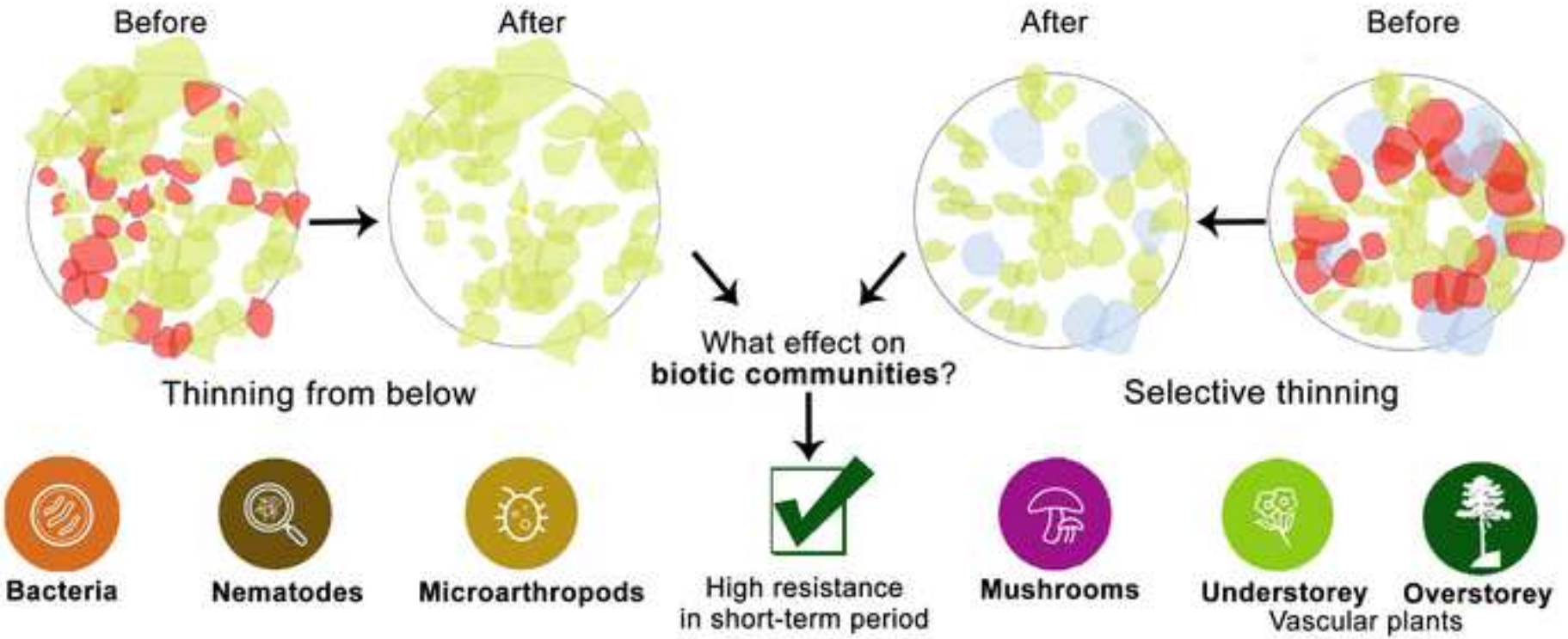
Graphical abstract



Silvicultural management does not affect biotic communities in conifer plantations in the short-term: A multi-taxon assessment using a BACI approach

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Abstract

Biodiversity maintenance is a key strategy for sustainable forestry in both above-ground and below-ground biotic communities. However, few studies applied continuous monitoring to analyse the responses of different taxonomic groups to silvicultural treatments. We studied the short-term effects of three silvicultural treatments (no thinning, thinning from below, and selective thinning) on taxonomic richness and composition in two *Pinus nigra* J.F. Arnold plantations in Tuscany (Italy). We conducted a 1 year before–3 years after control-impact (BACI) experiment with a complete randomized design and analysed the responses of five different taxonomic groups (bacteria, nematodes, microarthropods, mushrooms and vascular plants (overstorey and understorey), along with the patterns of different structural variables. The silvicultural treatments induced a sudden decrease of many parameters such as number of trees per hectare, basal area, and standing volume, with a direct impact on the Photosynthetic Active Radiation on the ground (PAR). Despite this, biological communities showed a high resistance to the tree thinning intensities. Indeed, none of the thinning treatments significantly affected the entire community in the short-term, neither regarding taxonomic richness nor composition. The different taxonomic groups showed a similar, low or null, sensitivity to forest management, and thus a high congruence in their responses.

