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Naturalness and Tree Composition Determine the Abundance of Rare and Threatened Orchids in Mature and Old-Growth *Abies alba* Forests in the Northern Apennines (Italy)

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Abstract: Forest *Orchidaceae* are important for European temperate forests, yet their distribution and abundance have so far interested limited research. In three pure or mixed silver fir stands in the Foreste Casentinesi National Park (NP) (Northern Apennines, Italy) we analysed how structural traits in mature and old-growth forests affected orchid communities in terms of abundance of the main genera, trophic strategy and rarity in the NP. We established three 20x60 m plots to quantify the structure of living and dead tree communities, including a set of old-growth attributes connected to large trees, deadwood, and established regeneration. In each plot, we measured the abundance of all orchid species and explored their behaviour according to trophic strategy (autotrophy/mixotrophy, obligate mycoheterotrophy), rarity within the NP, and threatened status according to the IUCN Red List. We used multivariate ordination and classification techniques to assess plot similarities according to forest structure and orchid communities and identify the main structural factors related to orchid community features. The main structural factors were used as predictors of orchid community traits. Forest composition (i.e. the dominance/abundance of silver fir) affected the presence of the main orchid genera: *Epipactis* were abundant in silver fir-dominated forests, *Cephalanthera* in mixed beech-fir forests. Interestingly, *Cephalanthera* could become limited even in beech-dominated conditions if fir regeneration was abundant and established. Old-growth attributes like the density of deadwood and large tree volume were important determinants of the presence of rare and mycoheterotrophic species. Our results provided a first quantitative description of forest reference conditions to be used in the protection and restoration of threatened and rare orchid species.

Keywords: terrestrial orchids; mycoheterotrophic orchids; mixotrophic orchids; primary forest; mature and old-growth; naturalness; forest restoration; conservation

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1. Introduction

The *Orchidaceae* is one of the largest plant families globally, with approximately 25,000 to 33,000 species [1,2] distributed across diverse environments worldwide [3]. It is the second-largest plant family in terms of species count, after the *Asteraceae* [4]. This family includes a significant number of endangered species, particularly among terrestrial orchids [5,6]. Human activities, such as habitat destruction and direct harm to populations, represent a major threat [7,8], while climate change further affects local species distribution [9,10]. Consequently, the conservation of these species and their habitats has become a global priority [11,12].

Forest biodiversity is widely recognized as being shaped by forest structure and management practices [13,14], which are often influenced by varying degrees of human intervention in forest ecosystems [15]. The relationships between forests and orchids have increasingly drawn the attention of researchers, who have explored various ecological aspects of these interactions [16–20]. In the case of temperate terrestrial forest orchids, their growth is known to depend not only on climatic and edaphic factors but also on biotic interactions [21–23]. These species depend on mutualistic mycorrhizal associations with fungi to absorb carbon from nearby trees, a process that is essential for their survival [24]. These interactions, whether obligatory or not, play a pivotal role in completing their life cycles and for some species, fungi are indispensable partners throughout all life stages [25,26]. The germination of orchid seeds, due to their lack of reserves, is entirely dependent on the establishment of mycorrhizal associations, which can range from highly specific to less [27]. Subsequently, during growth, plant carbon supplies may rely entirely on mycorrhizae (fully or obligate mycoheterotrophic species) or only partially by combining it with photosynthesis (partially mycoheterotrophic or mixotrophic species) [28]. Furthermore, the degree of mixotrophy can reduce to minimum and definitely shift towards autotrophy under certain conditions [29]. It can therefore be assumed that the more orchids rely on mycorrhizae to compensate carbon deficiencies (e.g. by establishing links with saprotrophic fungi) [30], the more they may be adapted, co-evolved, and specialized for forest ecosystems (e.g. non-photosynthetic orchid species). This underscores the intricate ecological interdependence of orchids within forest ecosystems, as well as the inherent challenges associated with studying these complex relationships.

It is well established that forest orchids are closely linked to light availability within forest ecosystems. Light is one of the primary physical factors influencing orchid distribution and abundance [3,31]. In forests, the amount of ground reaching light the ground can determine the presence of certain *Orchidaceae* species over others, depending on their ecology [32]. This phenomenon is linked to the ecological valence of each species with respect to the light factor [33–35]. Forest management practices often modify the canopy structure, thereby altering the amount of light that reaches the forest floor. Research indicates that human-induced disturbances, such as selective logging, can positively influence the growth of some forest orchids [36–38]. Conversely, studies have shown that practices like clear-cutting can negatively impact shade-tolerant species, potentially leading to their decline [39]. For strictly nemoral species, maintaining a closed canopy is essential for their survival [40]. Natural disturbance and the ecological processes connected to forest succession play a pivotal role in the survival of certain orchid species, yet these dynamics remain insufficiently explored [41,42]. Additionally, forest edge effects have been identified as significant factors influencing orchid distribution and occurrence [43]. Research on the ecology of orchids in complex habitats, such as old-growth forests, has seen some advancement in tropical regions [44,45]. However, this remains a largely underexplored area for terrestrial forest orchids in temperate climates [46].

In a forest ecosystem, other factors can be crucial. Soil characteristics play a fundamental role in influencing the abundance and distribution of *Orchidaceae* [3,23]. The type of geological substrate is important at both regional and local levels: changing in the availability of soil resources (like water and nutrients) across different substrates led to variations in the richness and composition of orchid species [47,48]. Soil physical and chemical properties are essential too [3,49], e.g. soil moisture and pH at the microsite scale, are of great interest as they not only affect species composition of orchids, but also their abundance [48]. Furthermore, the organic matter in the soil represents the main source of carbon and nutrients transferred to orchids through their mycorrhizal fungi [50]. It has been observed that the germination rate of orchids is positively correlated with the soil organic matter content [51]. The influence of these and other factors on population dynamics of forest orchids and their potential conservation implications has been only partially explored [21], leaving significant knowledge gaps.

Temperate orchids of open habitats have historically attracted greater attention from researchers, while forest orchids have remained relatively understudied. Genera such as *Ophrys* and *Orchis* have been extensively studied, particularly from systematic and ecological perspectives [52–54], even at a very fine taxonomic scale to the point of describing several possible hybrids [55–57]. Certain forest-specific orchids, especially those that are highly protected and threatened, like *Cypripedium calceolus* L. [58], have been the focus of numerous studies examining the impact of forest cover on their fitness and population dynamics [38,59,60]. However, other temperate forest orchid species, which face fewer threats, lack legal protection or are outside designated protected areas, have generally been less studied concerning the effects of changes in forest cover. This trend partly reflects a global research bias favouring open and semi-natural ecosystems [61] and also favouring "charismatic" and visually appealing flowering plants, which tend to attract more attention and funding in conservation science [62]. Some European forest orchid species exhibit a paucity of visually appealing morphological and chromatic characteristics, which often results in their inadvertent neglect by researchers. These observations underscore the necessity for further investigation into this group of species.

In the last decade, a growing interest has focused on Mature and Old-Growth (MOG) forests to identify their potential for biodiversity conservation as well as climate-change mitigation [63,64]. Besides primary forests, i.e. naturally-developing ecosystems representing undiscussed biodiversity sanctuaries [65], studying MOG naturalness-related attributes is crucial to better understand long term forests development and highlight pathways to ecological restoration in the so-called proforestation approaches [66]. The old-growth forest status provides unique features in terms of large tree dominance [67] and lifespan [68], deadwood and microhabitats [69], structural complexity [70]. Furthermore, the development of MOG into more advanced structural stages implies the onset of rare and complex patterns [71] and processes [72], favouring habitat conditions fundamental for the survival of highly specialised taxa [73,74]. Again, more research is still needed to fully describe and understand the casual relationships sustaining the presence of highly specialized forest species and the most advanced structural traits.

This research focused on orchid communities in pure and mixed forests with silver fir (*Abies alba* Mill.) at three sites within the "Parco Nazionale delle Foreste Casentinesi, Monte Falterona e Campigna" (National Park, NP) in the Northern Apennines (Italy). The study aimed to: (i) assess how forest structure, in particular old-growth features, influenced the prevailing traits of orchid communities in terms of dominant genera and trophic strategy; (ii) evaluate the ability of selected forest structure indicators to predict the selected traits of orchid communities; (iii) provide a first description of potential reference values of forest structural attributes to be used in species conservation and forest restoration.

2. Materials and Methods

2.1. Study Area

The "Parco Nazionale delle Foreste Casentinesi, Monte Falterona e Campigna" (hereafter referred to as the Park or NP) is an Italian protected area located in the Northern Apennines, specifically in the "Tosco-Romagnolo" Apennine, in Central Italy (Figure 1). It spans the border between the Emilia-Romagna and Toscana regions, covering parts of the provinces of Forlì-Cesena, Arezzo, and Firenze. The Park encompasses a total area of 36,400 ha. A key feature of the Park is the Apennine ridge, which runs from northwest to southeast, dividing the landscape into two main slopes. The highest points along this ridge are "Monte Falco" Mt. (1,657 m a.s.l.) and "Monte Falterona" Mt. (1,654 m a.s.l.). The three study sites are situated within the boundaries of the Park (Figure 1, Table 1). The first site, called Campigna (CMP), is located within the province of Forlì-Cesena and represents the northernmost site. Proceding to south, the other two sites are Camaldoli (CAM) and La Verna (VER), in the province of Arezzo. The geology of Campigna and Camaldoli is comparable, characterised by sandstones and arenaceous marls (Middle-Lower Miocene and

Palaeogene age, respectively), while La Verna can be distinguished for the presence of Middle-Lower Miocene skeletal limestones and calcarenites [75]. According to Pesaresi et al. [76], the areas fell within the temperate macrobioclimate and the temperate oceanic bioclimate, with a weak semicontinental subtype. The three sites exhibit a humid ombrotype and a supratemperate upper horizon [76]. At the average plot elevation (c. 1,090 m a.s.l.), the mean annual precipitation is 1,625 mm, and the mean annual temperature is 8.3 °C. From an ecological perspective, Campigna is mainly characterised by semi-natural forest of fir and beech, interspersed with small areas of conifer reforestations, Camaldoli is dominated by old coniferous reforestations undergoing spontaneous dynamics with patches of semi-natural fir or pure or mixed beech forests, and La Verna is characterised by beech and fir mixed stands [77]. A common feature of the three sites is their status as well-preserved forest which host high levels of specific diversity, comprising taxa often unique to the study areas and rarely found elsewhere in the Park [78–85]. In particular, our study sites fell within or were contiguous to habitats of interest to the Natura 2000 Network, classified under codes 9130 (*Asperulo-Fagetum* beech forests), 91M0 (Pannonian-Balkanic turkey oak-sessile oak forests), 9220* (Apennine beech forests with *Abies alba* and beech forests with *Abies nebrodensis*), and 9210* (Apennine beech forests with *Taxus* and *Ilex*) [86]. Additionally, these localities host numerous species of Community interest, including the beetles *Rosalia alpina* Linnaeus and *Osmoderma eremita* Scopoli, restricted to old-growth forest [87].

