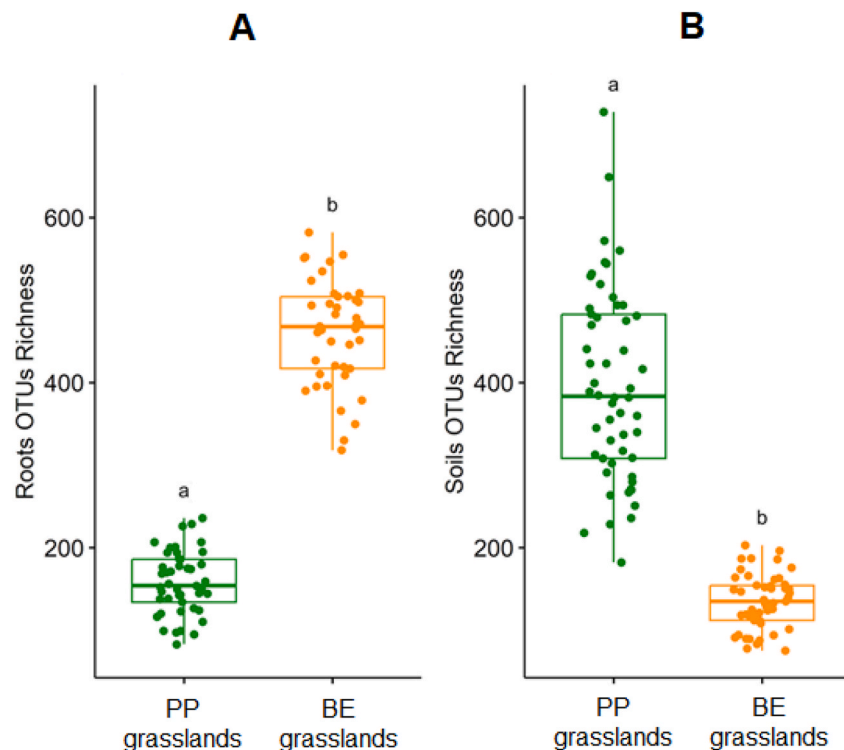


Fig. 1. OTU Richness in root (A) and in soil (B) fungal communities in *P. paniculata* (PP) and *B. erectus* (BE) grasslands. Non-parametric data, means compared using Kruskal-Wallis rank test. Lowercase letters indicate significantly different conditions (chi-squared = 63.624, df = 1, p -value = 1.5×10^{-15} for Fig. 1A and chi-squared = 75.754, df = 1, p -value $< 2.2 \times 10^{-16}$ for Fig. 1B).

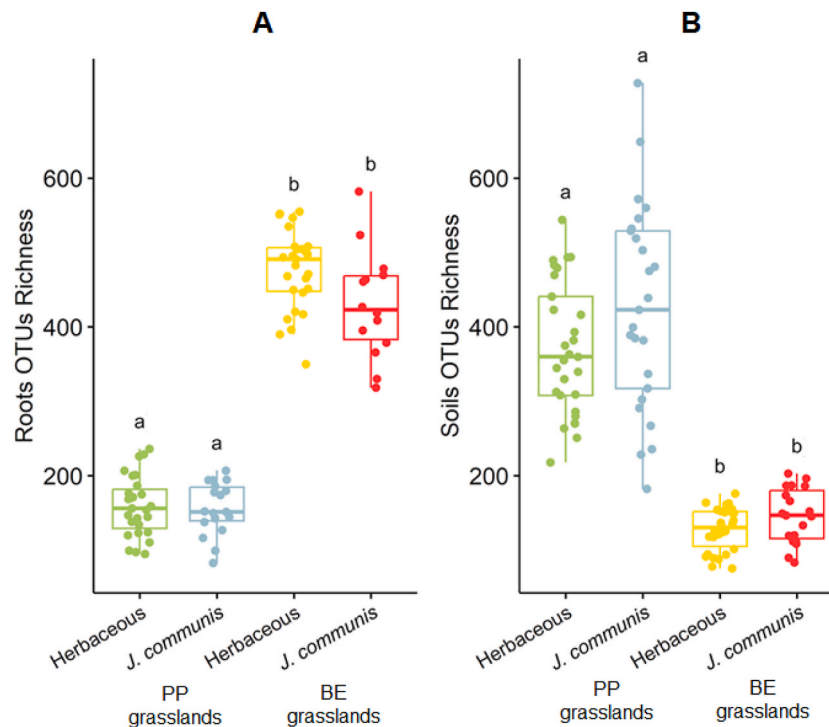


Fig. 2. Comparison of root OTU richness (A) and soil OTU richness (B) of fungal communities between herbaceous and *J. communis* in *P. paniculata* (PP) and *B. erectus* (BE) grasslands. Non-parametric data, means compared using Kruskal-Wallis rank test and Dunn's test for nonparametric data. Lowercase letters indicate significantly different conditions (ANOVA and Tukey HSD Test; ANOVA: p -value $< 2 \times 10^{-16}$ for Fig. 2A and Kruskal-Wallis rank sum test and Dunn test for Pairwise comparisons; Kruskal-Wallis chi-squared = 76.96, df = 3, p -value $< 2.2 \times 10^{-16}$ for Fig. 2B).

and Mucoromycota were more abundant in *B. erectus* than in *P. paniculata* grassland (Fig. S3A–C). For soil fungi, a higher abundance of Chytridiomycota in *P. paniculata* grassland was found (Fig. S3D–G).