Keywords: biodiversity monitoring, black pine forests, rehabilitation, stand structure, surrogate, thinning

1. Introduction

Planted forests are about 7% of world's forests (FAO 2020). These are mainly represented by pure and even-aged coniferous plantations, thanks to the low ecological requirements and high profitability of many conifer species (Kenk and Guehne, 2001). Such extensive plantations have a relatively low ecological value, compared to uneven-aged and mixed stands (Felton et al., 2010; Diaci et al., 2019; Deng et al., 2020). Thus, there were several attempts to convert non-native coniferous monocultures to mixed forests dominated by native species, with contrasting results (Knoke et al., 2008; Felton et al., 2010; Deng et al., 2020; Li et al., 2020; Kremer et al., 2021). The European black pine (*Pinus nigra* J.F. Arnold) is a long-lived, fast-growing and pioneer conifer (Kenk and Guenhe, 2001; Vallauri et al., 2002; Enescu et al., 2016; Olsson et al., 2020). It is native to the Mediterranean area, and it is widely used in reforestation practices both in Europe and worldwide (Enescu et al., 2016; Mikulová et al., 2019). The species has been extensively planted across European mountains in the XIX and XX centuries. In many European countries, such as Italy, the species was also used to cover bare soils that were overexploited by agricultural practices and later abandoned, when people moved from rural areas to cities (Marchi et al., 2020). According to the latest Italian National Forest Inventory, black pine forests cover almost 235,000 hectares in the country (2.7% of the whole national forest extension), mainly as pure and even-aged stands (Gasparini and Tabacchi, 2011). Despite the common use of the species across different ecological regions and administrative boundaries, its silviculture has often been neglected over the last decades. In this context, different studies showed that black pine plantations outside the species' native range are more prone to fire and biological invasions (Mikulová et al., 2019). Furthermore, they are affected by several biotic and abiotic stressors such as insect infestations and, more recently, to diseases fostered by climate change (Corona et al., 2009). Consequently, the rehabilitation of stands with a low compositional and structural heterogeneity is crucial to achieve a sustainable management of *P. nigra* planted forests (Muscolo et al., 2017; Diaci et al., 2019).

The rotation period of a planted forest can range between 40 and 90 years, depending on which timber product is needed. Most of Italian black pine plantations are currently too young to have experienced a full-rotation period. Silvicultural management is uncommon in black pine plantations, since the harvested wood generally has a low economic value (Cantiani and Chiavetta, 2015; Marchi et al., 2017). In Italy and elsewhere, the most frequently used silvicultural treatment in *P. nigra* plantations is thinning from below, which is applied to 45-65 year old stands. Such a treatment induces an almost homogeneous increase of the light radiation on the ground, affects the dominant layer by determining discontinuity in the crowns and thus affects the microclimate to a great extent and in a non-spatially homogeneous way (Landi et al., 2020). Recently, an alternative thinning system has been tested in Italy. This system uses the principles of selective thinning to remove the competitors of selected trees, freeing the crowns of "candidate" plants from competition for light (i.e., a positive selection). It recently proved to be effective in increasing stability and

biodiversity in middle-aged (45-50 years old) black pine planted forests, thanks to the reactivity and pioneer character of black pine itself (Cantiani et al., 2010; Cantiani, 2016). In addition, selective thinning was showed to enhance most of the main ecosystem services provided by planted forests in the Italian Apennines (Marchi et al., 2018).

Thinning of *Pinus* plantations, or coniferous plantations in general, may increase plant species richness (Osorio et al., 2009; Marchi et al., 2018). Nevertheless, an excessive thinning may induce relevant changes in plant communities, with results that are not always environmentally positive (Maccherini et al., 2019; Deng et al., 2020). Similarly, recent studies highlighted how the structure of microbial communities and their functional role in forest soils might be sensitive to land-use change and forest management practices (Amoo and Babalola, 2019; Isobe et al., 2020). Fungal communities as well may respond in a negative way to thinning interventions (Muller et al., 2007; Lin et al., 2011; Baral et al., 2015; Lin et al., 2015; Maghnia et al., 2017; Tomao et al., 2020), even though to different extents depending on the intensity of cutting (Bonet et al., 2012). By contrast, several authors reported that thinning did not determine relevant losses in richness and abundance of both nematodes and microarthropods (Huhta et al. 1967; Peck and Niwa, 2005).

The analysis of the responses of different taxonomic groups to forest management is essential to understand the effects of the latter on biodiversity, and to develop species-based surrogates useful for a sustainable management of forest ecosystems (Lindenmayer et al., 2014; Sabatini et al., 2016; Zara et al., 2021). Nevertheless, ~~the~~ most of the recent studies on monitoring rehabilitation or restoration of pine stands are based on the response of a single taxonomic group, usually vascular plants (Vrška et al., 2016; Mikulová et al., 2019; Deng et al., 2020).