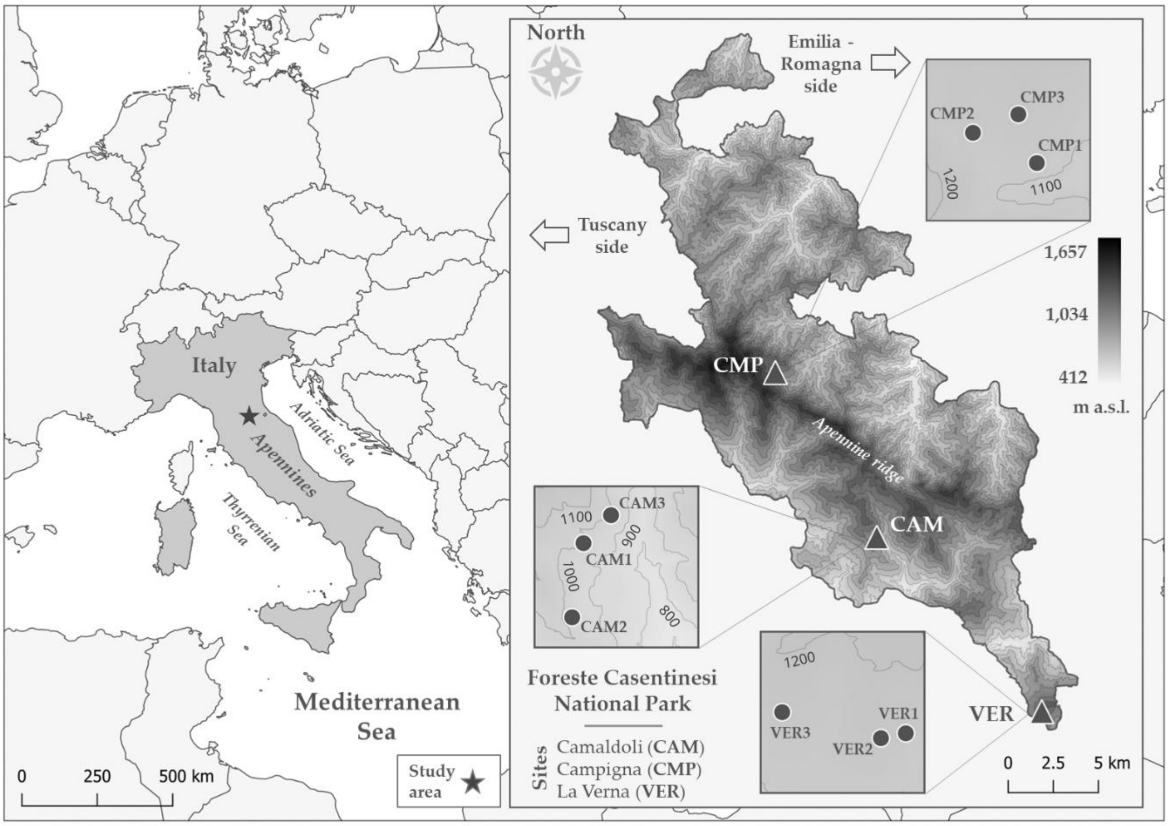

Figure 1. The location of (left) the study area and (right) the three study sites (CAM, CMP, VER) with each plot within the boundary of the National Park.

51 species of *Orchidaceae*, grouped into 17 genera [83,88], are included among the 1,172 native taxa recorded in the Park [85]. As a result of the extensive forested area covered by woods and forests in the NP (over 80%), many of the existing orchid species are typical plants of forest environments. For example, species such as *Neottia nidus-avis* (L.) Rich., *Cephalanthera damasonium* (Mill.) Druce, and *Epipactis helleborine* (L.) Crantz occur in over 60% of the Park's territory, divided into equal-sized 2

km cells [83]. Some species are even rarer, such as *Epipogium aphyllum* Sw., *Epipactis placentina* Bongiorni & Grünanger, and *E. greuteri* H.Baumann & Künkele [85].

Table 1. Summary of site features. Coordinates refer to the WGS84 system. Mean (min – max) values of elevation, slope and aspect were provided.

Site	Code	Latitude (°N)	Longitude (°E)	Elevation (m a.s.l.)	Slope (%)	Aspect (°)
Camaldoli	CAM	43.79151	11.81329	982 (956 – 1,000)	14 (9 - 20)	125 (81 - 185)
Campigna	CMP	43.87225	11.74016	1,135 (1,118 – 1,159)	22 (15 - 30)	172 (152 - 195)
La Verna	VER	43.70830	11.93217	1,153 (1,144 – 1,161)	19 (17 – 28)	130 (100 - 180)

2.2. Field Sampling of Forest Structure and Orchid Species Diversity

At each site, three replicated 20 m x 60 m plots (1200 m^2) were established in forest ecosystems at comparable elevation (Table 1). Each plot was set with a short side on the forest margin and then developed with its long side into the forest, parallel to the main slope. Plot coordinates endpoints were collected using a Garmin GPSMAP 66i. In each plot, we quantified living and dead tree structures. For all living trees with height > 1.3 m and Diameter at Breast Height (DBH) ≥ 2.5 cm, we recorded the species and measured DBH using a caliper. Height (H) was measured with a Haglof Vertex IV on trees sampled to represent 20-cm DBH classes. To quantify the establishment of tree regeneration we counted the number of trees all trees with DBH < 12.4 cm and falling in one of the following height (H) classes: 1, <10 cm; 2: 11–50 cm; 3: 51–150 cm; 4: 151–300 cm; 5: >300 cm).

The presence and absolute abundance (i.e., number of stems) of orchid species were recorded for each plot. When it was not possible to identify a specimen at the species or subspecies level (especially in plants without flowers of *Cephalanthera* and *Epipactis*), it was assigned to the appropriate higher taxonomic group and labeled as not classified species (NC). The taxonomic nomenclature follows Bartolucci et al. [89].

2.3. Quantifying Forest and Orchid Community Traits

Site-level H-DBH curves were calculated by merging data from the three plots using the Naslund equation [90]. Tree volume was calculated using measured DBH and predicted H according to species allometries available in Tabacchi et al. (2011). The dead biomass components were divided into Standing Dead Trees (SDT; DBH ≥ 10 cm) and Coarse Woody Debris (CWD; median diameter $D_{0.5}$ ≥ 10 cm and length ≥ 1 m). On each SDT we measured DBH and H, and calculated the volume using the cylinder formula. On each CWD, $D_{0.5}$ and length served to calculate volume according to the cylinder formula. Each deadwood piece was assigned to a decomposition stage ranking 1 (intact shape and consistency) to 5 (advanced decomposition with loss of original shape and consistency) according to Rubino & McCarthy [92].

To quantify the degree of old-growthness of each forest plot, we calculated a set of Structural Indicators (SI)[70,72]: stand structural complexity, measured with the DBH coefficient of variation (CV_{DBH}); established regeneration (frequency of trees with DBH < 12.5 cm); the density of large trees (DBH > 50 cm and DBH > 70 cm), large CWD ($D_{0.5}$ > 50 or 70 cm) and large SDT (DBH > 50 or 70 cm). Additionally, mean and density-weighted decay value were calculated for both CWD and SDT.

The Index of Regeneration (IR) was calculated as the weighted sum of regeneration density multiplied by the mean height value of each class (1 – 5). All indicators were also calculated separately for: ABAL (*Abies alba*), FASY (*Fagus sylvatica* L.), and OTBR (other broadleaves). All SI values were reported in Tables S1.

The forest Orchid Community (OC) dataset comprises 119 records collected across all plots. Orchids were grouped by genus and species, specific codes were assigned and their main features were synthesized in Table 2. Based on existing literature, genera and species were classified into two distinct categories according to their primary trophic regime: fully mycoheterotrophic species (code MH) and autotrophic/mixotrophic species (code AUMX). The species classified as fully mycoheterotrophic include *Epipogium aphyllum* and *Neottia nidus-avis* [93–95]. The species belonging to the genera *Cephalanthera*, *Epipactis*, and *Dactylorhiza* exhibit a more complex situation: these genera include both species in which mixotrophy, to varying degrees, is well documented [26,28,96–98], and species that occupy a trophic continuum between autotrophy and mixotrophy, which are still under investigation [99–101]. From a distributional perspective, orchids species were classified as widespread (code WIDE) or narrow range and rare (code NARROW) within the study area, according to Pica & Laghi [83]. Additionally, the IUCN Red List category assigned to each species was included [102]. Finally, the Ellenberg indicator values for each species, as derived from Pignatti et al. [103] and Tichý et al. [104], were also reported (Table 2).

Table 2. *Orchidaceae* species recorded in all sites, classified by: genus, trophism (MH, Fully Mycoheterotrophs; AUMX, Autotrophs/Mixotrophs), distribution within the National Park (WIDE, Widespread Species; NARROW, Narrow Range and Rare Species), IUCN Red List assessment (LC, Least Concern; NT, Near Threatened; EN, Endangered), Ellenberg indicator values (L, Light; T, Temperature; K, Continentality; F, Moisture; R, Soil Reaction; N, Nutrients). Codes corresponding to genera and species are provided.