The comparison of the root and soil fungal communities in *P. paniculata* grassland showed that the abundances of the different fungal phyla associated with herbaceous or *J. communis* were similar except for Chytridiomycota in soil (Fig. S4). In *B. erectus* grassland, variations in abundance were detected but were limited to root-associated Ascomycota and Basidiomycota (Fig. S4).

Moreover, the composition of root fungal communities associated with herbaceous or *J. communis* did not show significant differences in the abundances of Basidiomycota, Ascomycota and Mucoromycota according to *J. communis* cover whether in *P. paniculata* or in *B. erectus* grasslands (Fig. S5–S7). Nevertheless, root fungal phyla varied significantly along specific *J. communis* cover gradients notably for Basidiomycota associated with *J. communis* in *B. erectus* grasslands and for Ascomycota associated with herbaceous in *P. paniculata* grasslands (Fig. S5A; S5D), but these differences were not amplified across increasing *J. communis* cover. The same patterns were observed for the four phyla of soil fungal communities including Chytridiomycota phylum (Fig. S5–S8).

3.5. Composition of herbaceous fungal communities at OTU level

The composition of fungal communities was analyzed at OTU level to characterize quantitative and qualitative variations of both root and soil fungal communities in herbaceous plants due to the presence of *J. communis*.

Regarding quantitative analysis, Venn diagram identified 284 root OTUs representing between 43.5% and 61% of the abundance of OTUs associated with plant roots (Fig. 5A) of which 25 OTUs were the most abundant (Table 1).

In *P. paniculata* grassland, among these abundant OTUs, 8 OTUs representing a total abundance of 32%, varied in herbaceous plants in

presence of *J. communis* (Table 1A): *Mycopan scabripes* an ericoid mycorrhizal fungus (ErMF) was significantly more abundant in herbaceous plants without *J. communis* while 7 OTUs consisting of saprotrophs (*Mycena citrinomarginata*, and *Incrupila aspidii*), saprotroph/endophyte (*Cladophialophora* sp), ErMF (*Leptodophora orchidicola* and *Leohumicola minima*) and coniferous pathogens and animal (*Gremmenia infestans* and *Exophiala equina*, respectively) were more abundant in herbaceous plants in the presence of *J. communis*.

In *B. erectus* grassland (Table 1B), 4 of the 25 most abundant OTUs (representing a total abundance of 16%) varied in the presence of *J. communis*; an ErMF (*Hyaloscypha hepaticicola*) was significantly more abundant in herbaceous plants without *J. communis*, while one saprotroph (*I. aspidii*), one saprotroph/endophyte (*Cladophialophora* sp) and one OTU belonging to Helotiaceae family were more abundant in herbaceous plants in the presence of *J. communis*.

For the soil fungal communities, 265 ubiquitous OTUs were characterized representing between 33% and 61.2% of the total abundances (Fig. 5B), of which 14 of these OTUs were the most abundant (i.e., $>0.5\%$). In *P. paniculata* grassland (Table 1C), among these most abundant OTUs, the four OTUs, that tolerate stress or are involved in plant stress protection (*Solicoccozyma aerea*, *Tetracladium* sp, *Elasticomyces elasticus* and *Extremus* sp), which were significantly different (representing 28% in abundance), were more abundant in the herbaceous soils in the presence of *J. communis*, while in the *B. erectus* grassland (Table 1D), only the saprotroph *Pleotrichocladium opacum* (representing 7% in abundance) was significantly less abundant in the herbaceous soils in the presence of *J. communis*.

Regarding qualitative analysis, in both *P. paniculata* and *B. erectus* grasslands, root fungal communities of herbaceous plants included a substantial number of OTUs specifically associated with the presence of *J. communis*: 697 OTUs in *P. paniculata* grassland (2.8 times more than shared OTUs) and 1373 in *B. erectus* grassland (1.6 times more than shared OTUs) (Fig. 6A and B).