In this work, we carried out a multi-taxon analysis to quantify the effects of different thinning practices on biodiversity in two black pine plantations of Tuscany (Central Italy), where black pine was widely introduced for silvicultural purposes outside its native range (Pignatti et al., 2017-2019). We used data coming from the SelpiBioLife (LIFE13 BIO/IT/000282) project, started in 2014 and ended in May 2019. The aim of the project was to assess the effects of different silvicultural practices on structural features, soil microclimate, and soil biodiversity and functioning in *P. nigra* planted forests (Cantiani, 2016). ~~Previously~~In a previous paper about cross-taxon relationship in the year before management application (Barbato et al., 2019), the analysis of recorded SelpiBioLife biotic data ~~recorded in the year before management application~~ revealed a robust covariation in species diversity among different groups of organisms (bacteria, vascular plants, mushrooms, ectomycorrhizae, mycelium, carabids, microarthropods), except for nematodes, which were highly mediated by environmental variables (Barbato et al., 2019). Here, we considered a wide range of organisms as well, belonging to four kingdoms: Bacteria, Fungi (mushrooms), Animalia (nematodes and microarthropods), and Plantae (vascular plants, both understorey and overstorey). Three different treatments (no thinning, thinning from below, and selective thinning) were applied to black pine stands. Then, we conducted a before-

after control-impact (BACI) experiment with continuous monitoring over 4 years (5 years for fungi), to assess the effects of management on biodiversity.

To the best of our knowledge, this is the first multi-taxon, continuous monitoring study assessing the effects of forest management practices on biodiversity using a BACI approach. Our main aim was to test whether species richness and composition of different biotic communities are affected by the treatments, and to highlight if silvicultural practices may cause significant biodiversity changes (either positive or negative) in the investigated communities.

2. Material and methods

2.1 Study areas

The study was carried out in two black pine plantations in Tuscany (central Italy) (Figure 1).

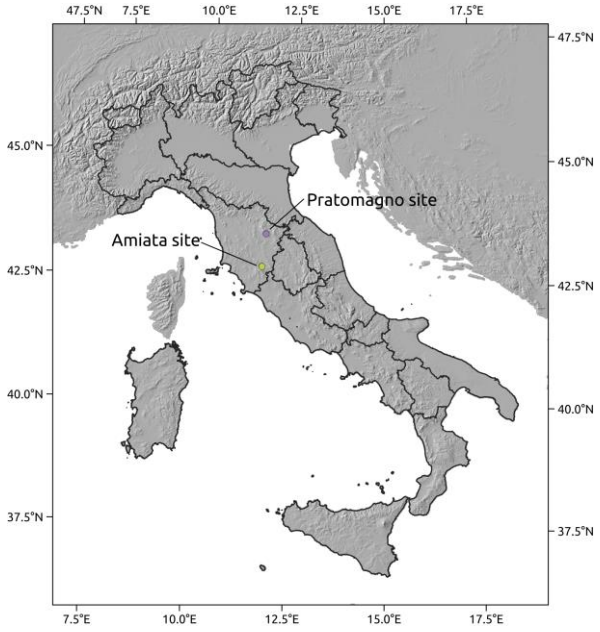


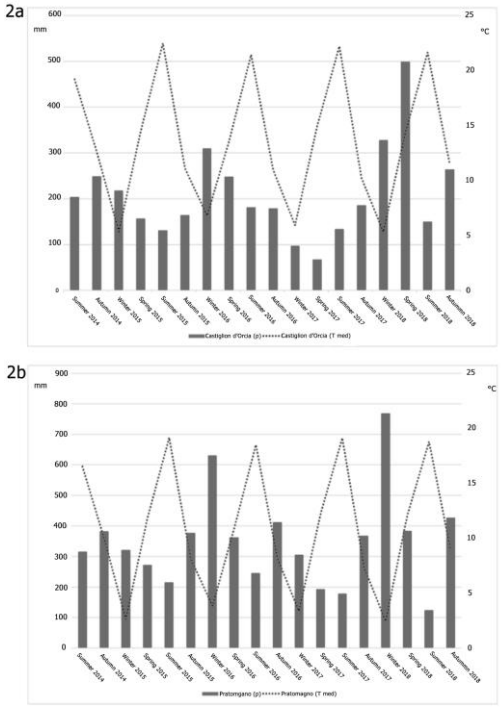
Figure 1. Study area. Location of Amiata and Pratomagno study sites in Tuscany and Italy.

The first plantation (Amiata) is in southern Tuscany (Castiglion d'Orcia, Siena; 42°56'8''N, 11°38'13''E, mean elevation 780 m a.s.l.). It was dominated by 44-year-old black pines (91.3% and 97.3% of total trees/hectare and of basal area/hectare, respectively), accompanied by >10% of *Quercus pubescens* Willd. and *Quercus cerris* L. (Cantiani, 2016). The second plantation (Pratomagno) is in north-eastern Tuscany (Arezzo; 43°35'55.3"N, 11°42'33.9"E, mean elevation 960 m a.s.l. It was dominated by 57 years-old black pines (83.4% of total trees/hectare and 86% of total basal

area/hectare), with scattered *Abies alba* (especially at higher elevations), and occasionally broad-leaved species such as *Fagus sylvatica*, *Fraxinus ornus*, and *Quercus cerris* (Cantiani, 2016).