Genus	**Code**	**Trophism**	**Species**	**Code**	**NP distribution**	**Red List**	**Ellenberg Indicator Values**					
							L	T	K	F	R	N
Cephalanthera	CEPHA	AUMX	*C. rubra* (L.) Rich.	CERU	WIDE	LC	3.0	5.0	5.0	4.0	8.0	3.0
			C. longifolia (L.) Fritsch	CELO	WIDE	LC	4.0	5.0	5.0	3.0	8.0	3.0
			C. damasonium (Mill.) Druce	CEDA	WIDE	LC	2.0	5.0	4.0	4.0	7.0	4.0
						Mean	**3.0**	**5.0**	**4.7**	**3.7**	**7.7**	**3.3**
Epipactis	EPIPA	AUMX	*E. helleborine* (L.) Crantz	EPHE	WIDE	LC	3.0	5.0	5.0	5.0	7.0	5.0
			E. greuteri H.Baumann & Künkele	EPGR	NARROW	EN	2.0	6.0	-	7.0	7.0	6.0
			E. microphylla (Ehrh.) Sw.	EPMI	WIDE	NT	7.0	7.0	4.0	3.0	6.0	2.0
			E. leptochila (Godfery) Godfery	EPLE	NARROW	LC	3.0	6.0	3.0	4.0	9.0	4.0
			E. purpurata Sm.	EPPU	NARROW	LC	2.8	5.8	-	5.5	7.3	5.3
			E. exilis P.Delforge	EPEX	NARROW	LC	-	-	-	-	-	-
						Mean	**3.6**	**6.0**	**4.0**	**4.9**	**7.3**	**4.5**
Neottia	NEOTT	MH	*N. nidus-avis* (L.) Rich.	NENA	WIDE	LC	2.0	5.0	5.0	5.0	7.0	5.0
Dactylorhiza	DACTY	AUMX	*D. maculata* (L.) Soó s.l.	DAMA	WIDE	LC	7.0	5.0	5.0	5.0	-	4.0
Epipogium	EPIPO	MH	*E. aphyllum* Sw.	EPAP	NARROW	LC	1.0	4.0	5.0	5.0	3.0	9.0

To quantify the orchid community features and compare plots, we chose the following indicators: genera and species number; genera and species absolute abundance; genera and species relative abundance; trophic group relative abundance; distribution range relative abundance. Statistical analyses were performed using the relative abundances of the taxonomic (genus), trophic, and distributional levels, indicated by the letter "p" following the variable (CEPHAp, EPIPAp, NEOTTp; MHp, AUMXp; WIDEp, NARROWp). Species grouping was necessary due to the frequent zero or low values of some species in several plots. Scarcely represented genera (*Epipogium* and *Dactylorhiza*) were included only in trophism and distribution analysis.

2.4. The Relationships Between the Orchid Community and Forest Structure

A Principal Component Analysis (PCA) was used to identify the main structural indicators to describe stand/plot differences and identify the candidate predictors for the orchid community. The initial set of 102 variables was divided into thematic groups describing tree composition, regeneration, large trees, standing, and lying deadwood (Table S1).

To remove redundant or ineffective variables, we proceed with a two-step method [72]: (i) with the first PCA, we filtered out variables with loading < 50% of the absolute maximum axis loading [105,106]; (ii) the remaining variables were passed again through a PCA, to select the best ones per each group/subgroup, i.e. those with the highest loading (satisfying the 50% threshold). In both PCAs we retained the minimum number of axes explaining ≥ 2/3 of the overall variance (i.e. 2; [105,106]). The initial set of 102 structural indicators was first reduced to 77 and then to 32.

To further illustrate how plots grouped according to similarities in either structural or orchid community features, we classified them using Ward's clustering algorithm and Pearson's distance [72].

To select potential predictors of orchid species community features according to structural indicators, we ran a final PCA incorporating both SI and OC values to highlight correlations between the two datasets. The best SI, i.e. the one per subgroup with the highest loading, were retained as candidate predictors for the different descriptors of the OC. For each OC variable, we performed univariate or bivariate linear regressions choosing the 1-2 strongest SI, and the final model was chosen according to AIC. All models were checked for linear model assumptions [107,108].

Data were analysed and processed using QGIS 3.32.3 software [109], R software [110] and RStudio [111].

3. Results

3.1. Analysis of Forest Structure, Living and Dead Biomass

According to their DBH distributions, Campigna (CMP) and Camaldoli (CAM) hosted mainly pure even-aged silver fir stands, with angiosperms locally established in smaller size classes, while La Verna (VER) was an uneven-aged beech-dominated stand with fir and minor presence of other angiosperms (Figure 2, Table 3). Despite its lower stature (35 m compared to 40 m in CAM and CMP), VER had the largest trees (Table 3), either silver fir or beech (Figure 2). Pure forests (CAM and CMP) showed a cathedral-like canopy of silver fir, but locally (i.e. CMP2, CAM2) plots exhibited a two-layered structure with an understory layer dominated by beech and other angiosperms. In the regeneration layer, the most established species was fir, especially at CAM, while at CMP both the establishment and the presence of beech was higher (Figure 3, Figure S1). In VER, the multilayered structure of the mixed stand was accompanied by higher values of the regeneration index, with half of IR made of fir and the other half angiosperms (Figure 3, Figure S1). VER3 had the most complex structure, with higher density of both very large and small trees, especially fir (Figure 2), and the highest IR values detected (Figure 3, Figure S1).

Figure 2. DBH distribution (relative frequency) by site/plot. Bar color represented tree composition (ABAL, *Abies alba*; FASY, *Fagus sylvatica*; OTBR, other broadleaves). Beside each site's code, numbers indicate the corresponding plots.

Table 3. Summary of the structural characteristics, Index of Regeneration, deadwood and species composition (ABAL, *Abies alba*; FASY, *Fagus sylvatica*; OTBR, other broadleaves) for each site. Values: site mean and, when parentheses are present, plot's minimum-maximum.

		CAM	**CMP**	**VER**
Forest type		Pure silver fir	Pure silver fir	Mixed beech
Living Trees	Stature (m)	33.4	37.2	30.9
	DBH (cm)	38.7 (34.9 – 44.3)	45 (39.5 - 49)	22.1 (19.6 – 25.1)
	Density (stem ha^{-1})	483 (458 – 508)	469 (358 – 583)	772 (666 – 933)
	Basal Area (m^2 ha^{-1})	65 (55.4 – 75.4)	83.4 (75.6 – 87.3)	45.2 (43.3 – 48.6)
	Tree Volume (m^3 ha^{-1})	958.9 (772 – 1,137.6)	1,345 (1,246 – 1,404.8)	584 (481.1 – 726.5)
	Large Tree Volume (%)*	5.5	14.4	21.1
Tree Species composition (% Volume)	ABAL	98.8	98.7	24.7
	FASY	1	0	63.7
	OTBR	0.2	1.3	11.6
Tree Index of Regeneration (%)	Small (IR1-2)	96.5	64.6	34.8
	Intermediate (IR3)	0	4.1	0.2
	Established (IR4-5)	3.5	31.3	65
Total Deadwood	Density (stem ha^{-1})	202.8 (108.3 – 391.7)	166.7 (75 – 283.3)	83.3 (33.3 – 158.3)
	Volume (m^3 ha^{-1})	76.6 (10.6 – 172.4)	24.6 (14.4 – 36.5)	42.2 (4.4 – 93.5)
Coarse Woody Debris (CWD)	Density (stem ha^{-1})	166.7 (0 – 391.7)	136.1 (66.7 – 100)	50 (33.3 – 75)
	Volume (m^3 ha^{-1})	19.1 (0 – 46.7)	10.1 (5.1 – 15.1)	34.8 (3.4 – 93.5)

Standing Dead Tree (SDT)	Density (stem ha^{-1})	36.1 (0 – 108.3)	30.6 (8.3 – 41.7)	33.3 (0 – 83.3)
	Volume (m^3 ha^{-1})	57.5 (0 – 172.4)	14.5 (4.2 – 31.4)	7.3 (0 – 20.9)

*DBH > 70 cm.

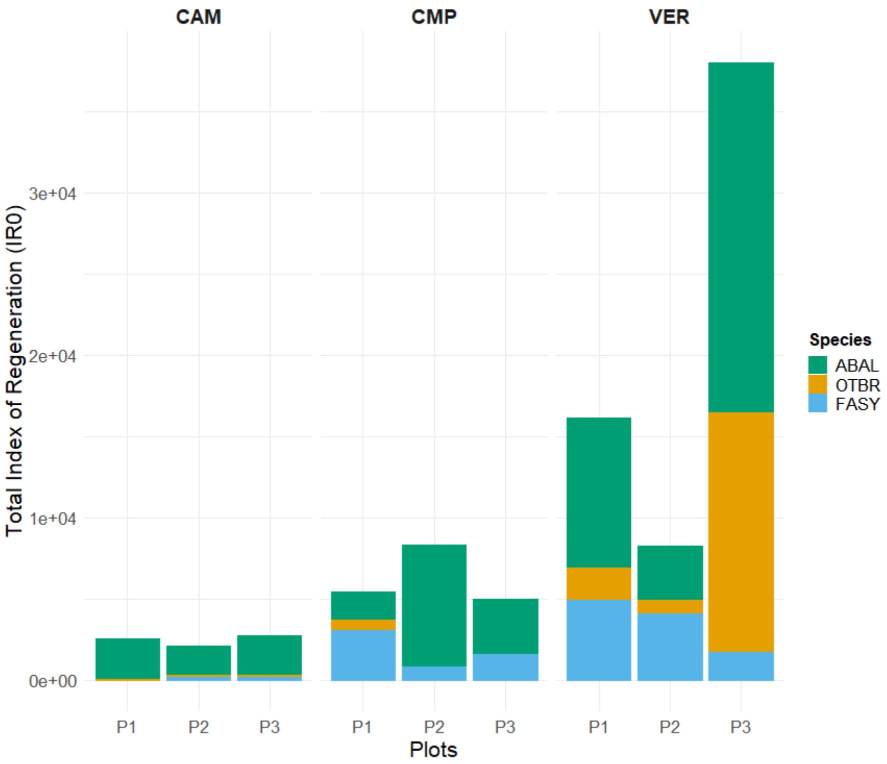

Figure 3. Total Index of Regeneration (IR0) according to tree composition (ABAL, *Abies alba*; FASY, *Fagus sylvatica*; OTBR, other broadleaves). IR1-5 separately reported in Figure S1.