In soil fungal communities of herbaceous plants, 1201 OTUs were

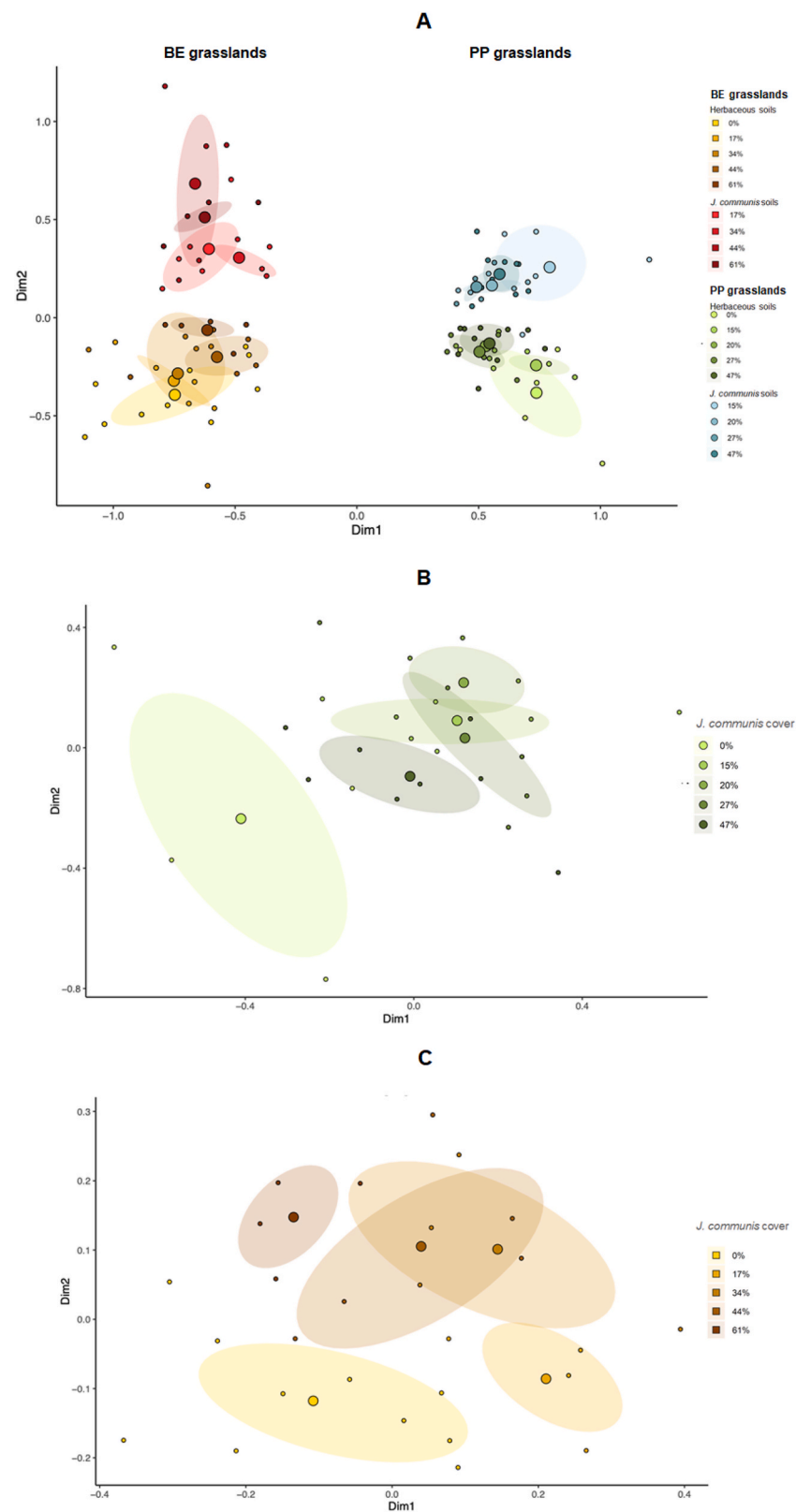


Fig. 3. Non-metric multidimensional scaling (NMDS) ordination of root fungal communities between *P. paniculata* (PP) and *B. erectus* (BE) grasslands (A) and within *P. paniculata* (B) and *B. erectus* (C) grasslands along *J. communis* cover. Fig. 3A (stress value: 0.13; R²: 0.98), Fig. 3B (stress value: 0.19; R²: 0.96), Fig. 3C (stress value: 0.17; R²: 0.97).