2.2 Climatic, edaphic, and geological features

We retrieved data on precipitation and temperature for the study years, relatively to the closest climatic stations: Castiglione d'Orcia (Amiata), 544 m a.s.l., and Pratomagno, 695 m a.s.l. (Regione Toscana, 2021) (Figure 2a,b). Seasonal mean temperatures were comparable across the years of the experiment in both the study sites. On the contrary, the total seasonal rainfall had a fluctuating pattern and was particularly low in spring and summer 2017 at both sites (Figure 2a,b). In the Amiata site (Fig. 2a), the average annual temperature was 12.5°C (max: 21.7 °C in July; min: 4.5 °C in January) and the mean annual rainfall was 687 mm, with November being the rainiest month. In the Pratomagno site (Fig. 2b), the mean annual temperature was 10.5°C (max: 19°C in July; min: 1.5°C in January) and the mean annual rainfall was 997 mm, with an absolute peak in autumn and a relative peak in spring.



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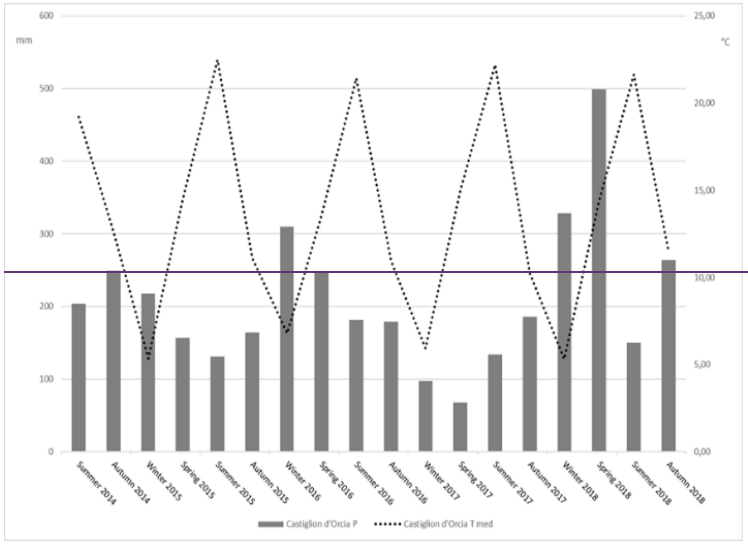


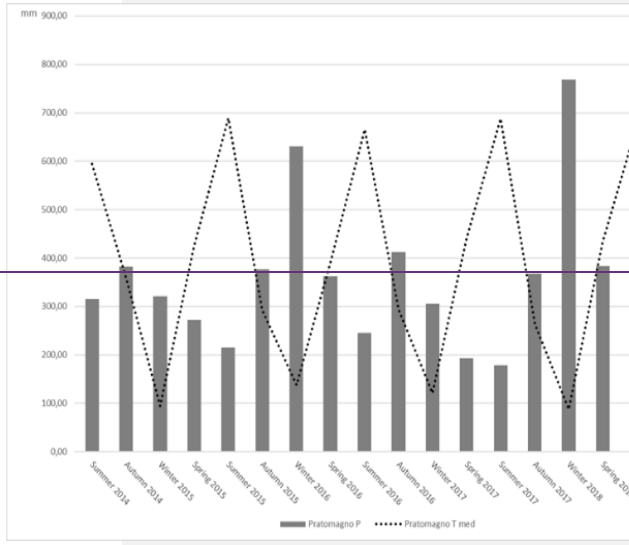


Figure 2a,b. Climatic data. Total seasonal rainfall (p) and seasonal mean temperature (T med) for the two sites (Fig. 2a, Castiglion d'Orcia – Amiata; Fig. 2b ~~and~~ Pratomagno) in the years of observation (2014-2018).

In the Amiata site, geological substrates are characterized by clay, calcareous, and marly lithofacies. Soils are deep and rich in organic matter (average content: 4.7%) in the topsoil "A" horizons (Barbato et al., 2019).

In the Pratomagno site, lithotypes are represented by quartz-feldspar sandstones, alternating with siltstones and argillite. The average organic matter in the topsoil "A" horizons is 6.8% (Barbato et al., 2019). Soils are generally moderately deep, and locally deeper due to strong erosion.

