

Article

Shifting Diversities in the Anthropocene: Impact of Alien Species on Plant and Macrofungal Diversity in Native Forests

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Abstract

This study evaluates the ecological impact of *Robinia pseudoacacia* L. (black locust) invasion on native chestnut (*Castanea sativa* Mill.) groves on Mount Amiata (Central Italy), focusing on both plant and macrofungal community dynamics. Surveys were conducted over a three-year period (2022–2024) across 16 plots to assess shifts in taxonomic alpha diversity, species richness, and trophic guild structure. Our results demonstrate that while *R. pseudoacacia* stands exhibit a higher Shannon–Wiener index for plants, native chestnut groves host significantly greater species richness and higher taxonomic distinctiveness across both biological groups. A major shift in fungal functional structure was observed with chestnut-dominated plots characterized by a predominance of ectomycorrhizal species (58.3%), whereas invaded stands were heavily dominated by saprotrophic fungi (73.4%). Non-metric Multidimensional Scaling (NMDS) further confirmed a clear separation in community composition between the two forest types, indicating that *R. pseudoacacia* invasion leads to a homogenization of the forest biota and a potential decline in ecosystem health, as evidenced by the sharp reduction in mycorrhizal diversity. These findings highlight the importance of monitoring macrofungal communities as sensitive bioindicators of the ecological degradation caused by invasive woody species.

Keywords: black locust; chestnut; flora; funga; ecological changes; Italy

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1. Introduction

Black locust (*Robinia pseudoacacia* Fabaceae Lindl.) was introduced into Europe at the beginning of the 17th century from North America [1] and is one of the most planted woody species in the world [2,3]. Its widespread cultivation stems from its rapid growth, high-quality durable timber, and versatility in reforestation, particularly for the remediation of disturbed or erosion-prone sites. Beyond its economic value in honey production and biomass energy, it has recently gained attention for its potential in atmospheric CO₂ sequestration [3,4]. Despite these benefits, *R. pseudoacacia* is recognized as one of the 40 most invasive woody angiosperms worldwide [5]. In Europe, it is classified as highly

invasive [6,7] and is included in the blacklists of alien species for Italy [8]. The substitution of native forests by pure *R. pseudoacacia* stands significantly alters ecosystem dynamics. Invasion leads to shifts in species composition by modifying soil nutrient availability—specifically through nitrogen fixation—and altering light conditions [9–11]. Consequently, native forest communities are more diverse than in pure *R. pseudoacacia* stands, suggesting that its invasion also causes homogenization of the forest plant biota [12].

In the Mediterranean landscape, chestnut groves (*Castanea sativa* Mill.) represent a vital socio-economic and ecological resource. They provide essential timber (coppice and high forest), chestnut and non-wood forest products, such as mushrooms and honey. Furthermore, they offer critical ecosystem services, including fire protection, soil erosion control, and the maintenance of high levels of biodiversity, particularly regarding fungal communities that sustain the forest's health and productivity [13,14].

The development of fungal fruiting bodies is governed by a complex interplay of climatic factors [15], forest structure [16–18], soil properties [19], presence of pollutants [20,21] and nutrient availability (e.g., nitrogen and water status) [22–24]. Ectomycorrhizal (ECM) species, parasitic and saprotrophic species, play a fundamental role in nutrient cycling [25,26]. ECM species are especially crucial for tree vitality, as they establish symbiotic relationships that enhance water and nutrient uptake while increasing host resistance against pathogens [27–29]. Conversely, saprotrophs together with bacteria and other organisms facilitate the decomposition of organic matter into simpler elements. Fungal parasitism, however, should not be considered solely as a degradative factor but rather as an important regulatory mechanism. By reducing the dominance of certain host species, it can facilitate the establishment of pioneer or less competitive species, thereby contributing to the maintenance and enhancement of biodiversity.

On Mount Amiata, a volcanic massif in Central Italy, chestnut groves are a defining feature of the landscape and a cornerstone of the local economy through mushroom and chestnut harvesting. *R. pseudoacacia* is currently present and is locally encroaching into chestnut groves, particularly in disturbed or marginal stands. Previous studies on plant communities have highlighted a reduction in species richness and a shift in floristic composition in black locust stands compared to native forests, including those dominated by chestnut [12,30]. However, the effects of *R. pseudoacacia* on the understory vegetation remain a matter of debate, as several studies have reported conflicting results [3,30,31]. Moreover, the compositional variability observed in invaded sites may be driven by intrinsic habitat characteristics rather than by the presence of the non-native species alone [32]. Black locust, as a nitrogen-fixing species, can significantly influence the nitrogen cycle in forest soils [30,33]. Consequently, changes in soil nutrient availability may lead to shifts in the composition of microbial communities, with potential effects on ecosystem functions and structure [30,34]. Among soil biota, fungi play a key role in biogeochemical cycles and in mediating interactions among organisms within forest ecosystems, making them particularly relevant for understanding the ecological consequences of black locust invasion.