Deadwood presence was highly variable in terms of density and volume between- and within-sites (Figure 4). Deadwood density was on average around 150 elements per ha and less than 48 m^3 ha^{-1}, and originated mainly from silver fir at CAM-CMP and beech at VER. CMP showed high density but low volume of deadwood (particularly in CMP2), especially CWD. CAM had comparable patterns but can be distinguished for exceptionally high SDT volume in CAM2, characterized by many large standing dead fir trees. VER3, instead, distinguished for having a few but large CWD.

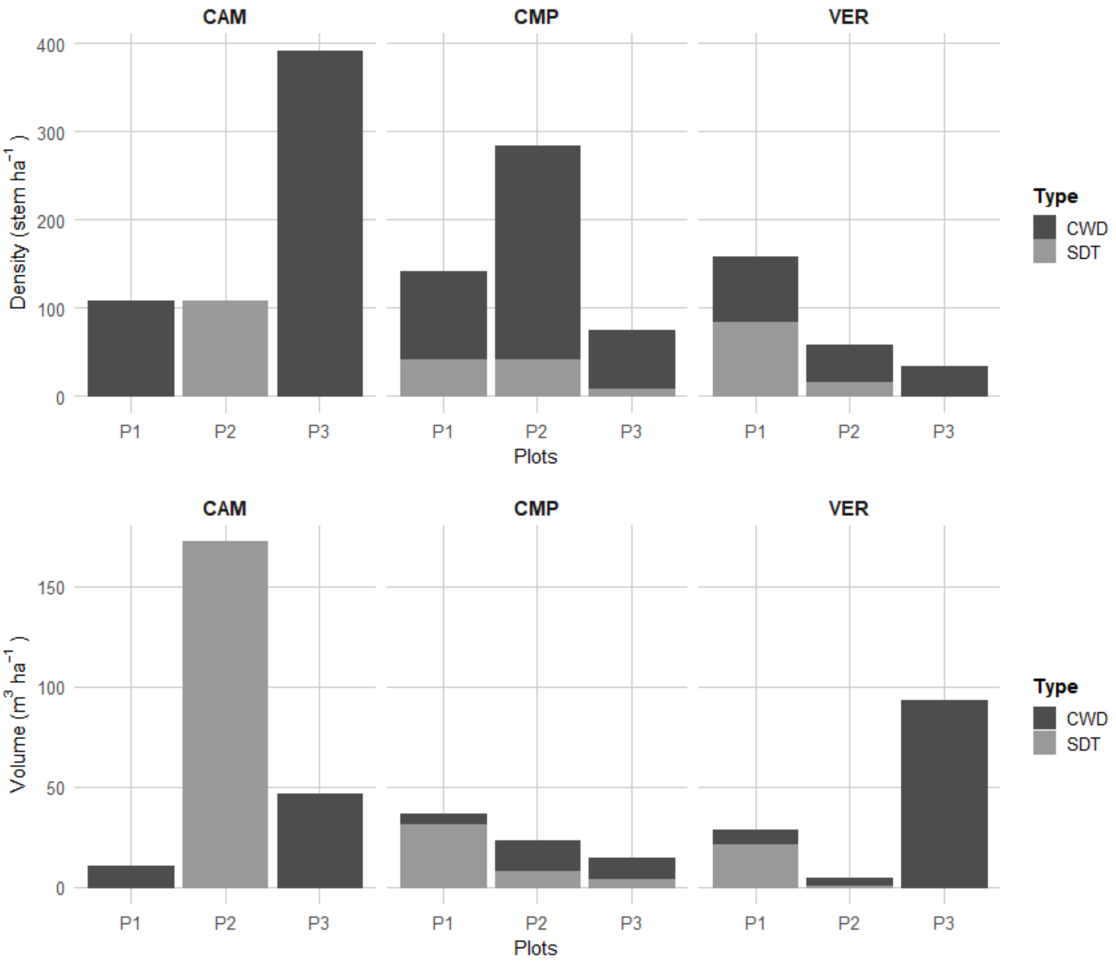

Figure 4. Deadwood density (stem ha^{-1}) and volume (m^3 ha^{-1}) across sites and individual plots (CWD, Coarse Woody Debris; SDT, Standing Dead Trees).

Among all the 102 Structural Indicators (SI) initially calculated to describe forest structure and their old-growthness, a final set of 32 SI were selected through the PCA screening (Figure S2) to group plots in terms of similarity in structural features (Figure 5).

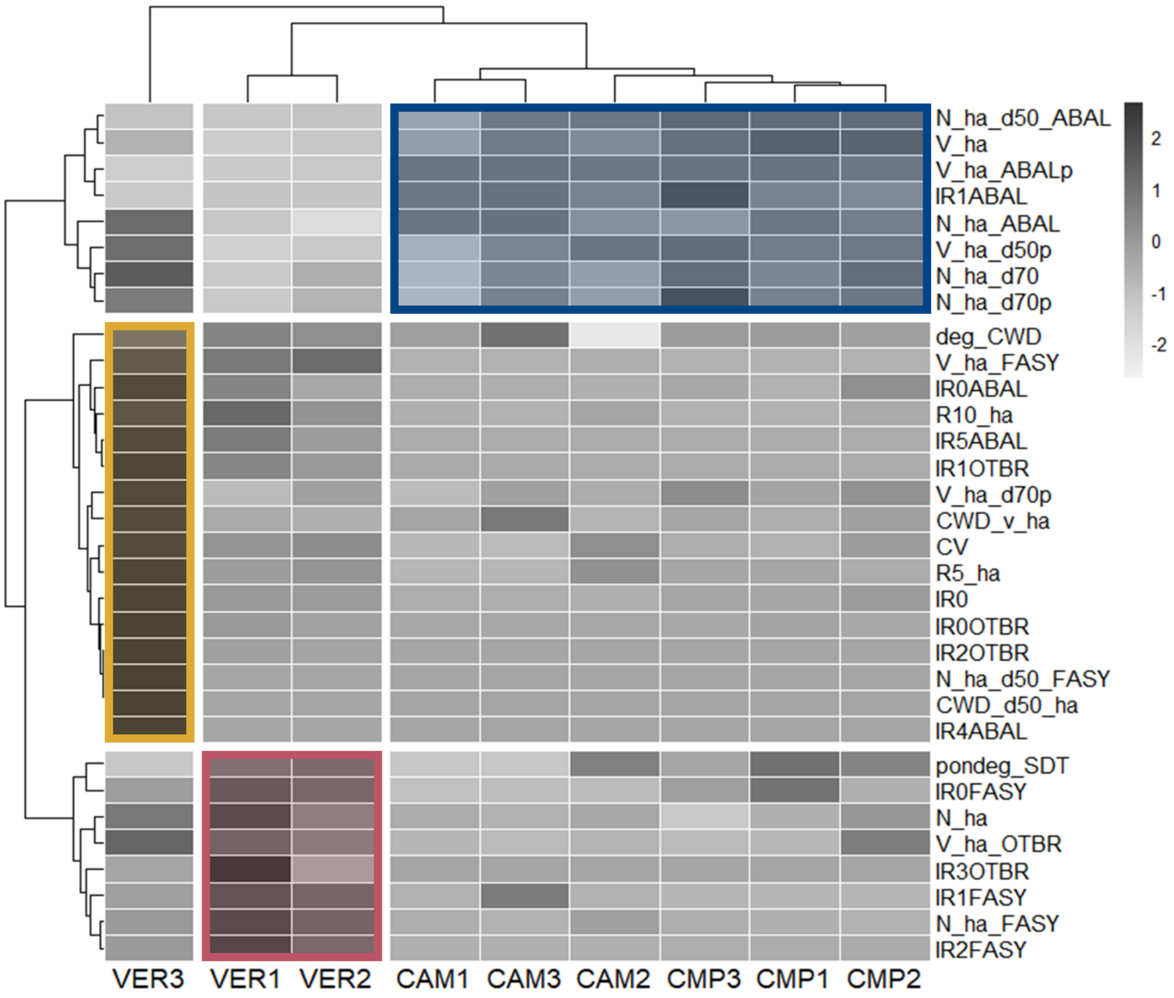

Figure 5. Correlation matrix ordered according to the hierarchical clustering of plots based on selected Structural Indicators (SI). Boxes delineated the main three clusters grouping the plots: higher old-growthness (yellow; VER3); cathedral-like fir forest (blue; CAM 1-3, CMP 1-3); mixed beech stands (red; VER 1-2).

One group (yellow), including only VER3, was characterized by beech dominance and the highest levels of structural complexity and old-growthness: the DBH coefficient of variation (CV); volume and density of large trees (DBH > 70 cm, FASY with DBH > 50 cm); CWD volume with advanced decomposition stages. Furthermore, there was a substantial established regeneration of silver fir (IR4 and IR5, R5 and R10) and smaller cohorts of other broadleaf species (IR0OTBR). Plots VER1 and VER2 (red) were characterized by beech abundance (N_ha_FASY) and high biomass of other broadleaves (V_ha_OTBR), alongside younger (IR1 and IR2FASY) and intermediate (IR3FASY) regeneration often dominated by beech. Lastly (blue), all CMP and CAM plots were dominated by silver fir with cathedral-like structure, i.e. high density of large trees, associated with small silver fir regeneration (IR1ABAL).