2.3 Experimental design and silvicultural treatments

To test the effects of thinning on biodiversity in *P. nigra* plantations, we carried out a before-after control impact (BACI) experiment over 4 years of monitoring (5 years for fungi – 1 year before, 3-4 years after). The monitoring activity started in 2015 (2014 for fungi) and ended in 2018. In such studies, the BACI approach is especially useful because it allows attributing changes in community diversity to an impact, rather than to natural variability (Stewart-Oaten et al., 1986).

A complete randomized design was established in 2014 in the two plantations. In each site, we selected a stand of 20 ha dominated by black pine, being as homogeneous as possible in terms of features like basal area, tree density, and gaps in the canopy cover (Barbato et al., 2019). In each stand, nine squared macroplots of 1 ha were marked on the ground. Three circular plots were randomly selected in each macroplot, with a total of 27 plots per site, within which we

recorded biological (10 m radius plots or 1 ha macroplot) and structural (15 m radius plots) data (Cantiani and Marchi, 2017). Three different treatments were applied in July 2015 (Marchi et al., 2018):

a) control, no thinning (C).

b) Thinning from below (TB), which removes only dominated, small, or standing dead trees below the main canopy layer, aiming at concentrating the growth on the remaining trees. This is the silvicultural treatment most adopted in Apennine pine forests.

c) Selective thinning (ST), aiming at favouring a certain number of trees with good phenotypic features (target trees or crop trees), to guarantee a high degree of mechanical stability already in the juvenile phase (Klädtke, 2002). Thus, crop trees are those that are wished to reach the end of the rotation cycle, favoured by the removal of their competitors. We selected on average 100 crop trees/hectare.

2.4 Forest features

In each site, the following variables were measured (Landi et al., 2020):

a) Diameter at Breast Height (DBH), i.e., the diameter at a height of 1.30 m, recorded using a diameter measurement tape.

b) Total height, i.e., the height of the first living whorl and the height at maximum crown width, using Vertex III.

c) Mean crown radius, assessed using the vertical sighting method, as the quadratic mean of eight crown radius (Pretzsch et al., 2015).

d) Total Photosynthetically Active Radiation on the ground (PAR, i.e., the total amount of light available for photosynthesis), evaluated using ceptometers (AccuPAR model PAR-80 e LP-80 - Decagon Devices Inc., Pullman, WA, USA).

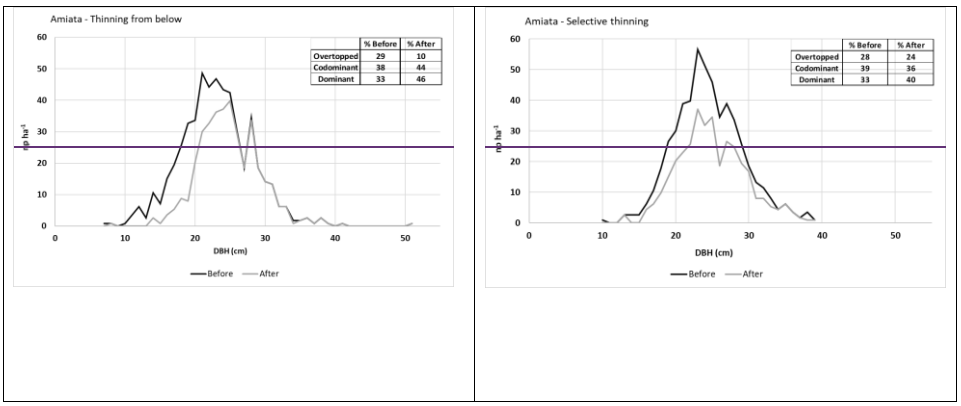
e) Crown radius at crown base, used to calculate the total crown volume, i.e., the space occupied by the canopy (Pretzsch, 2009).

Then, the main dendrometric variables were calculated, such as the total basal area per plot and the total standing volume per plot. The volumes of single trees were calculated using the most recent equations from the Italian national forest inventory (Tabacchi et al., 2011). Each tree was progressively numbered and geo-referenced using a polar coordinate system (horizontal distance from the centre of the circular plot and degrees from the north) by means of FieldMap® technology, and then converted into metric planar coordinates (Cantiani and Marchi, 2017). Descriptive statistics about the main structural variables measured in the year 2015, before and immediately after silvicultural treatments, are shown in Table 1.