Previous studies on the fungal communities of nearby fir forests [17,35,36] have shown that community composition is strongly influenced by silvicultural practices, lithology, and soil nitrogen level factors that potentially can be modified by the arrival of black locust. Nevertheless, in chestnut-dominated forests, most previous studies have focused on plant community or on soil or on fungal communities' descriptions, while controlled investigations explicitly linking plant diversity, fungal community diversity, and the effects of allochthonous species invasion remain limited. This study aims to fill this gap. The main objective of this study is to evaluate the impact of *R. pseudoacacia* invasion on the epigeal plant and macrofungal communities within native chestnut groves. Specifically, we assess differences in taxonomic alpha diversity, species abundance, and trophic group distribution by comparing three years of sampling data between native

chestnut groves and invaded stands, and we aim to quantify the ecological shifts induced by this invasive species.

2. Materials and Methods

The study was conducted on the southwestern slope of Mount Amiata, an orographically isolated volcanic massif in southern Tuscany (Italy) that is, with its 1738 m above sea level, the highest peak of this meridional part of the region [37].

The study area (Figure 1), located within the municipality of Santa Fiora (GR) at an elevation of 800–850 m a.s.l., is geologically characterized by quartz-latic volcanic and rheoignimbrites dating to 300,000–280,000 years ago (<https://www.igmi.org/> (accessed on 28 March 2025)). The local climate is defined as perhumid according to the Thornthwaite classification [38], with a Mediterranean-Tyrrhenian regime [39].



Figure 1. Location of the study area in Tuscany, showing the distribution of sampling plots. Red points indicate black locust groves, and green points indicate chestnut groves.

During the 2022–2024 study period, the Santa Fiora station [40] recorded an average annual temperature of 12.0 °C and cumulative precipitation of 847.3 mm. Notably, 2023 was characterized by higher autumn temperatures than 2022 and 2024, while rainfall distribution varied, peaking in September/October in 2022 and 2024, and in November during 2023 (Figure 2).

In September 2022, 16 plots of 100 m² (10 × 10) were selected: eight dominated by a native *C. sativa* and eight by invasive *R. pseudoacacia*. Plots were selected for maximum physiognomic homogeneity to minimize microclimatic bias (Figure 1; Table 1).

Table 1. Coordinates of the sampling plots. Eight plots are in black locust-dominated stands (left) and eight in chestnut-dominated stands (right). Plots labelled **R** indicate black locust dominance, whereas plots labelled **C** indicate chestnut dominance.

Plot	Coordinates	Plot	Coordinates
R1	N 42.84049 E 11.57915	C1	N 42.84053 E 11.57793
R2	N 42.84068 E 11.57890	C2	N 42.84091 E 11.57799
R3	N 42.84088 E 11.57836	C3	N 42.84151 E 11.57796
R4	N 42.84120 E 11.57856	C4	N 42.84011 E 11.57888
R5	N 42.85013 E 11.57014	C5	N 42.84964 E 11.57052
R6	N 42.84896 E 11.57095	C6	N 42.85276 E 11.57016
R7	N 42.84874 E 11.57039	C7	N 42.85276 E 11.57016
R8	N 42.85466 E 11.57725	C8	N 42.85445 E 11.57682

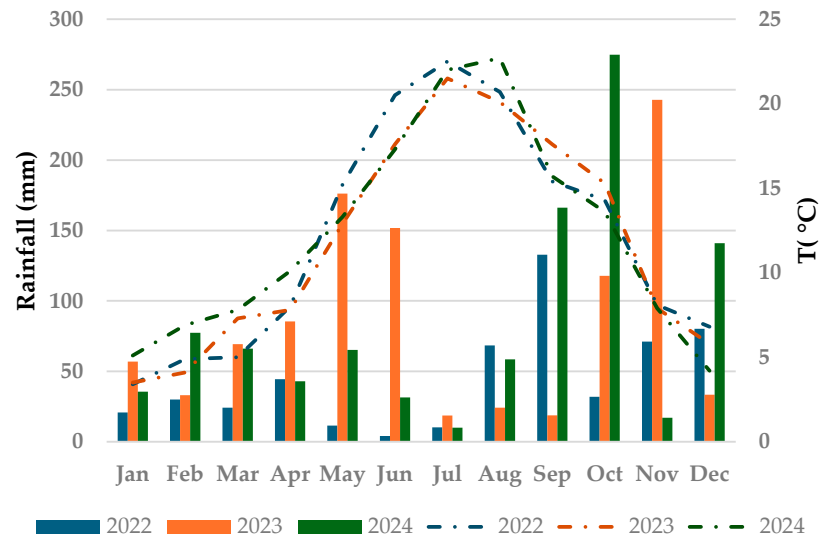


Figure 2. Monthly precipitation and average temperatures recorded during the study period (2022–2024). Vertical bars indicate total monthly precipitation (left axis); the dashed line represents the mean monthly temperature (right axis).