3.2. Analysis of Forest Orchid Communities

The forest orchid community showed that, in terms of relative abundance, the genera *Epipactis* and *Neottia* are ubiquitous and the genus *Epipogium* is exclusive of a single site (CMP1; Figure 6). In terms of species, some are rare and exclusively located in individual plots, i.e. *Epipactis microphylla* at CAM3; *E. leptochila*, *E. purpurata*, *Cephalanthera damasonium* and *Epipogium aphyllum* at CMP1; *E. exilis* at VER1 (Figure S3). Each plot contained an average of 3 species, with CMP1 reaching a maximum of 8 species. On average, there were 2 genera per plot, with a maximum of 4 genera coexisting. The average absolute abundance was around 13 individuals per plot, ranging from 54 individuals (CMP1) to 2 (CMP3).

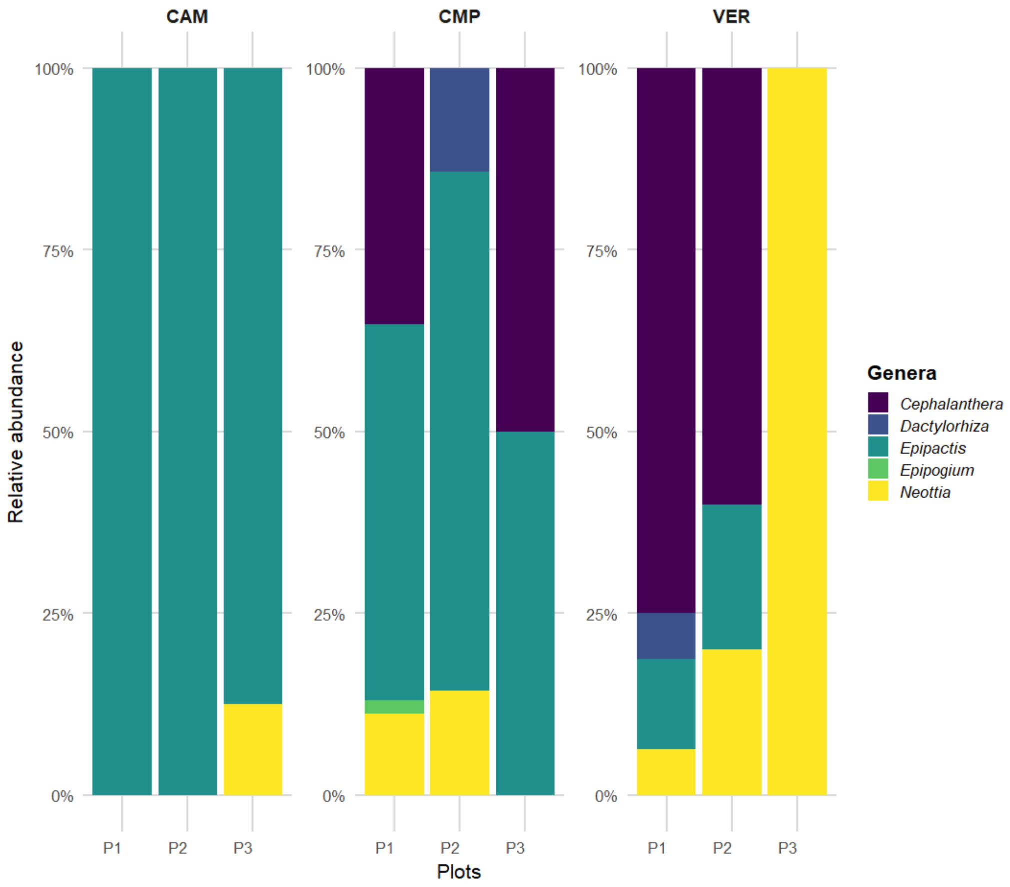

Figure 6. Relative abundance and distribution of *Orchidaceae* genera across sites and plots.

The analysis of the orchid community in terms of trophic strategy (Figure S4) or distribution range (Figure S5) revealed that the most abundant species were mixotrophic and with a wide range. Mycoheterotrophic and narrow distribution species were present at all sites, often with lower abundance (except for VER3). A correlation matrix, ordered according to hierarchical clustering, was conducted to better understand the grouping of the orchid community and plots (Figure 7).

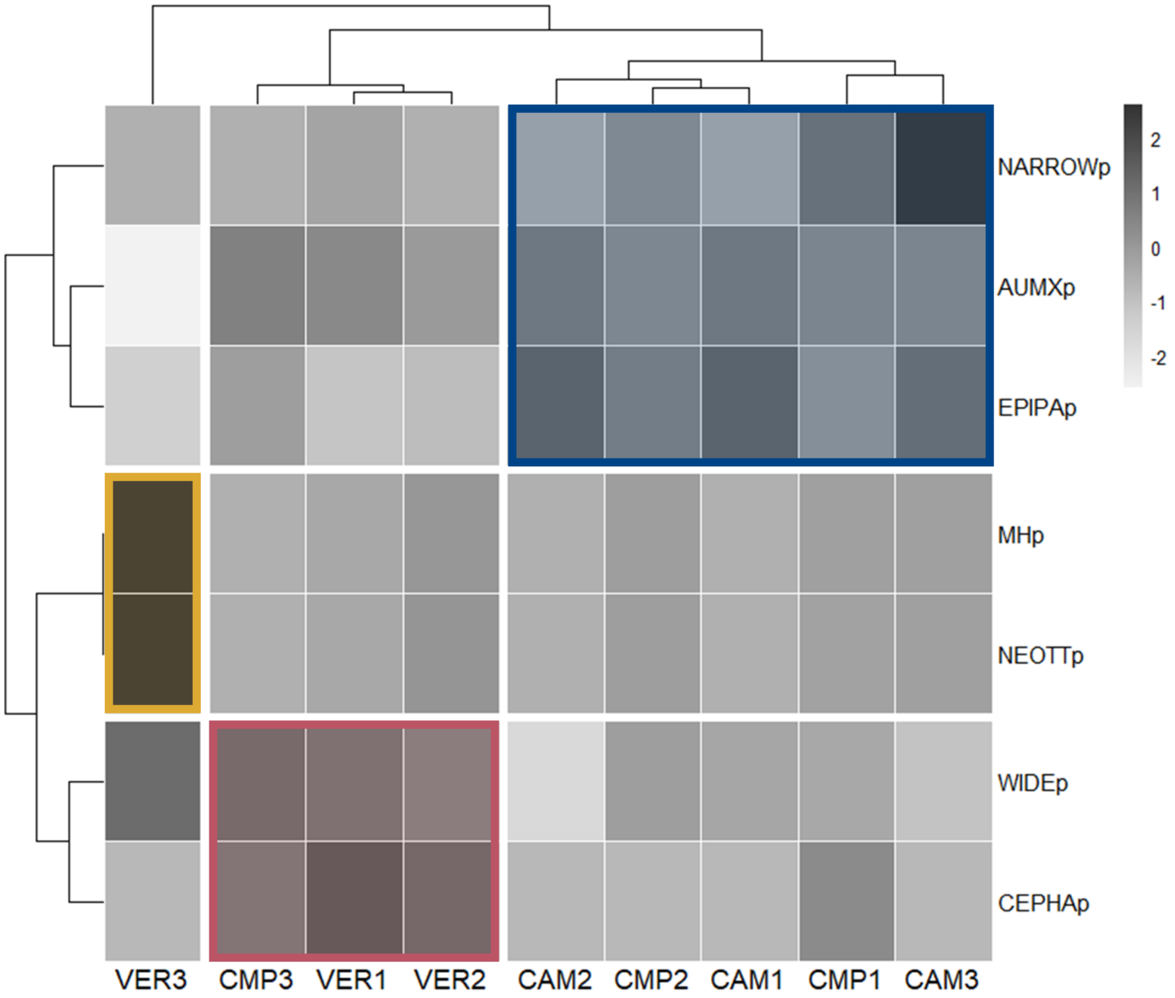

Figure 7. Correlation matrix ordered according to the hierarchical clustering of plots based on the Orchid Community (OC). All orchid variables were calculated as percentage (p) values.

Interestingly, plot clustering according to similarity in orchid communities well reflected the classification made with the structural indicators (SI; Figure 5). The cluster with VER3 (yellow; old-growthness, beech dominance) had a higher presence of mycoheterotrophs (MHp), all represented by the genus *Neottia* (NEOTTp). Most plots (blue; CMP 1-2 and CAM 1-3), including cathedral-like silver fir stands, were characterized by a high abundance of the genus *Epipactis* (EPIPAp) and, only in some plots (especially CAM3), by a higher percentage of narrow range species (NARROWp). Differently from the SI classification, orchid community clustering associated CMP3 (cathedral-like silver fir stands) to VER 1-2 (red; mixed forests with high beech density and regeneration), due to the presence of widely distributed orchids (WIDEp) and the abundance of the genus *Cephalanthera* (CEPHAp). The last two clusters shared also the highest frequency of autotrophic/mixotrophic species (AUMXp).

3.3. Forest Structure and Orchid Community Relations

The joint PCA analysis (Figure 8) made on selected structural indicators and orchid community metrics arranged plots primarily along an increasing gradient of old-growthness (PC1, 64.5% total variance) and then along species composition (PC2, 23.8% total variance). Composition varied from mixed forests dominated by beech to pure silver fir forests. Along the PC1 axis, positive PC1 loadings (VER3) featured higher values of structural complexity and old-growth attributes (e.g., coefficient of variation, deadwood volume, large tree volume) where mycoheterotrophs species (MH), i.e. *Neottia* (NEOTTp), prevailed. Under these conditions, high broadleaved regeneration was accompanied by advanced silver fir regeneration (Figures 2 and 3), whose shadow depressed the establishment of autotrophic-mixotrophic species (AUMXp). Negative PC1 loadings characterized even-aged, biostatic forests with low regeneration (CMP and CAM plots). On the PC2, these plots with negative loadings were dominated by silver fir with a higher frequency of *Epipactis* (EPIPAp) and narrow range species (NARROWp). These were juxtaposed to mixed forests with abundant beech and high beech regeneration (positive loadings, VER 1-2), where the genus *Cephalanthera* (CEPHAp) is widely distributed (WIDEp).