		Before treatment					*After treatment*					*Percent removal*		
Site	**Treatment**	**N trees** *n ha^{-1}*	**Basal area** *m^2 ha^{-1}*	**Standing Volume** *m^3 ha^{-1}*	**Quadratic mean** *cm*	**Mean height** *m*	**N trees** *n ha^{-1}*	**Basal area** *m^2 ha^{-1}*	**Standing** *m^3 ha^{-1}*	**Quadratic mean** *cm*	**Mean height** *m*	**N trees** *%*	**Basal area** *%*	**Standing Volume** *%*
AMIATA	*Thinning from below*	971	42.3	357.6	23.7	17.9	675	34	290.8	25.3	18.3	30.4	19.7	18.7
	Selective thinning	971	47.4	446.4	24.9	18.2	638	32.3	309.2	25.4	12-8.4	34.3	31.9	30.7
PRATOMAGNO	*Thinning from below*	1085	72.6	722.3	29.3	19.1	695	56.1	582.9	32.1	19.9	35.9	22.6	19.3
	Selective thinning	1056	66.6	586.6	28.6	18.9	731	47	412.6	28.6	19	30.8	29.4	29.7

Table 1. Descriptive statistics on the main dendrometric variables measured in 2015, before and immediately after the application of silvicultural treatments.

The density distributions and the social ranking of the analysed stands, before and immediately after the application of silvicultural treatments, are shown in Figure 3. Although the proportion of harvested trees in the two study areas was similar for TB and ST treatments (30.4% to 35.9% - Table 1), the two thinning systems had a different impact on the social classes. Namely, the TB treatment had a more intense effect on the dominant trees (overtopped layer) without any influence on the dominant layers (the tallest trees), while the ST treatment had a more extensive effect, with an influence on all the social classes (Figure 3).



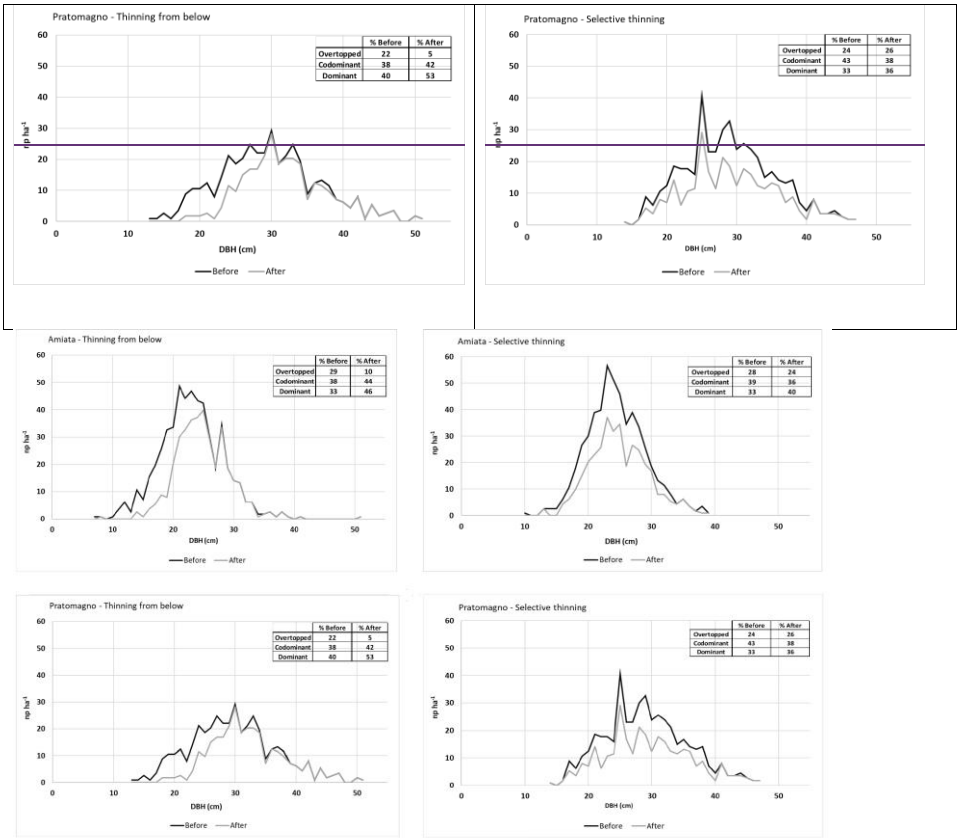


Figure 3. Density distributions and social ranking of the analysed stands, measured in the year 2015, before (black line) and immediately after (grey line) the application of silvicultural treatments.

2.5 Biodiversity sampling

Five different taxonomic groups (bacteria, nematodes, microarthropods, mushrooms and vascular plants) were sampled at plot or macroplot level in the years from 2015 to 2018 (2014 to 2018 for fungi). The organisms were identified to the species (vascular plants and fungi), genus (bacteria), family (nematodes), or order level (microarthropods).