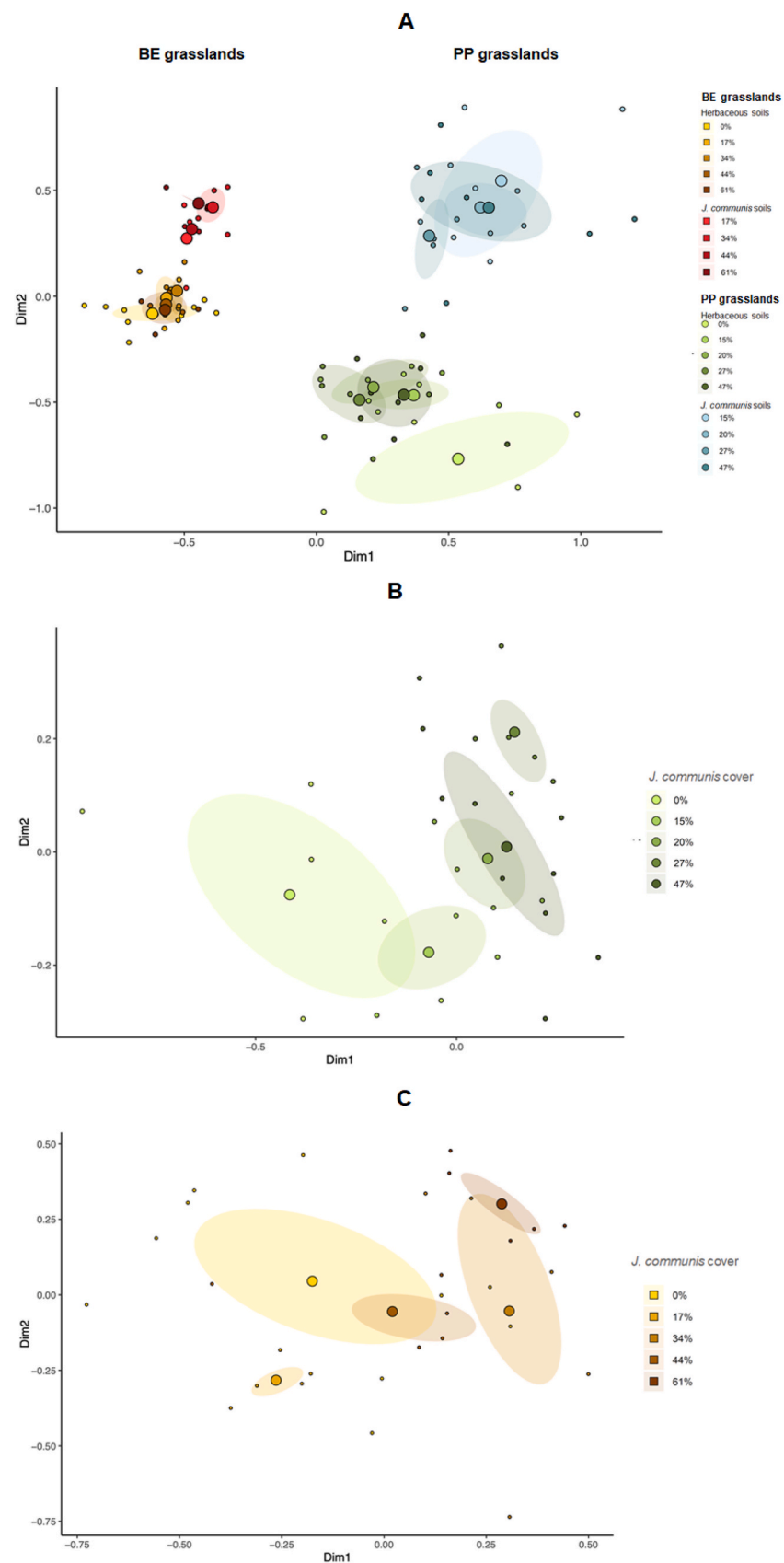


Fig. 4. Non-metric multidimensional scaling (NMDS) ordination of soil fungal communities between *P. paniculata* (PP) and *B. erectus* (BE) grasslands (A) and within *P. paniculata* (B) and *B. erectus* (C) grasslands along *J. communis* cover. Fig. 4A (stress value: 0.14; R²: 0.98), Fig. 4B (stress value: 0.2; R²: 0.94), Fig. 4C (stress value: 0.19; R²: 0.95).

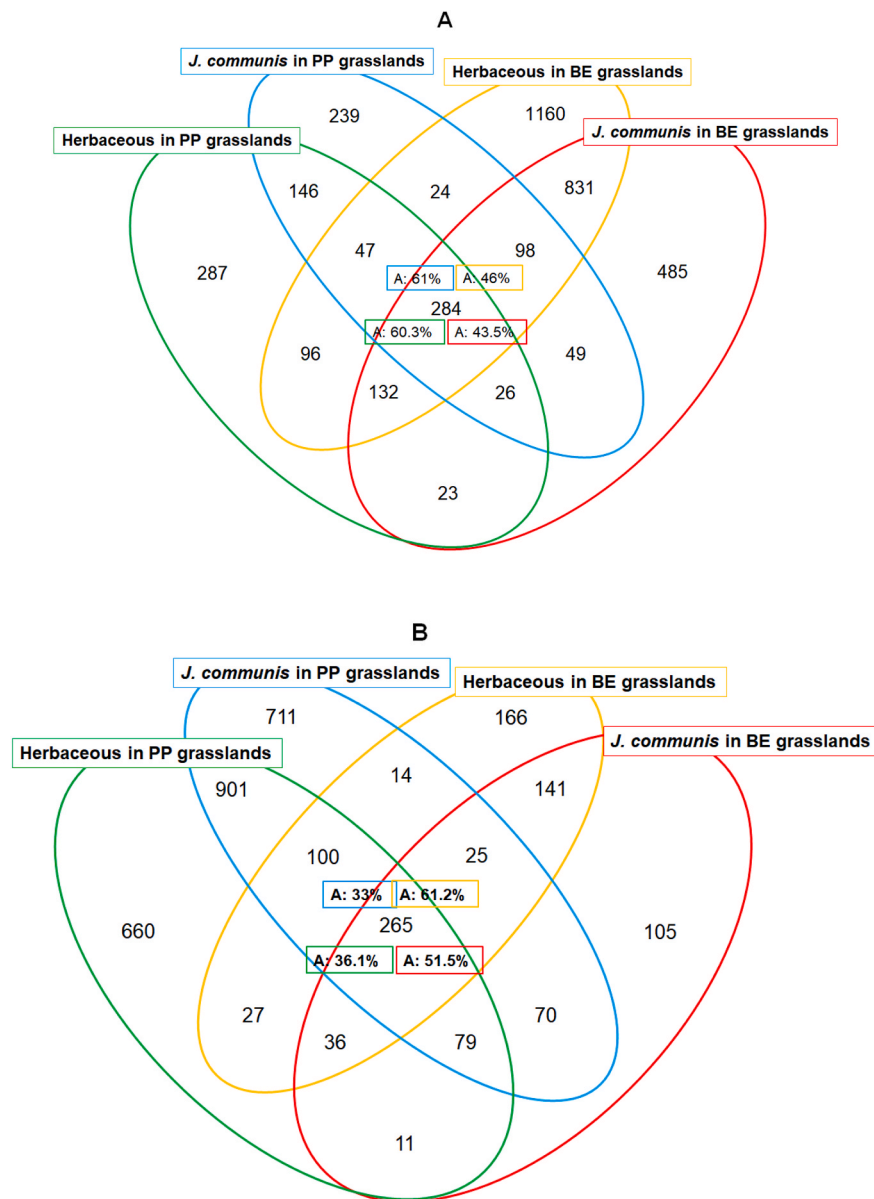


Fig. 5. Distribution of root (A) and soil (B) OTUs present in *Juniperus* and herbaceous samples in *P. paniculata* (PP) and *B. erectus* (BE) grasslands. The numbers of OTUs shared between different conditions and the number of OTUs shared between all conditions (ubiquitous OTUs) are indicated. The percentages of ubiquitous OTU abundance in each condition are boxed. A means abundance.

specifically associated with *J. communis* presence in *P. paniculata* grassland (1.6 times more than shared OTUs), whereas in *B. erectus* grassland, only 280 such OTUs were detected (1.2 times fewer than shared OTUs) (Fig. 6C and D).