To determine the presence and abundance of plant species in each plot, vegetation was surveyed in Autumn 2022 and Spring 2023. During the samplings, the tree, shrub and herbaceous species present in the plots were identified and recorded and their abundance was assessed based on the percentage coverage of the plot area.

Parallel to the botanical surveys, mycological sampling was conducted monthly during the spring and autumn seasons from 2022 to 2024. All sporocarps visible to the naked eye (at least 1 mm or larger mm) were collected and quantified according to the methodology proposed by [41], as adapted for Mediterranean ecosystems by [42].

Although sporocarp surveys may not fully capture belowground fungal diversity [43–45], they remain a globally recognized standard for documenting forest fungal biodiversity [46–48]. The identification of fungal species was done partially on fresh material directly on the field, and partially on fresh and dried samples at the mycology laboratory of the Life Science Department of the University of Siena, by using standard morphological methods supported by general analytical keys and monographic references [35] with nomenclature following CABI list (www.indexfungorum.org (accessed on 28 November 2025)). The exsiccata are preserved in the *Fungarium of Herbarium Universitatis Senensis* (SI-ENA) and registered in the ‘anArchive’ database (www.anarchive.it (accessed on 30.04.2025)). Each species was classified in different trophic categories following [49] and based on field direct observations

Plant and fungal community data processing involved the calculation of taxonomic alpha diversity metrics, including species richness (SR), the Shannon–Wiener index (H'), and taxonomic distinctiveness, the latter estimated according to the methodology proposed by [50]. Community structure and similarities between plots were explored via Non-metric Multidimensional Scaling (NMDS) based on species composition matrices [51,52]. Statistical significance between the two forest types was assessed using the non-parametric Kruskal–Wallis test [53], as the data did not meet normality assumptions. All analyses were performed using the vegan package [54] within the RStudio 2023.12.1+402 statistical environment [55].

3. Results

Table S1 (Supplementary Material) shows the plant species recorded during the samplings in the study period. In the plots dominated by black locust, 62 species were counted. *R. pseudoacacia* and *Sambucus nigra* L. represent the most abundant entities in the arboreal and shrub layer, respectively. The herbaceous species with the greatest coverage were *Urtica dioica* L., *Salvia glutinosa* L. and *Hedera helix* L. (Table S1). In the plots dominated by chestnut, 68 species were counted. *Castanea sativa* and *Acer campestre* L. represent the most abundant entities in the arboreal and shrub layer. The herbaceous species with the greatest coverage were *Festuca heterophylla* Lam., *Hieracium murorum* L., *Pteridium aquilinum* (L.) Kühn. and *Rubus ulmifolius* Schott. Several seedlings of *A. campestre* and some seedlings of *Abies alba* Mill. were observed in various plots too (Table S1), but their ground cover was not considered significant.

The analysis of the plant communities shows significant differences for the plots dominated by *R. pseudoacacia* compared to those dominated by *C. sativa*. Specifically, the Shannon index is lower in the chestnut plots compared to the black locust plots. However, this evidence is reversed if only the number of species is taken into consideration (Figure 3A,B).

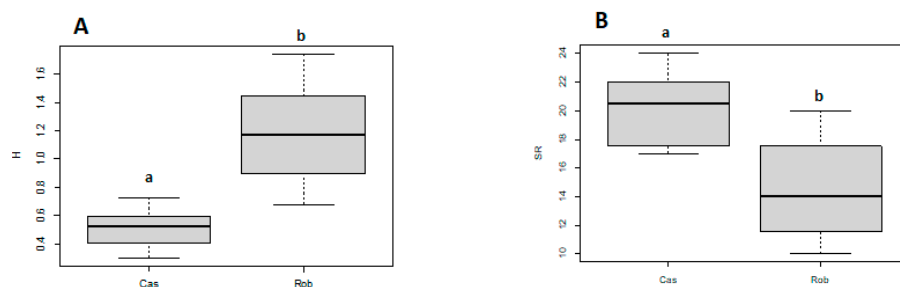


Figure 3. (A,B). Box plot showing variations in the Shannon diversity index (A) and plant species richness (B) in plots dominated by *C. sativa* (Cas) and *R. pseudoacacia* (Rob). Different letters (“a” and “b”) indicate statistically significant differences ($p < 0.05$).

The plant community under black locust shows a taxonomic distinctiveness below the average. However, the observed Δ^+ values are within the range of expected values except for the plot R2 (Figure 4), which seem to host taxonomically poorer plant communities. Unlike the black locust groves, the plots dominated by chestnut show taxonomic distinctiveness (Δ^+) values generally above the average and in any case always within the expected values or higher (C1).