Bacteria were sampled from the soil at the end of May. Five different soil sub-samples were randomly collected from the topsoil (0-20 cm) of each macroplot and homogenized for laboratory analyses. After sieving at 2 mm, total DNA was extracted from 0.5 g of soil using the commercial kit FastDNA™ SPIN Kit for Soil (MP Biomedicals, Santa Ana, California, USA). The structural characterization of the bacterial community was carried out by means of a high-throughput sequencing approach with Miseq Illumina technology (IGA Technology Services s.r.l., Italy), targeting the

16S DNA ribosomal genes with primers 515F and 806R (Caporaso et al., 2012). The structure of the bacterial community was reported as the relative abundance of each taxon, expressed as percentage/0.5 g of soil. Data were processed by using the standard pipeline of QIIME2 (Bolyen et al., 2019).

Nematodes were sampled at the end of May, through three soil samples randomly collected in each macroplot with a hand auger, at a depth of 15 cm in the top layer of bulk soil, after removing surface residues. Samples were then pooled to form a composite sample. Individuals were extracted from 100 ml of soil using the cotton-wood filter method for 48 hours at room temperature (≃ 20°C), counted, and identified.

Microarthropods were sampled at the end of May in each plot, through three soil samples randomly collected using a special 10 cm^3 corer for mesofauna sampling. Soil samples were extracted using modified Berlese-Tullgren funnels and the standard methodology (Parisi et al., 2005). For each sample, the number of individuals was recorded.

Mushrooms were sampled in each plot, where all the above-ground epigeous fruit bodies larger than 1 mm were counted (Arnolds, 1981). The sampling was performed every two weeks during the period of highest fungal production (autumn), and once in spring. Species identification was performed with the usual morphological techniques, general analytical keys, and monographs.

Understorey and overstorey vascular plants were recorded in each plot yearly, in late spring-early summer. The percentage cover of each understorey vascular plant species was visually estimated in the field. The proportion of ground coverage of overstorey plant species was calculated in each plot by processing in QGIS the eight crown radii measured for each tree.

2.6 Statistical analyses

To standardize the observation scale of different taxa, we aggregated all the data collected at the plot level to the macroplot level. For this purpose, we calculated the mean percentage covers as regards vascular plants (separately for overstorey and understorey), whereas we used the sum method for the remnant groups. We obtained a total of 18 sampling units (macroplots), 9 for each site. *P. nigra* occurrence and abundance data were excluded from the analyses.

First, we tested the effect of time (year of observation - fixed, four levels), site (fixed, two levels), and their interactions on macroplot's forest structural features (total height, basal area, mean height, crown volume, standing volume, Quadratic Mean Diameter -QMD-, dominant height, and PAR), separately for each treatment. Then, we tested the effect of the period of observation (fixed, two levels: before (B) and after (A) management), treatment (fixed, three levels: C, ST, and TB), time (year of observation - random nested in period, five levels for fungi or four levels for the other taxa), and site (fixed, two levels) on taxonomic richness and community composition (occurrence and abundance values for each taxon) of the different groups of organisms. Following, we performed uni/multivariate permutational analyses of

variance (PERMANOVA - Anderson, 2001), checking for significant interactions between the period of observation (BA) and the treatment (C, ST, TB), which would imply a press impact of the treatment application (Underwood, 1994). PERMANOVA was based on a Bray–Curtis dissimilarity matrix, calculated from the untransformed bacteria data and ln(x+1)- the transformed abundance data for the other taxa. Regarding the univariate analyses, we calculated an Euclidean distance matrix derived from untransformed data. The analyses were performed using the PERMANOVA routine in the PRIMER v6 computer program (Clarke and Gorley, 2006), including the add-on package PERMANOVA + (Anderson et al., 2008). All tests were performed with 999 permutations of residuals under a reduced model (Anderson and Ter Braak, 2003), considering an alpha level of 0.05. Significant terms relevant to the hypotheses were investigated through *post hoc* pair-wise comparisons using PERMANOVA *t*-test and 999 permutations.

The effects of different treatments on species composition were also checked through the Principal Response Curve technique (PRC, van den Brink and ter Braak, 1998). This technique, derived from the redundancy analysis (RDA) constrained ordination method, plots the principal components of the effects of TB and ST treatments along the period of observation, expressed as deviations from the control treatment (van den Brink and ter Braak, 1998). The significance of the PRC diagrams was tested by means of Monte Carlo permutation tests (999 permutations), permuting whole time series in the partial RDA from which the PRCs were obtained. The PRC analyses were performed in Canoco 5 (ter Braak and Šmilauer, 2012; Šmilauer and Lepš, 2014).

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3. Results

3.1 Forest structural features

None of the variables measured in the control macroplots significantly changed over the years of observation (Tables 2, 3). Tree density, crown volume and basal area significantly decreased in both TB and ST macroplots after management application (Table 2). The PAR increased in all the managed macroplots, while the standing volume decreased only in the ST treatment. The QMD increased in TB macroplots from 2015 to 2017 and stayed constant between 2017 and 2018 (Table 3). The interaction site × year was significant only for mean height values, thus indicating a similar pattern of change over the observation period for the two sites (Table 2).