4. Discussion

To date, there are no studies addressing the specific encroachment effects of a given shrub species on the belowground fungal communities in different types of grasslands, each dominated by a particular herbaceous species. We filled this gap by reporting on the effects of encroachment by one dwarf shrub species, *J. communis*, on both root and soil fungal communities across two subalpine grasslands. Within fungal communities structured by the identity of dominant herbaceous species, *P. paniculata* or *B. erectus*, we found that *J. communis* encroachment primarily affected community composition, exerting differential effects on root and soil fungal communities. Furthermore, neither community weighted mean plant functional traits in *P. paniculata* and *B. erectus*

grasslands nor climatic parameters, particularly temperature and precipitation, which are important climatic factors influencing fungal communities (Větrovský et al., 2019), differed significantly between the two sampling years, indicating that abiotic conditions were broadly comparable across years.

4.1. Grassland type rather than *J. communis* encroachment shapes root and soil fungal richness

Within each grassland, the root and soil fungal communities associated with herbaceous plants showed no significant differences in richness from those associated with *J. communis*. Additionally, the fungal communities of herbaceous plants did not vary with increasing *J. communis* cover. These findings suggest the lack of *J. communis* encroachment effects on the richness of root and soil fungal communities in these subalpine grasslands. Our results are consistent with those of Grau et al. (2019), who also found no variation of total fungal community richness along the succession from grassland to *J. communis* and

Table 1

Mean abundance and standard deviation (SD) of the most abundant ubiquitous fungi between herbaceous samples at 0% shrub cover and in the presence of any *J. communis* cover in *P. paniculata* (PP) grassland roots (A), in *B. erectus* (BE) grassland roots (B), in *P. paniculata* (PP) grassland soils (C) and in *B. erectus* (BE) grassland soils (D). Most abundant ubiquitous fungi are selected among roots or soils separately. They represent at least 0.5% of total roots or soils abundance respectively and are found in all grassland and vegetation types. Darker lines with bold p-values have significantly different mean abundances between *J. communis* cover conditions. Means were compared with ANOVAs or the Kruskal–Wallis rank sum test for non-parametric data.

A

	PP grasslands; root fungal communities				p-value
	Herbaceous (- <i>J. communis</i>)		Herbaceous (+ <i>J. communis</i>)		
	Mean	SD	Mean	SD	
<i>Leptodophora orchidicola</i>	0.0538	0.0351	0.1329	0.0468	0.0037
<i>Mycena citrinomarginata</i>	0.0023	0.0047	0.0901	0.1207	0.0459
<i>Gremmenia infestans</i>	0.0567	0.0325	0.1493	0.0512	0.0019
<i>Marasmius tricolor</i>	0.0000	0.0000	0.1452	0.2098	0.1152
<i>Lachnum</i> sp.	0.1957	0.2109	0.1222	0.1414	0.4868
<i>Pezicula ericae</i>	0.0328	0.0301	0.0413	0.0314	0.6197
<i>Incrupila aspidii</i>	0.0437	0.0162	0.109	0.0729	0.0290
<i>Herpotrichia juniperi</i>	0.0213	0.0246	0.0636	0.0642	0.1716
<i>Cladophialophora</i> sp. 1	0.0859	0.0208	0.0548	0.0412	0.1572
<i>Fusarium acuminatum</i>	0.0619	0.0772	0.0641	0.0318	0.3223
<i>Cyphellophora</i> sp.	0.0129	0.0257	0.017	0.0234	0.5487
<i>Minutisphaera aspera</i>	0.1217	0.0894	0.1448	0.0823	0.6120
<i>Mycopan scabripes</i>	0.2736	0.1907	0.1275	0.1155	0.0437
<i>Exophiala equina</i>	0.0561	0.0284	0.121	0.0537	0.0344
<i>Heteroconium</i> sp.	0.0293	0.0133	0.0823	0.0652	0.0759
<i>Hyaloscypha hepaticicola</i>	0.0429	0.0319	0.0582	0.0496	0.6820
<i>Phialophora foetens</i>	0.0014	0.0029	0.0206	0.0284	0.1284
<i>Lachnum carneolum</i>	0.0366	0.0494	0.0301	0.0527	0.7238
<i>Cladophialophora</i> sp. 3	0.0602	0.0073	0.1168	0.0412	0.0123
<i>Ilyonectria crassa</i>	0.0305	0.0239	0.0386	0.027	0.5816
<i>Helotiaceae</i> sp. 2 GM 14-28	0.0000	0.0000	0.0133	0.026	0.2605
<i>Mycena alnetorum</i>	0.0023	0.0047	0.0504	0.0879	0.1736
<i>Petrakia</i> sp.	0.1957	0.1257	0.1326	0.0866	0.2179
<i>Leohumicola minima</i>	0.0353	0.0240	0.0828	0.0318	0.0063
<i>Cladophialophora</i> sp. 2	0.0657	0.0274	0.0581	0.0373	0.4128