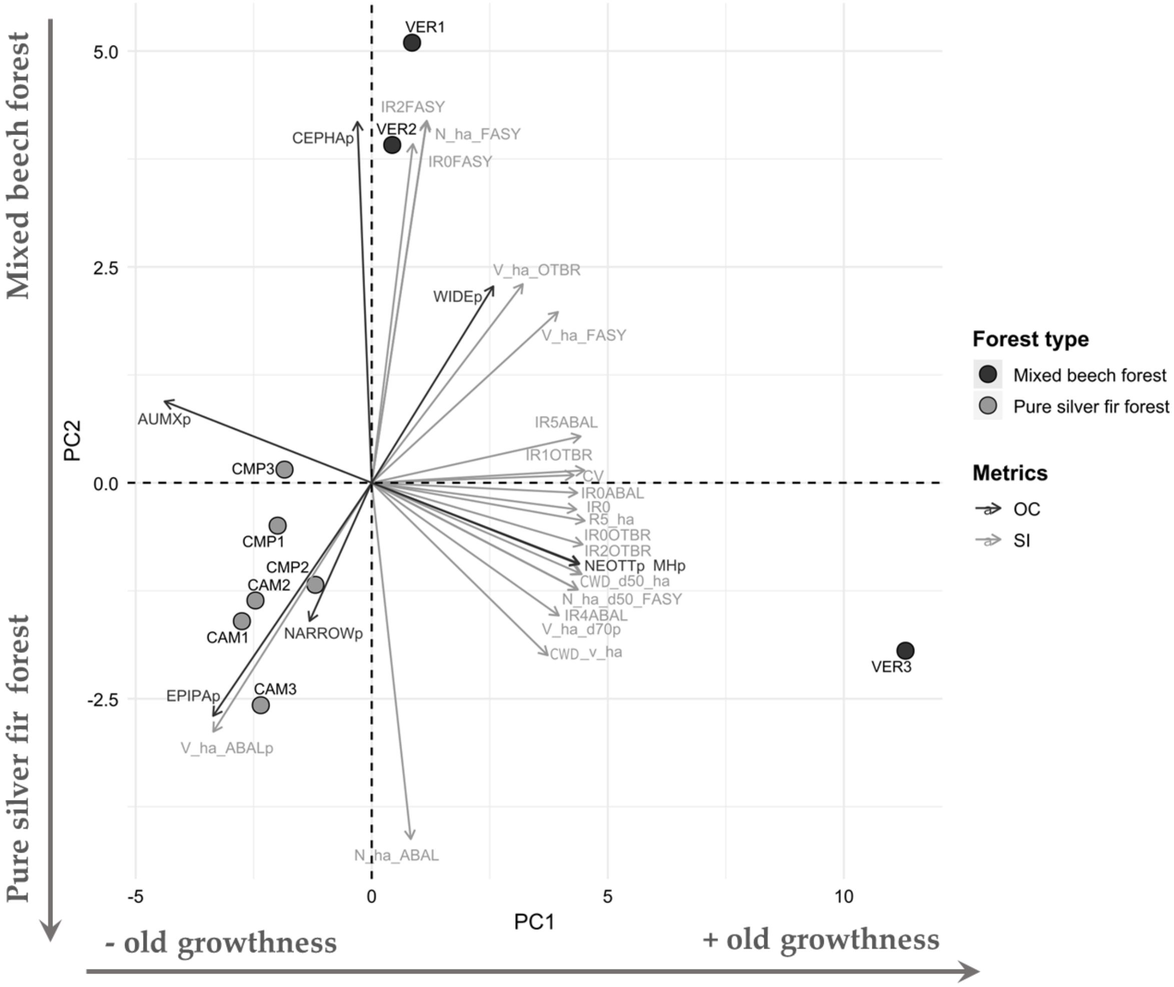

Figure 8. PCA loadings biplot illustrating the relationship between Structural Indicators (SI, gray arrows) and Orchid Community (OC, black arrows) metrics. Dots: plots coloured according to forest type. The PCA explained 88.3% of total variance.

3.3.1. Modelling the Orchid Community According to Forest Features

We developed simple or multiple linear regression models to predict the most important orchid community features using forest structure. Considering the main relationships between orchid communities and forest structure and composition highlighted above, and the most important aspects connected to orchid conservation and restoration (e.g. main genera, trophic strategy, rarity, narrow distribution, red list status; Table 2). However, considering that often single species had many zero values, we investigated how structural variables controlling the relative abundance of the following orchid community features: (1) plot abundance of genus *Epipactis* (6 species; 4 with narrow distribution; one endangered and one nearly-threatened species); plot abundance of genus *Cephalanthera* (3 species); plot abundance of mycoeterotrophs (2 species, one with narrow distribution); plot abundance of narrow distributed species (5 species). In each case, the selection of explanatory variables was made by selecting the one or two most highly correlated SI to that predictor.

The relative abundance of *Epipactis* was mainly predicted by silver fir dominance (Figure 9), confirming the strong dependency of the species of this genus from the conditions found in pure and mature fir stands, where their average abundance was 75% compared to 12% in mixed stands with less than 30% biomass made of fir (t-test: p-value = 0.002).

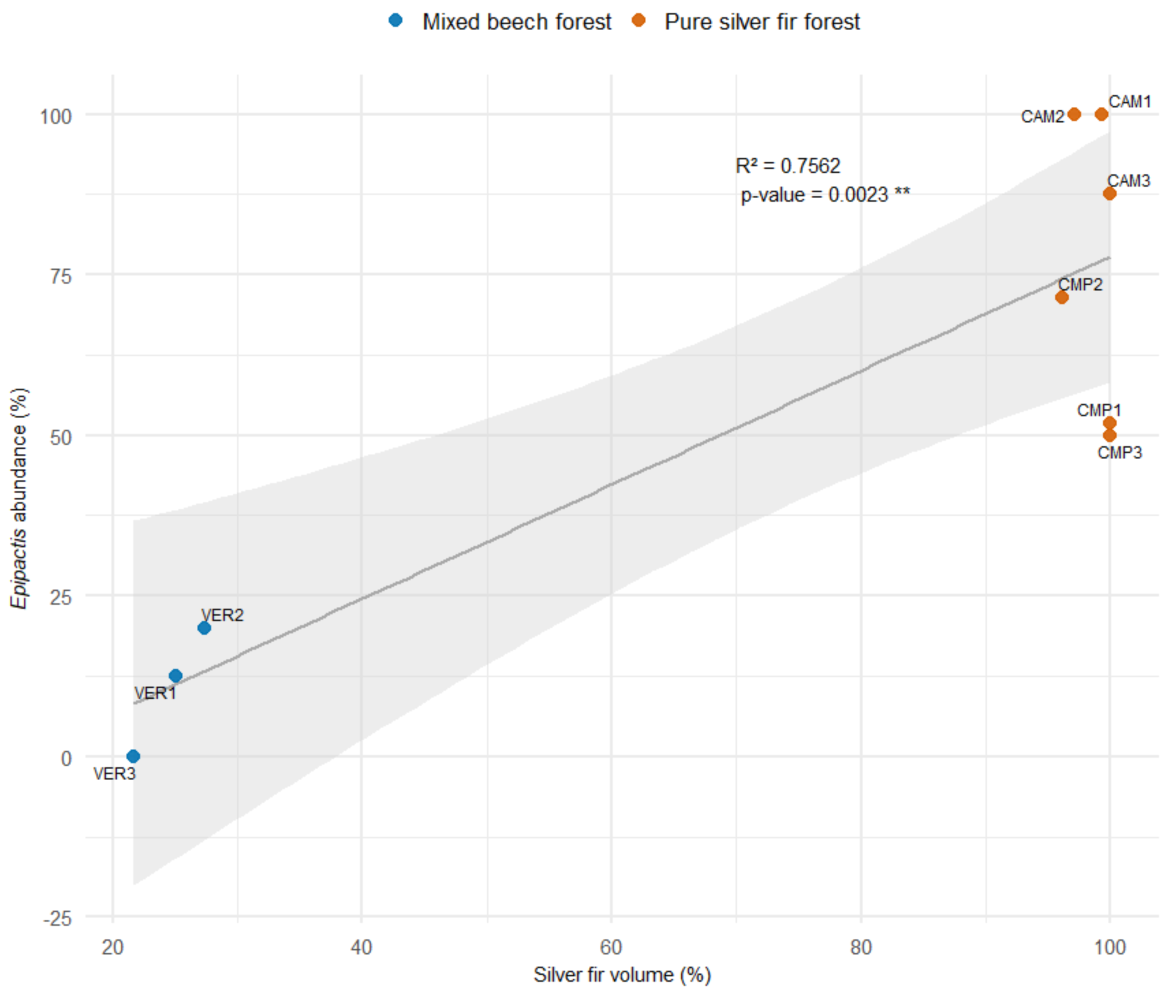

Figure 9. Linear regression modelling the relative abundance of the genus *Epipactis* with silver fir volume at the plot level.

Conversely, the genus *Cephalanthera* was negatively affected by the density of silver fir (Figure 10). Fir density below 250 stem ha^{-1} provided increases of *Cephalanthera* up to 50% of the overall diversity, while values exceeding 350 stem ha^{-1} *Cephalanthera* declined below 30%. This pattern held both for mixed forests (VER 1-2) and low-density fir stands (CMP3). Among mixed forest plot, VER3 was the only one without *Cephalanthera*: this plot had the highest density of established silver fir regeneration (Figures 2 and 3).