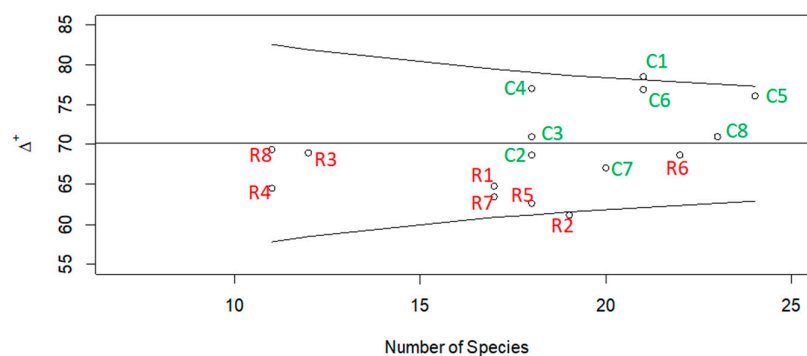


Figure 4. Average taxonomic distinctiveness (Δ^+) of plant communities (black lines represent the 95% confidence intervals). Green and red letters represent the plant species assemblages under *C. sativa* and *R. pseudoacacia*, respectively.

The Nonmetric multidimensional scaling (NMDS) analysis shows a clear separation of the chestnut and black locust grove plots according to the composition of the plant communities (Figure 5).

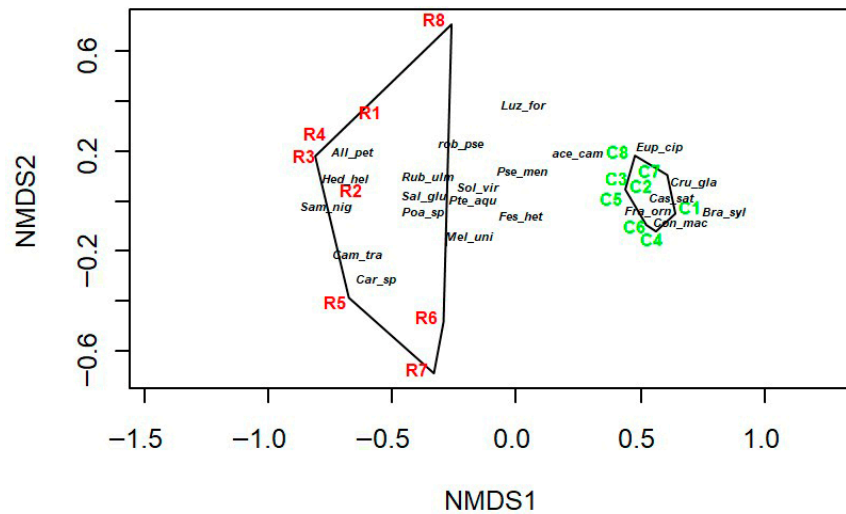


Figure 5. Non-metric Multidimensional Scaling (NMDS) ordination plot of plant communities. Red and green symbols represent *R. pseudoacacia* and *C. sativa* dominated plots, respectively. To enhance clarity, only dominant and characteristic species are labeled. Corresponding abbreviations are provided in Table S1.

Regarding the fungal component, a total of 309 taxa belonging to 52 families was identified (Table S2, Supplementary Material), with a prevalence of Cortinariaceae (35 spp.), Mycenaceae (35 spp.) and Russulaceae (29 spp.). The most representative genera were *Mycena* (28 spp.), *Cortinarius* (27 spp.) and *Russula* (21 spp.).

In the plots dominated by black locust, 154 species were recorded. The fungal community was largely composed of saprotrophic species (73.4%), including wood saprotrophs (Sw, 44.2%), humus saprotrophs (Sh, 48.7%), and litter saprotrophs (Sl, 6.2%). Parasitic species accounted for 5.2% of the total, while mycorrhizal species represented 16.9%. In plots dominated by *C. sativa*, 223 species were identified, with mycorrhizal fungi being the dominant functional group (58.3%). Saprotrophic species comprised 35.9% of the community, distributed among wood saprotrophs (Sw, 47.5%), humus saprotrophs (Sh, 45.0%), and litter saprotrophs (Sl, 7.5%). Parasitic species accounted for only 1.8% of the total (Figure 6; Table 2).

Table 2. Number of fungal and plant species recorded in each of the 16 sampling plots. The table also reports the total number of fungal species per trophic group (ECM—ectomycorrhizal species, S—saprotrophic species, P—parasitic ones) and the total fungal abundance (abb.) for each forest type (*Castanea sativa* and *Robinia pseudoacacia* stands).