B

	BE grasslands; root fungal communities				p-value
	Herbaceous (- <i>J. communis</i>)		Herbaceous (+ <i>J. communis</i>)		
	Mean	SD	Mean	SD	
<i>Leptodophora orchidicola</i>	0.1869	0.0335	0.2186	0.0564	0.1194
<i>Mycena citrinomarginata</i>	0.0761	0.0902	0.1132	0.1426	0.9600
<i>Gremmenia infestans</i>	0.1950	0.0783	0.2072	0.0723	0.6852
<i>Marasmius tricolor</i>	0.2086	0.1396	0.148	0.1258	0.2482
<i>Lachnum</i> sp.	0.1153	0.1402	0.2022	0.1247	0.0502
<i>Pezicula ericae</i>	0.0617	0.0492	0.048	0.0175	0.9200
<i>Incrupila aspidii</i>	0.0628	0.0301	0.1215	0.0346	0.0002
<i>Herpotrichia juniperi</i>	0.0520	0.0193	0.056	0.0254	0.9200
<i>Cladophialophora</i> sp. 1	0.0504	0.0242	0.0812	0.0344	0.0121
<i>Fusarium acuminatum</i>	0.0982	0.0486	0.0816	0.0223	0.2917
<i>Cyphellophora</i> sp.	0.1223	0.0851	0.0961	0.0552	0.3383
<i>Minutisphaera aspera</i>	0.1203	0.0866	0.1177	0.0981	0.9600
<i>Mycopan scabripes</i>	0.0202	0.0146	0.0175	0.0279	0.2570
<i>Exophiala equina</i>	0.1125	0.0200	0.1199	0.0402	0.5139
<i>Heteroconium</i> sp.	0.0881	0.0637	0.1231	0.0418	0.1752
<i>Hyaloscypha hepaticicola</i>	0.1386	0.1200	0.0519	0.0488	0.0067
<i>Phialophora foetens</i>	0.1713	0.0950	0.135	0.0875	0.3219
<i>Lachnum carneolum</i>	0.0692	0.0477	0.1168	0.0666	0.0599
<i>Cladophialophora</i> sp. 3	0.0833	0.0176	0.0845	0.0275	0.9016
<i>Ilyonectria crassa</i>	0.0252	0.0123	0.0362	0.0192	0.1197
<i>Helotiaceae</i> sp. 2 GM 14-28	0.0503	0.0287	0.0895	0.0509	0.0355
<i>Mycena alnetorum</i>	0.0008	0.0025	0.0012	0.005	0.7402
<i>Petrakia</i> sp.	0.0584	0.0574	0.048	0.045	0.4978
<i>Leohumicola minima</i>	0.0690	0.0486	0.0605	0.0482	0.6514
<i>Cladophialophora</i> sp. 2	0.0268	0.0258	0.0331	0.0232	0.2914

C

	PP grasslands; soil fungal communities				p-value
	Herbaceous (- <i>J. communis</i>)		Herbaceous (+ <i>J. communis</i>)		
	Mean	SD	Mean	SD	
<i>Cadophora orchidicola</i>	0.0406	0.0151	0.0582	0.0219	0.1008
<i>Solicoccozyma aeria</i>	0.0543	0.0283	0.0995	0.0423	0.0179
<i>Exophiala equina</i>	0.1021	0.0791	0.1249	0.0443	0.2253
<i>Pseudogymnoascus pannorum</i>	0.0515	0.0331	0.0715	0.0491	0.2987
<i>Cladophialophora</i> sp. 3	0.0864	0.0434	0.0846	0.0176	0.8174
<i>Rachicladosporium pini</i>	0.0282	0.0259	0.0465	0.0438	0.4529
<i>Coniochaeta ligniaria</i>	0.0414	0.0325	0.0546	0.0285	0.3649
<i>Pleotrichocladium opacum</i>	0.0588	0.0341	0.0628	0.0204	0.7258
<i>Gymnostellatospora alpina</i>	0.0336	0.0279	0.0527	0.0343	0.1333
<i>Tetracladium</i> sp.	0.0218	0.0273	0.1094	0.0807	0.0039
<i>Elasticomyces elasticus</i>	0.0340	0.0073	0.0905	0.0607	0.0111
<i>Ilyonectria crassa</i>	0.0574	0.0344	0.0456	0.0248	0.3726
<i>Extremus</i> sp.	0.0143	0.0101	0.0383	0.0226	0.0111
<i>Mortierella</i> sp.	0.0008	0.0018	0.0007	0.0023	0.5124

D

	BE grasslands; soil fungal communities				p-value
	Herbaceous (- <i>J. communis</i>)		Herbaceous (+ <i>J. communis</i>)		
	Mean	SD	Mean	SD	
<i>Cadophora orchidicola</i>	0.0600	0.0190	0.0716	0.0291	0.2633
<i>Solicoccozyma aeria</i>	0.1700	0.0907	0.1272	0.0914	0.0946
<i>Exophiala equina</i>	0.0396	0.0191	0.0543	0.0223	0.1036
<i>Pseudogymnoascus pannorum</i>	0.0477	0.0221	0.0361	0.037	0.0938
<i>Cladophialophora</i> sp. 3	0.1082	0.0727	0.14	0.083	0.3126
<i>Rachicladosporium pini</i>	0.0934	0.0897	0.1087	0.0773	0.6323
<i>Coniochaeta ligniaria</i>	0.1193	0.0699	0.1811	0.0953	0.0807
<i>Pleotrichocladium opacum</i>	0.1985	0.0986	0.1026	0.0621	0.0197
<i>Gymnostellatospora alpina</i>	0.0931	0.0596	0.0986	0.0788	0.7918
<i>Tetracladium</i> sp.	0.0190	0.0276	0.0779	0.0818	0.0623
<i>Elasticomyces elasticus</i>	0.0190	0.0237	0.0204	0.0274	0.9810
<i>Ilyonectria crassa</i>	0.0427	0.0161	0.0437	0.0264	0.6921
<i>Extremus</i> sp.	0.0367	0.0484	0.0337	0.0504	0.9634
<i>Mortierella</i> sp.	0.0527	0.0409	0.0661	0.0527	0.4877

J. sabina shrubland but contrast with that reported by Sun et al. (2019) showing the differential influence of shrub species on fungal alpha diversity, suggesting that, additional to grassland type, fungal community richness is sensitive to shrub species.