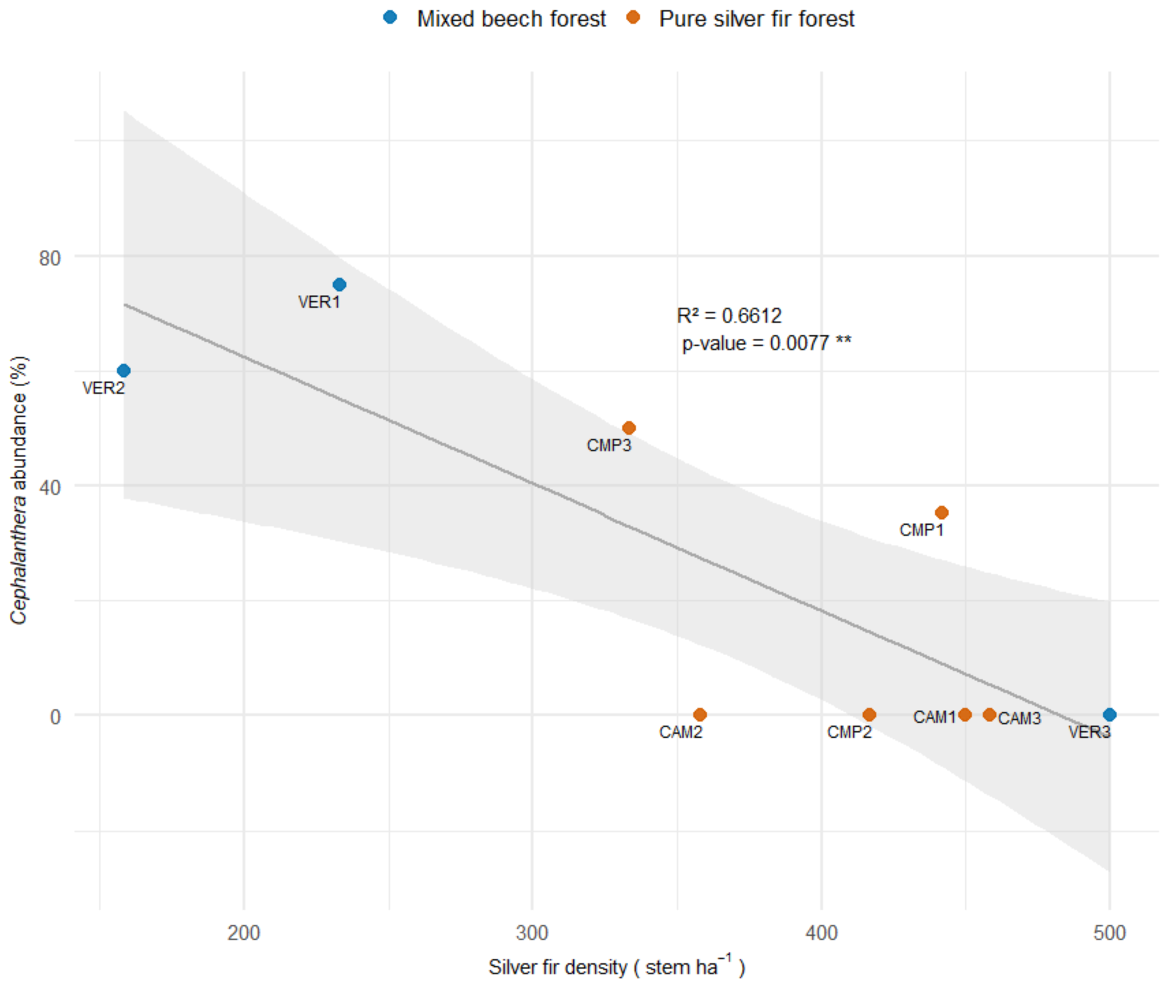

Figure 10. Linear regression modelling the relative abundance of genus *Cephalanthera* and the density of silver fir at the plot level.

The abundance of fully mycoheterotrophic species (*Neottia nidus-avis* and *Epipogium aphyllum*, MH) was directly predicted by the percentage of volume in large trees (DBH > 70 cm) (Figure 11). Mycoheterotrophic species accounted for 0 to 20% of the orchid community if the large tree volume was less than 15%, and increased to 100% in the case with the largest shares (50%). Furthermore, their relative abundance is directly related to by the Coefficient of Variation (CV) and the CWD volume (Figure S6).

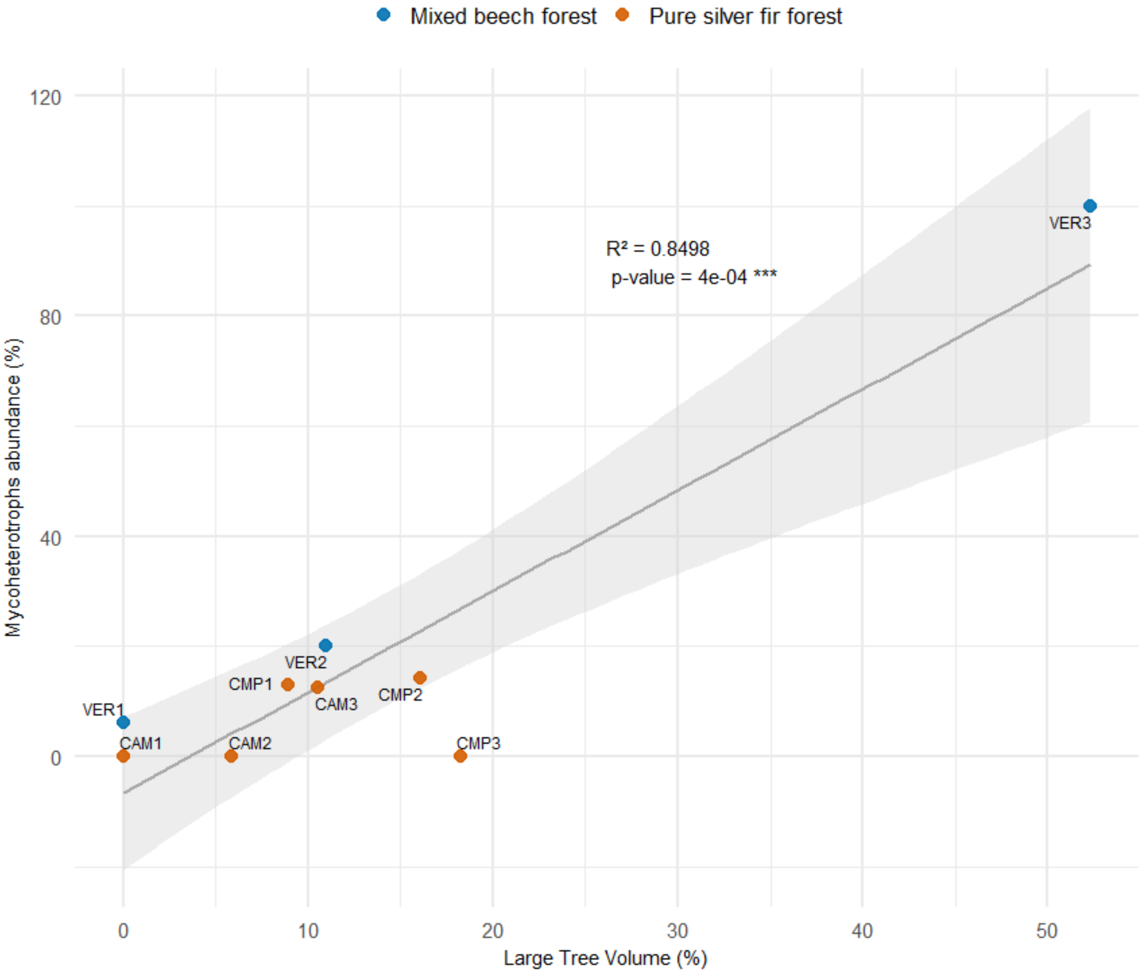

Figure 11. Linear regression modelling the relative abundance of fully mycoheterotrophic species (*Neottia nidus-avis* and *Epipogium aphyllum*) and the volume of large trees (DBH > 70 cm).

Orchid species with limited distribution (*Epipactis exilis*, *E. greuteri*, *E. leptochila*, *E. purpurata* and *Epipogium aphyllum*) were directly related to the cumulative deadwood density (CWD + SDT; Figure 12). The highest values were reached in pure silver fir forest plots, with the maximum in CAM3.

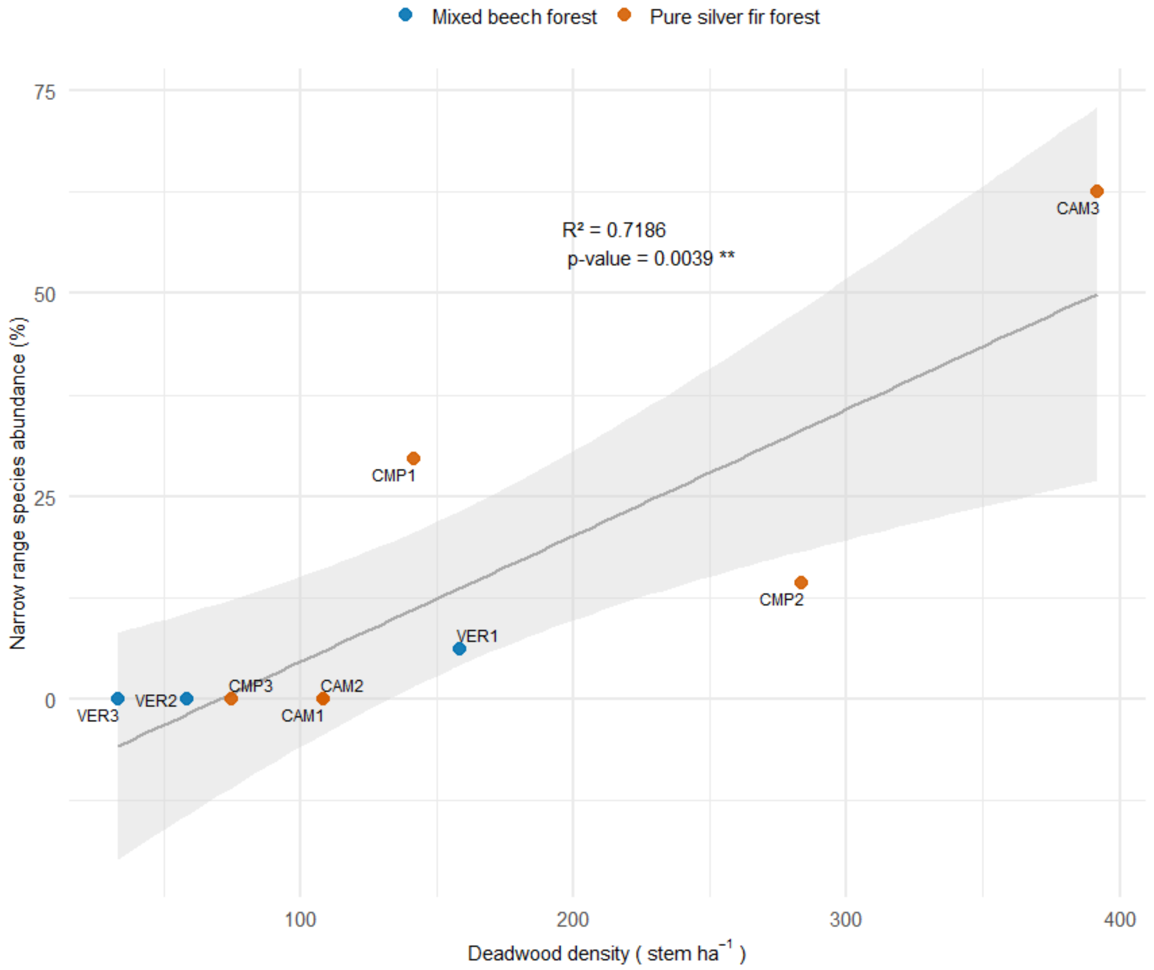

Figure 12. Linear regression modelling the relative abundance of narrow range species and the density of total deadwood (CWD + SDT).