Plot	Forest Type	No. Plant	No. Fungi	ECM sp.	S sp.	P sp.	Total abb.
C1	<i>Castanea</i>	21	59	44	14	1	436
C2	<i>Castanea</i>	17	50	26	23	1	602
C3	<i>Castanea</i>	17	66	39	26	1	2009
C4	<i>Castanea</i>	18	63	32	31	0	3383
C5	<i>Castanea</i>	24	69	44	25	0	2146
C6	<i>Castanea</i>	21	41	33	8	0	256
C7	<i>Castanea</i>	20	54	36	17	1	1472
C8	<i>Castanea</i>	23	64	28	35	1	1556

RI	<i>Robinia</i>	12	40	4	34	2	12,067
R2	<i>Robinia</i>	16	50	7	39	4	2210
R3	<i>Robinia</i>	12	52	7	43	2	2368
R4	<i>Robinia</i>	10	45	6	35	4	2899
R5	<i>Robinia</i>	18	33	5	27	1	1955
R6	<i>Robinia</i>	20	34	11	21	2	453
R7	<i>Robinia</i>	17	24	9	13	2	190
R8	<i>Robinia</i>	11	21	4	17	0	327

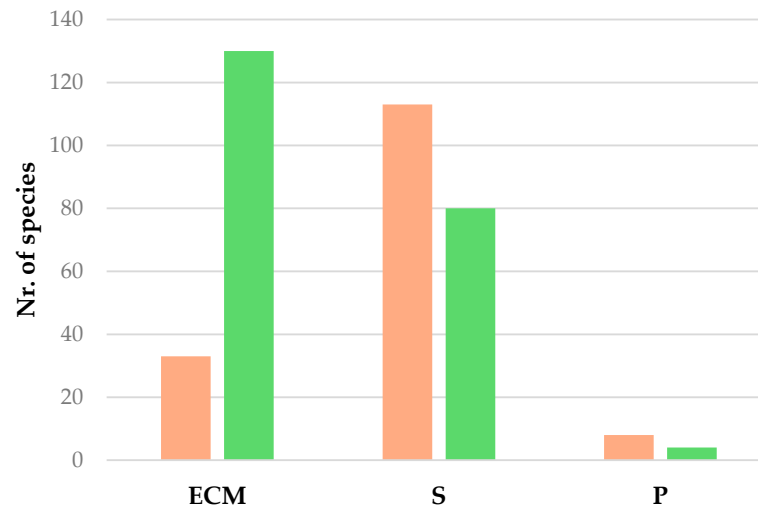


Figure 6. Repartition in trophic groups of macrofungal species recorded in black locust (orange columns) and chestnut (green columns) plots. ECM indicates ectomycorrhizal species, S saprotrophic species, P parasitic ones.

Several species were recorded across multiple years of sampling (Table S2), including *Amanita citrina* Pers., *A. rubescens* Pers., *Armillaria mellea* (Vahl) P. Kumm., *Artomyces pyxidatus* (Pers.) Jülich, *Auricularia auricula-judae* (Bull.) Quél., *Cantharellus cibarius* Fr., *Coprinellus micaceus* (Bull.) Vilgalys, Hopple & Jacq. Johnson, *Tremella globispora* D.A. Reid, *Rickenella fibula* (Bull.) Raithelh., as well as several species of the genera *Crepidotus* (*C. Caspari* Velen., *C. cesatii* (Rabenh.) Sacc. *C. subverrucisporus* Pilát) and *Mycena* (*M. galopus* (Pers.) P. Kumm., *M. inclinata* (Fr.) Quél., *M. leptcephali* (Pers.) Gillet, *M. niveipes* (Murrill) Murrill, *M. vitilis* (Fr.) Quél.). In contrast, some species were detected in only one year (*A. phalloides* (Vaill. ex Fr.) Link, *Russula emetica* (Schaeff.) Pers., *Tricholoma saponaceum* (fr.) P. Kumm., *Lactarius zonarius* (Bull.) Fr.), while others were recorded in two sampling years (*Clitocybe phaeoptalma* (Pers.) Kuyper, *Lactarius zonarius*, *Russula cyanoxantha* (Schaeff.) Fr., *Russula foetens* Pers., *Hebeloma crustuliniforme* (Bull.) Quél.). Notably, a high number of carpophores of *Crepidotus cesatii*, *C. caspari*, *Lanzia echinophila* (Bull.) Korf, and *Mycena vitilis* were observed.

The cumulative species accumulation curve over the three-year sampling period showed an asymptotic trend (Figure 7). Species richness varied among years (Table S2), with the highest number recorded in 2022 (192 species), followed by 2024 (146 species) and 2023 (97 species).

At the genus level, three main patterns were identified: (i) species-rich and highly productive genera, such as *Crepidotus*, *Mycena*, and *Russula*; (ii) species-rich but weakly productive genera, including *Lactarius*, *Inocybe*, and *Amanita*; and (iii) species-poor but highly productive genera, such as *Laccaria*, *Marasmius*, and *Scleroderma*.