However, regardless of fungal groups (root or soil), grassland-specific variations in fungal richness were observed. These variations could probably be linked, to the identity and functional traits of the dominant herbaceous species at each grassland. In particular, previous studies, conducted in the same grasslands as those investigated in the present study, showed that *P. paniculata* exerts allelopathic effects that reduce arbuscular mycorrhizal fungal communities in both its own roots and those of neighboring plants (Viard-Créat et al., 2009; Binet et al., 2013). This could explain the lower richness observed in root fungal communities compared to soil communities in *P. paniculata*-dominated grasslands, as its allelopathic effects appear to specifically target root fungal communities. Furthermore, the observation that root and soil fungal communities responded differently to plant characteristics, particularly the inverse relationship between the richness of one group and that of the other, was consistent with the findings of Zhang et al. (2022), who reported that root and soil fungal communities exhibited

distinct responses to plant traits.

4.2. Grassland type and *J. communis* encroachment drive contrasting patterns of root and soil fungal beta-diversity

The beta-diversity analyses revealed grassland-specific effects on both root and soil fungal communities, consistent with previous studies (Pellissier et al., 2014) and with patterns observed in fungal community richness. These identity effects were reflected in the turnover of fungal communities, resulting in differences in the relative abundance of fungal phyla between the two grasslands. Interestingly, the impact of grassland type differed significantly between root and soil fungal communities: phyla that were less abundant in one fungal group tended to be more abundant in the other fungal group. We suggested that this inverse pattern helped maintain overall phylum-level abundance stability across the two fungal groups.

In contrast to fungal richness, increasing *J. communis* encroachment effects on fungal beta-diversity were also observed in each grassland. These effects were reflected in variations that led to discontinuities in fungal communities along its cover gradient. Moreover, *J. communis*