4. Discussion

Forest structure could be related the presence of orchid species in various ways, which must be considered individually. The structure of the stands can be broken down and analyzed into two main components: species composition and old-growthness. The relations between forest orchids and transects should be examined by comparing structural metrics with the groups of species associated with them.

4.1. Tree Composition and Orchid Diversity

Orchid species diversity was highly variable among plots (1 to 8 species) but reached high values both in pure and mixed silver fir forests. In particular, one pure silver fir plot (CMP1) hosted the highest number of orchid species found (8). In general, beech forests host most European forest orchid species [3,112], but a significant number can also be present in conifer forests [3] . It has been observed that, in contexts dominated by broadleaf trees, forest orchids avoid conifer-dominated zones [113], attributing their absence to substrate acidification caused by needle litter [113,114]. The high widespread presence especially of *Epipactis* sp.pl. in our silver fir-dominated sites is therefore noteworthy.

The abundance of the main genera of our orchid communities (*Epipactis* and *Cephalanthera*, 9/12 species found) were anticorrelated and highly dependent on tree composition and light availability. *Epipactis* was the main genus in silver fir-dominated cathedral-like forests, reaching 100% when fir exceeded 90% dominance and preferring even-aged, biostatic tree communities without established

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regeneration and relatively scarce light conditions. Like other forest orchid species, *Epipactis* responds differently to light conditions [3]. Canopy closure has been observed to reduce the reproductive success of *E. helleborine* [115], even if it's possible that shaded conditions promote the production of heavier seeds and therefore higher seedling survival rates [116]. *Epipactis* species are also associated with a wide range of fungal species [26,29,98,117–119]. The initial germination process of *Epipactis* does not necessarily depend on the presence of specific symbionts, and *Epipactis* species with different ecological requirements share similar mycorrhizal partners during germination [120]. A high presence of ectomycorrhizal symbionts associated with silver fir has been confirmed, even in very small areas [121]. Furthermore, large, mature plants of different species can promote high diversity in fungal communities associated with their roots [122], even if simply co-occuring in nearby habitats [119].

Cephalanthera species responded positively to conditions characterized by greater light availability associated to structural heterogeneity and the dominance of beech both in the dominant and regeneration layer, with scarce fir regeneration. In southeastern England, *C. damasonium* was able to colonize rapidly young beech plantations [123]. *C. longifolia* has high photosynthetic efficiency and can benefit from higher light intensity conditions [124]. In *C. damasonium*, carbon gain can increase by 52% when transitioning from a more illuminated forest habitat (open pine forest) to a darker one (dark beech forest) [125–127]. Increased light exposure can lead *Cephalanthera* species (*C. damasonium* and C. *longifolia*) to transition from partially mycoheterotrophy to autotrophy, thereby balancing the use of naturally available carbon [128]. Light response can also affect physiological and reproductive efficiency [3], i.e. reductions in dormancy and the number of flowers due to low light conditions (*C. longifolia* [129,130]) or the reduced fruit set rates caused by dense tree and shrub cover (*C. rubra* [131]).

4.2. Trophic Strategies in Orchids and Old-Growth Attributes

Fully mycoheterotrophic species increased with old-growth features, i.e. higher dominance of large trees (DBH > 70 cm), deadwood (CWD) volume and structural complexity. The best predictions factor was the amount of downed deadwood, confirming that these orchids depend on ground deadwood for carbon [30,118,132], differing in their life cycle from orchids associated with leaf-litter-decaying fungi [133]. Mycoheterotrophs were favoured by deadwood in the form of large logs with advanced decomposition stages (Table 4), which confirmed their preference for habitat integrity [134]. Their presence in several pure fir plots and their prevalence in multilayered forests with established fir regeneration and lower light availability at the ground (i.e. VER3) confirmed the shade tolerance of mycoheterotrophic species [135].

Rare species within the NP responded positively to the overall deadwood density (CWD + SDT), with substantial increases when density was > 100 elements per ha. The total deadwood counted per hectare includes both downed deadwood (CWD) and standing deadwood (SDT) at various decay stages. Contrarily to mycoheterotrophs, rare species were more related to medium/small size deadwood (no need of large, decomposed logs). Almost all rare orchids were autotrophic/mixotrophic *Epipactis*, but included also the mycoheterotroph *Epipogium aphyllum* (only at CMP1). Overall, rare species are present in cathedral-like even-aged silver fir forests (promoting *Epipactis*) with prevailing self-thinning / self-pruning producing small deadwood (promoting *Epipogium aphyllum*). Mycorrhizal formation within specific microhabitats may be difficult and influence orchid abundance [136] and the rarity of terrestrial orchids can become both the cause and consequence of high mycorrhizal specialization [137]. Nevertheless, the biological differences of our rare taxa demonstrated the need for further research to investigate separately and with more detail the ecology of rare species.

According to the variety of the ecology and the trophic strategies possessed by orchid species in forest with silver fir in the National Park, an ensemble of approaches must be implemented to maximize the conservation and promote the restoration of orchid diversity. Specifically, maintaining or restoring tree communities with mixed rather than pure silver fir, with even-aged or complex structure, and with different degree of old-growth attributes may lead to the presence and abundance of some taxa rather than others (Table 4).

The presence of mycoheterotrophs (MH) may be promoted by allowing the development of old-growth conditions that generate large trees (> stems ha^{-1}), a tree community with high coefficient of variation (>) and, especially, substantial deadwood accumulation (> m^3 ha^{-1}) (Table 4). Rare species (localized distribution within the NP) are mainly represented by *Epipactis* sp.pl. and are, therefore, favoured in cathedral-like pure silver fir forests (770-1140 m^3 ha^{-1}, 90-100% silver fir). These conditions were also associated to high density of small deadwood (medium/small size CWD and SDT, c. 200-400 per ha), a factor probably favouring the mycoheterotroph *Epipogium aphyllum*. Such conditions also allowed for hosting the only two globally threatened (e.g., *Epipactis microphylla*, *E. greuteri*) orchids in our dataset, stressing once again the important of conserving pure mature fir stands.

As a preferential habitat for *Epipactis* sp.pl., which comprised 50% orchid species found in this study, pure silver fir mature forests conservation is key in the area. The other main genus, *Cephalanthera*, needed instead opposite conditions, i.e. lower silver fir density (< 350 stems ha^{-1} focus on composition), a significant proportion of beech in the canopy and in the regeneration layer. Forest with a more complex, multilayered structure with sufficient light availability would promote the presence of *Cephalanthera*, especially in areas where *Cephalanthera* species are threatened [138,139].

Table 4. Reference Values of forest attributes (rounded) related to the occurrence and relative abundance of the studied orchid community.

Forest features	**Large Trees**	**CWD**	**Deadwood**	**Silver fir**		**Preferred Forest Structure**
Values	**% Volume**	**Volume (m^3 ha^{-1})**	**Density (stem ha^{-1})**	**% Volume**	**Density (stem ha^{-1})**	
Mycoeterotrophic species (abundance > 25%)	> 20	> 25	-	-	-	Complex, old-growth structure with decaying CWD
Narrow range species (abundance > 25%)	-	-	> 230	-	-	Mature, self-thinning/self-pruning pure fir
Cephalanthera (abundance > 50%)	-	-	-	-	< 250	Angiosperm dominant, scarce fir regeneration
Epipactis (abundance > 50%)	-	-	-	> 70	-	Mature, pure fir

An approach targeting the enhancement of the overall orchid biodiversity, both in terms of abundance and dominance, the ideal forest structure should include a mosaic of structural phases allowing for the presence of mature patches dominated by fir (favouring *Epipactis*) with small deadwood associated to density-dependent mortality (favouring *Epipogium*), uneven-aged patches dominated by angiosperms (favouring *Cephalanthera*) alternated to degradation patches with disturbance-induced mortality of dominant trees generating large deadwood (favouring *Neottia*). Such a structure characterizes natural temperate forests with a mixed disturbance regime characterized by gap-dynamics, that could ideally benefit the entire ecological spectrum of the orchid diversity described [140] In any case, the conservation of orchid communities is closely tied to natural forest processes and the preservation of older forest areas, and the attributes associated to forest age both in mature and old-growth tree communities, such as self-thinning or snag/log abundance [41].

In summary, localized changes in the vertical structure and texture of forests can profoundly impact the presence of certain species. Implementing targeted management practices may facilitate the conservation of specific forest orchid species or groups, depending on conservation goals. This need highlights valuable opportunities for future research that could refine forest management practices, emphasizing the preservation of species that are critically threatened on both European and global scales. Nevertheless, further in-depth studies are essential to better understand the ecology of forest orchids within woodland environments.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Figure S1: Indices of Regeneration (IR1-5); Figure S2: PCA of Structural Indicators (SI); Figure S3: Orchid species abundance; Figure S4: Trophic regime abundance; Figure S5: NP distribution abundance; Figure S6: Mycoeterotrophs linear regressions; Table S1: List of Structural Indicators (SI).

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