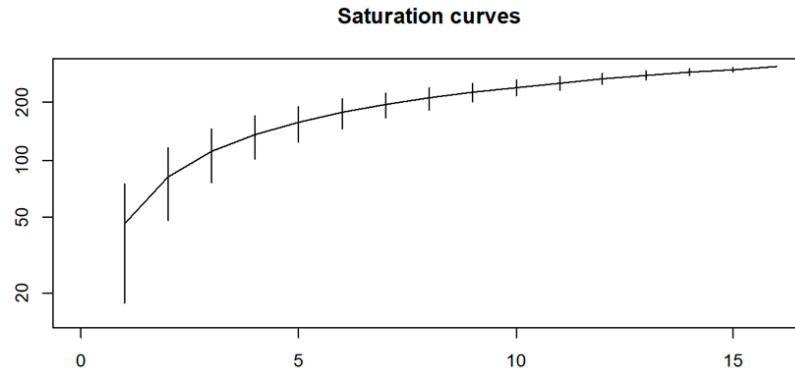


Figure 7. Cumulative number of macrofungal species recorded over the three-year study period (2022–2024) across the 16 study plots, including eight plots dominated by *R. pseudoacacia* and eight plots dominated by *C. sativa*.

Statistical analyses indicated that while the fungal Shannon index did not differ significantly between the two communities, species richness was significantly higher in the chestnut-dominated stands (Figure 8A,B). Similar to the plant communities, the fungal assemblages associated with black locust exhibited lower-than-average taxonomic distinctiveness, with nearly all plots (except R1) falling outside the expected range (Figure 9). Conversely, chestnut plots (except C7) remained above the average and statistically robust.

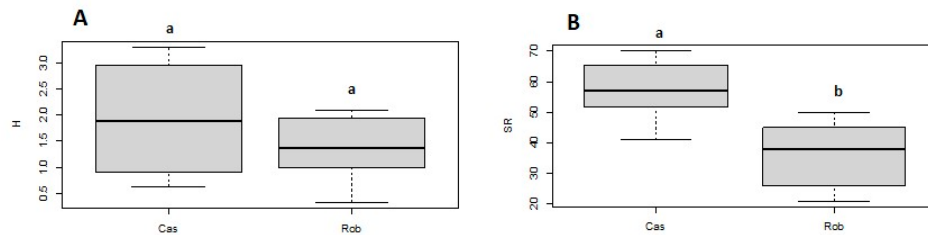


Figure 8. Box plot showing variations in the Shannon diversity index (A) and macrofungal species richness (B) in plots dominated by *C. sativa* (Cas) and *R. pseudoacacia* (Rob). Different letters (“a” and “b”) indicate statistically significant differences ($p < 0.05$).

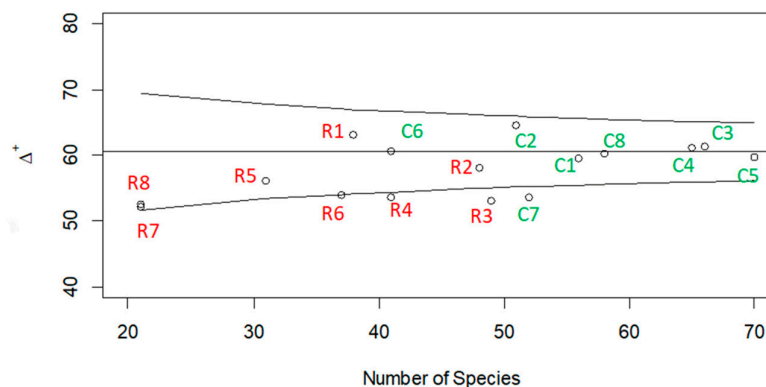


Figure 9. Average taxonomic distinctiveness (Δ^+) of macrofungal communities. Green and red letters represent the fungal species assemblages under *C. sativa* and *R. pseudoacacia*, respectively.

Finally, NMDS analysis confirmed a clear differentiation of fungal communities between the areas invaded by *R. pseudoacacia* and the native chestnut groves (Figure 10), faithfully reflecting the separation patterns observed in the botanical component.