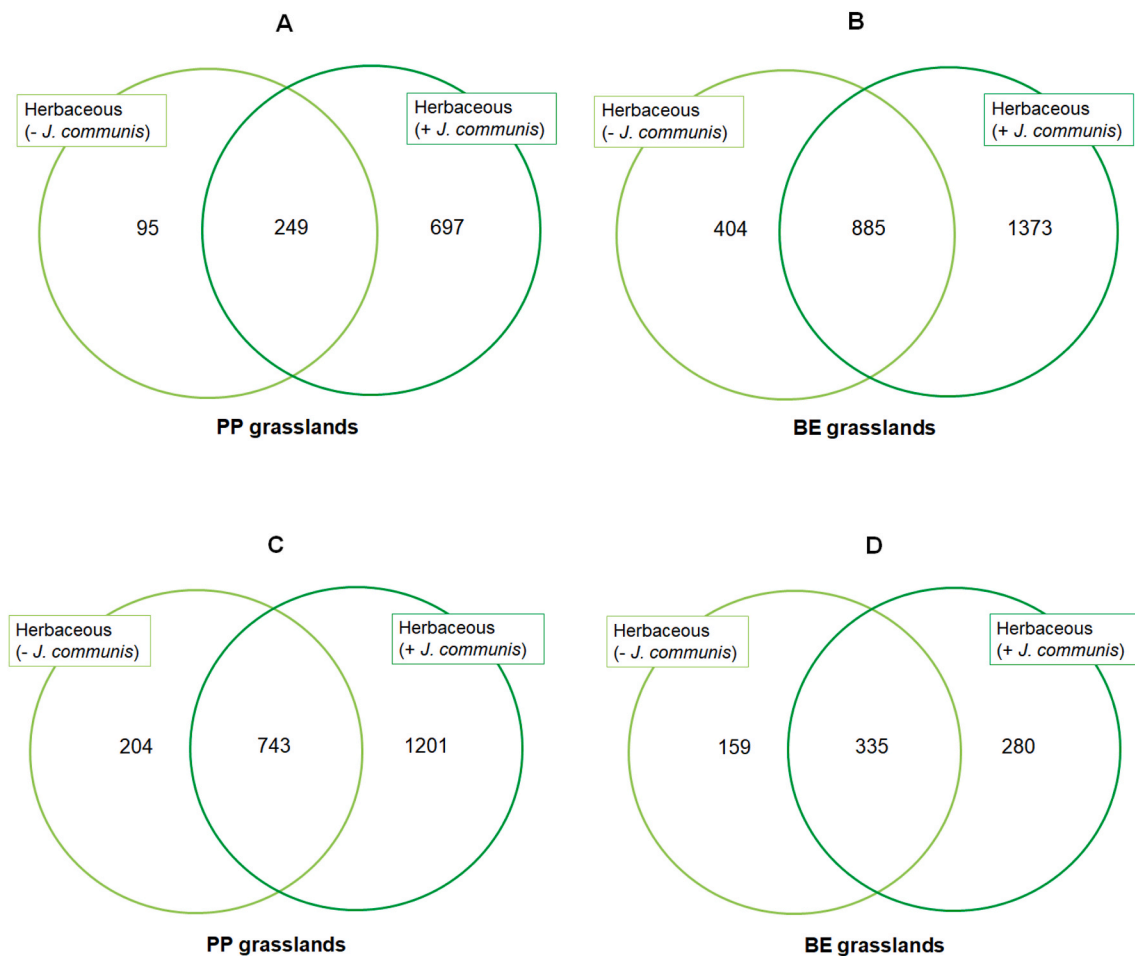


Fig. 6. Distribution of root (A, B) and soil (C, D) OTUs present in herbaceous samples in presence/absence of *J. communis* in *P. paniculata* (PP) and *B. erectus* (BE) grasslands. We compared the OTUs present in the samples of herbaceous without *J. communis* with those present in the samples of herbaceous invaded by *J. communis*, also considering, if any, the discontinuities of the fungal communities highlighted by beta diversity. The numbers and abundances of OTUs shared between herbaceous with and without *J. communis* are indicated. A means abundance.

effects were grassland-specific, indicating that fungal communities respond differently depending on the dominant host plant species, likely due to distinct plant–fungal interaction patterns. Previous study, performed in the same grasslands as those investigated here has shown that *P. paniculata* tends to associate preferentially with endophytic rather than arbuscular mycorrhizal fungi in its roots (Mouhamadou et al., 2011), which may explain the differential responses to *J. communis* encroachment observed between the two grasslands.

Additionally, effects of *J. communis* density in each grassland differed significantly between root and soil fungal communities, which is consistent with previous findings indicating that these two fungal groups responded differently to biotic and abiotic factors (Zhang et al., 2022; Fu et al., 2022).

4.3. Grassland-dependent effects of *J. communis* encroachment on belowground fungal OTU composition

OTUs varied in response to *J. communis* presence within each grassland type. At the quantitative level, among the most abundant ubiquitous OTUs shared by herbaceous plants and *J. communis*, those significantly impacted by its presence reached abundances of 32% and 28% in *P. paniculata* and *B. erectus* grasslands, respectively. Likewise, from a qualitative perspective, the number of herbaceous OTUs specifically associated with the presence of *J. communis* was higher (on average 2.3 times greater than the number of shared OTUs) except for soil OTUs in *B. erectus* grassland (1.2 times lower). These quantitative

and qualitative findings indicate that *J. communis* encroachment has a significant impact on belowground fungal community composition as reported in the Pyrenees (Grau et al., 2019). Interestingly, both the number of impacted OTUs and their abundance varied quantitatively depending on the grassland type: more OTUs (at least twice more) with higher abundances (at least twice higher) were affected in *P. paniculata* grasslands, in both roots and soils in comparison to *B. erectus* grasslands. Qualitatively, *J. communis* encroachment exerted positive effects on soil and root OTUs in *P. paniculata* grasslands (the number of specific root or soil OTUs was higher than the number of shared OTUs), whereas in *B. erectus* grasslands, it had negative effects on the number of OTUs, but only in the soil. According to our hypothesis, these results indicate that the effects of *J. communis* encroachment on fungal community composition differ according to grassland type and fungal group (Zhang et al., 2022; Fu et al., 2022; Lepinay et al., 2024). These *J. communis* encroachment effects concerned important functional groups including saprotrophs, endophytes, ErMF and pathogens.

5. Conclusions

Colonization by *J. communis* in two subalpine grasslands dominated by herbaceous species with contrasting functional traits did not affect the richness of root and soil fungal communities. However, *J. communis* encroachment influenced beta-diversity and composition. These effects, reflected in the structuring of root and soil fungal communities along the *J. communis* cover gradient, varied between the two grasslands and the

fungus groups. They led to quantitative and qualitative changes of belowground fungal communities including saprophytic, endophytic, ericoid and pathogenic fungi known to be critical to ecosystem functioning. Although sampling was conducted over two consecutive years under similar climatic conditions, interannual variability may shape fungal community dynamics over longer temporal scales. Consequently, long-term, multi-year monitoring combined with targeted functional analyses will be crucial to more fully capture temporal variability and to improve our understanding of shrub-driven alterations in soil fungal assemblages.

CRedit authorship contribution statement

Marie-Noëlle Binet: Conceptualization, Investigation, Methodology, Writing – review & editing. **Christelle Gonindard-Melodelima:** Methodology, Writing – original draft. **Camille Marchal:** Methodology, Writing – original draft. **Karl Grigulis:** Investigation, Methodology, Writing – original draft. **Marie-Pascale Colace:** Investigation, Methodology. **Elena Tello-García:** Methodology. **Lucía Laorden-Camacho:** Methodology. **Sophie Pérignon:** Methodology. **Frédéric Laporte:** Methodology. **Manon Paul-Traversaz:** Methodology. **Georg Leitinger:** Funding acquisition, Writing – original draft. **Sandra Lavorel:** Conceptualization, Funding acquisition, Investigation, Validation, Writing – review & editing. **Bello Mouhamadou:** Conceptualization, Investigation, Supervision, Validation, Writing – review & editing.

Declaration of competing interest

All authors declare that they have no conflicts of interest regarding this research.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.rhisph.2026.101309>.

Data availability

Data are submitted in the Genbank database.

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