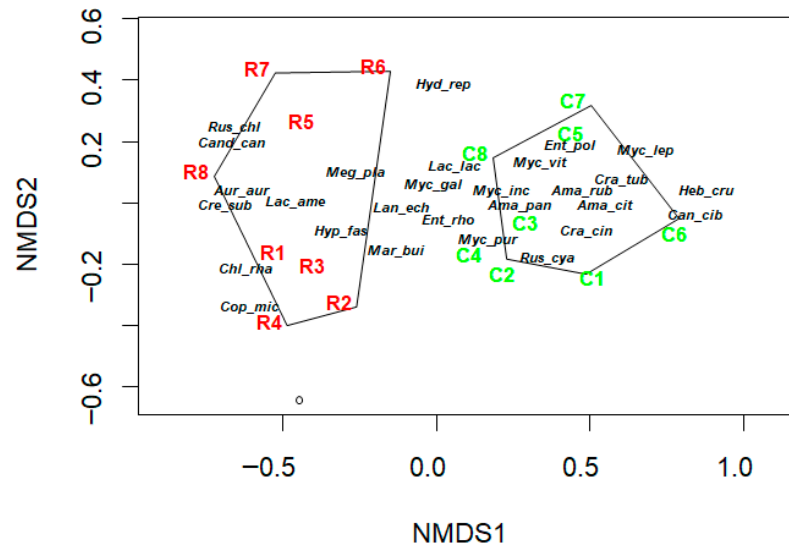


Figure 10. Non-metric Multidimensional Scaling (NMDS) ordination plot of macrofungal communities. Red and green symbols represent *R. pseudoacacia* and *C. sativa* dominated plots, respectively. To enhance clarity, only dominant and characteristic species are labeled. The species reported in the graph are the most abundant in chestnut groves, in black locust groves and in both of kind forest. Corresponding abbreviations are provided in Table S2.

4. Discussion

The comparison between black locust-dominated stands and native chestnut groves revealed marked differences in both vascular plant and macrofungal community structure, highlighting the pervasive ecological effects of *R. pseudoacacia* invasion. Although total plant species richness was only moderately lower in black locust plots than in chestnut stands, the compositional and functional attributes of the communities differed substantially, indicating that species counts alone may underestimate invasion-driven biodiversity alterations [56–58].

Given the volcanic origin of Mount Amiata, the flora of the chestnut groves is poorer than other Apennine areas of the same altitude and latitude. In this area there is the prevalence of helio-xerotolerant plants [37]. In the botanical component, black locust stands exhibited a higher Shannon–Wiener diversity index despite significantly lower species richness. This apparent paradox likely reflects a more even distribution of a limited number of taxa rather than a truly diverse assemblage, a pattern frequently reported in invaded ecosystems [59,60]. In contrast, native chestnut groves supported higher species richness, but were characterized by stronger dominance patterns, particularly in the woody and herbaceous layers. The prevalence of nitrophilous and disturbance-tolerant species such as *Urtica dioica* and *Sambucus nigra* under black locust suggests that changes in soil chemistry, light availability, and litter quality—well-documented effects of *R. pseudoacacia*—play a key role in reshaping understory vegetation [3,30,61].

These compositional shifts were further emphasized by taxonomic distinctiveness analyses, which revealed that black locust plots were generally taxonomically impoverished, whereas chestnut stands maintained phylogenetically broader and more balanced plant assemblages. Reduced taxonomic distinctiveness is increasingly recognized as a hallmark of biological invasions, reflecting biotic homogenization processes driven by environmental filtering and competitive exclusion [60,62,63]. The clear separation observed in NMDS ordinations confirms that these differences are not merely quantitative but reflect fundamentally distinct floristic identities.

Patterns observed in the mycological component closely mirrored those of the vascular plants, reinforcing the notion of strong plant–fungus coupling in forest ecosystems [64–66]. The overall fungal richness recorded over the three-year monitoring period was high, and the asymptotic species accumulation curve suggests that sampling effort was sufficient to capture the majority of taxa present [67]. The reduction of fruit bodies in 2023 is linked to the exceptionally low rainfall and high temperatures recorded that year. Numerous studies demonstrate that fruit set production is strongly governed by water availability, particularly in ectomycorrhizal fungi [68–71]. Periods of drought can reduce the development of active mycelium and prevent the growth of fruiting bodies [72], an effect that is set to increase with climate change [73]. Higher fruiting levels in 2022 and 2024, however, indicates a certain resilience of local fungal communities. Temporal variability in annual richness likely reflects inter-annual climatic fluctuations affecting fruiting phenology rather than structural changes in the communities, as widely documented in long-term fungal monitoring studies [74,75].

At the genus level, the identification of contrasting richness–productivity strategies underscore the functional heterogeneity of fungal assemblages and cautions against equating sporocarp abundance with ecological importance [76,77]. Genera such as *Mycena* and *Crepidotus* combined high species richness with high productivity, whereas ectomycorrhizal-rich genera such as *Inocybe* and *Lactarius* showed lower fruiting output, consistent with their cryptic belowground biomass and conservative reproductive strategies [64,65].

The most striking contrast between forest types emerged in the structure of fungal trophic group. Black locust stands were overwhelmingly dominated by saprotrophic fungi, whereas chestnut groves supported mycorrhizal taxa as the primary functional group. This shift is consistent with the limited ectomycorrhizal compatibility of *R. pseudoacacia* and its tendency to promote rapid litter decomposition, favouring saprotrophs over symbiotic fungi [3,78,79]. The high prevalence of saprotrophic taxa in the black locust grove is consistent with the pioneer and nitrogen-fixing nature of black locust, which tends to favour decomposers capable of exploiting N-rich, high-turnover substrates [80–82]. Conversely, the high percentage of ectomycorrhizal fungi in chestnut stands reflects the nature of *C. sativa* as a strongly mycorrhizal forest species [83]. Ectomycorrhizal fungi are known to form complex and highly specific underground networks, contributing to the greater functional diversity and resilience observed in chestnut stands [84,85]. The reduction in mycorrhizal diversity under black locust has important ecological implications, as it may constrain nutrient-cycling pathways and limit the regeneration potential of native tree species [86,87]. Numerous studies have documented a decline in fungal diversity due to the presence of *R. pseudoacacia*, a non-native species that alters soil properties by increasing nitrogen availability and modifying litter quality [9,30,88,89].

Despite the lack of significant differences in fungal Shannon diversity between forest types, the significantly higher species richness and taxonomic distinctiveness in chestnut stands indicate that native forests sustain more complex and resilient fungal assemblages. As observed for plants, fungal communities associated with black locust showed reduced taxonomic distinctiveness, suggesting functional and evolutionary homogenization driven by invasion [65,90]. In the chestnut grove, taxonomic distinctiveness is within the expected values and is higher than average, indicating a more complex community with more varied evolutionary lineages, as found in other ecosystems dominated by native mycorrhizal tree species [91]. The congruent separation of plant and fungal communities in NMDS ordinations highlights the integrative impact of *R. pseudoacacia* on forest ecosystems, affecting multiple trophic levels simultaneously. Data confirms that *R. pseudoacacia* can significantly reduce fungal biodiversity, both in terms of richness and taxonomic complexity [12]. The prevalence of saprotrophic fungi and the reduction of ectomycorrhizal fungi in black locust stands are not just changes in composition but indicate a profound

alteration of fundamental ecosystem processes. Ectomycorrhizal fungi are crucial for soil carbon sequestration and nutrient uptake by the host. Their decline, combined with the increase in saprotrophs, significantly alters decomposition dynamics and the entire soil food web. In nitrogen-enriched environments, saprotrophic fungi tend to dominate, leading to faster litter decomposition (reducing organic carbon accumulation) and lower nutrient retention compared to mycorrhizal-dominant systems [15,92]. In this context, the greater diversity and stability of chestnut groves underscore the role of native tree species in the conservation of mycological biodiversity and the resilience of forest ecosystems.

5. Conclusions

This study demonstrates that the invasion of *R. pseudoacacia* profoundly alters both vascular plant and macrofungal communities when compared with native *C. sativa* forests. Although overall diversity indices based on species evenness may suggest comparable levels of biodiversity, a more detailed analysis reveals consistent reductions in species richness, taxonomic distinctiveness, and functional organization in black locust-dominated stands. These findings highlight the limitations of relying on single diversity metrics and underscore the importance of integrating taxonomic, structural, and functional approaches when assessing invasion impacts.

Native chestnut groves supported floristically and mycologically richer communities, characterized by higher phylogenetic breadth and a dominance of ectomycorrhizal fungi, reflecting well-established plant–fungus mutualistic networks. In contrast, black locust stands were associated with simplified assemblages dominated by saprotrophic fungi and disturbance-tolerant plant species, indicating a shift toward faster nutrient cycling and reduced ecological specialization. The parallel responses observed in plant and fungal communities emphasize the tight coupling between above- and belowground biodiversity and suggest that invasive trees can drive ecosystem-wide homogenization.

The consistent separation of forest types in multivariate ordinations further confirms that *R. pseudoacacia* invasion leads to distinct and persistent alternative community states rather than transient compositional changes. From a management perspective, these results suggest that the conservation and restoration of native chestnut forests are crucial for maintaining taxonomically and functionally diverse biotic communities. Efforts aimed at controlling black locust expansion and promoting native tree regeneration may therefore contribute not only to the preservation of plant diversity but also to the recovery of complex and resilient fungal assemblages, ultimately supporting long-term forest ecosystem stability.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/f17030354/s1>, Table S1: Plant species recorded during sampling. Plots labeled C are chestnut-dominated, whereas plots labeled R are black locust-dominated. Values represent the total percentage cover within each plot; values < 1 indicate cover not exceeding 1%. Abbr., indicates the species abbreviations used in the NMDS analysis. Table S2: Summary of mycological sampling across 16 plots (8 *Robinia* and 8 *Castanea* forests). G.t., trophic group (M: ectomycorrhizal; P: parasitic; Sc: coprophilous; Sh: humic saprotrophic; Sl: litter saprotrophic; Sw: lignicolous). T.S., total samples; Rf, *Robinia* forest; Cf, *Castanea* forest; Abb., number of fruiting bodies recorded; Freq., frequency of occurrence; Abbr., species abbreviation (also used for NMDS analysis). All data refers to the three-year study period.

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