Source of variation	*df*	**Trees density**						**Basal area**						**Mean height**					
		Thinning from below		Selective thinning		Control		Thinning from below		Selective thinning		Control		Thinning from below		Selective thinning		Control	
		MS	*F*	*MS*	*F*	*MS*	*F*	*MS*	*F*	*MS*	*F*	*MS*	*F*	*MS*	*F*	*MS*	*F*	*MS*	*F*
Site (*Si*)	1	31031	1.81	53372	4.91	29041	0.53	2340.7000	**72.23*** **	410.9300	**12.63** **	2636.6	**26.77*** **	76.96580	**368.86*** **	0.11099	0.93	4.98767	**6.18***
Year	3	148570	**8.68*** **	169200	**15.55*** **	1364.2	0.02	141.5200	**4.37** **	379.3800	**11.66** **	1.9616	0.02	6.7445	**32.33*** **	4.02167	**33.86*** **	0.6605	0.82
Si× Year	3	1073.3	0.06	728.92	0.07	373.59	0.01	21.29860	0.66	22.69860	0.70	2.504959	0.02	3.05459	**14.60*** **	0.42188	**3.53***	0.0340	0.04
Residual	16	17107		10879		54351		32.4050		32.5410		98.4930		0.21086		0.12186		0.8050	
Total	23																		

278 * = P≤0.05; ** = P≤0.01; *** = P≤0.001.

Source of variation	*df*	**Crown Volume**						**Standing volume**						**Quadratic mean diameter (QMD)**					
		Thinning from below		Selective thinning		Control		Thinning from below		Selective thinning		Control		Thinning from below		Selective thinning		Control	
		MS	*F*	*MS*	*F*	*MS*	*F*	*MS*	*F*	*MS*	*F*	*MS*	*F*	*MS*	*F*	*MS*	*F*	*MS*	*F*
Site (*Si*)	1	619700000	**12.94** **	630780000	**12.61** **	288450000	1.95	466810	**144.90*** **	32572	**9.79** **	265840	**22.03*** **	325.22	**136.12*** **	122.42	**66.63*** **	183.48	**20.11** **
Year	3	256450000	**5.35** **	486680000	**9.73** **	82321000	0.56	6955.6	2.16	24913	**7.49** **	1403.4	0.12	10.805	**4.52** **	3.393	1.85	0.832793	0.09
Si × Year	3	4019800	0.08	54279000	1.08	3824600	0.03	2019.5	0.63	1506.8	0.45	190.76	0.02	0.23219	0.10	0.10344	0.06	0.0155	0.00
Residual	16	47903000		50010000		147600000		3221.6		3325.8		12065		2.39892		1.84373		9.1226	
Total	23																		

279 * = P≤0.05; ** = P≤0.01; *** = P≤0.001.

Source of variation	*df*	**Dominant height**						*df*	**PAR**					
		Thinning from below		Selective thinning		Control			Thinning from below		Selective thinning		Control	
		MS	*F*	*MS*	*F*	*MS*	*F*		*MS*	*F*	*MS*	*F*	*MS*	*F*
Site (*Si*)	1	112.42	**1089.2*** **	0.16081	1.01	5.1746	**9.74** **	1	0.0046	0.26	0.0352	2.87	0.01085	2.27
Year	3	0.0720	0.70	0.04379	0.24	0.0405	0.08	2	0.0646	**3.63***	0.13287	**10.48** **	0.00000	0.00
Si × Year	3	0.010568	0.05	0.00423	0.03	0.003850	0.01	2	0.0145	0.81	0.0135	1.10	0.0029	0.79
Residual	16	0.10321		0.165955		0.5312		30	0.02178		0.0123		0.0037	
Total	23					5.1746		35						

280 * = P≤0.05; ** = P≤0.01; *** = P≤0.001.

281 **Table 2.** Results of permutational analyses of variance (PERMANOVA) for dendrometric parameters.

Treatment	Year	N trees per ha (Tree density)	Crown volume	Basal area	Mean height	Photosintetically Active Radiation (PAR)	Standing volume	Quadratic mean diameter (QMD)	Dominant height
		nr ha-1	*m^3 ha^{-1}*	*m^2 ha^{-1}*	*m*	*µmol/(m^2s)*	*m^3 ha^{-1}*	*cm*	*m*
	2015	1034	45006$_{\pm}$40	52$_{\pm}$90	18$_{\pm}$04	0$_{\pm}$13	466$_{\pm}$07	25$_{\pm}$78	20$_{\pm}$80
Control	2016	1025	37781$_{\pm}$92	53$_{\pm}$79	18$_{\pm}$63		494$_{\pm}$72	26$_{\pm}$12	20$_{\pm}$87
	2017	1008	37421$_{\pm}$11	53$_{\pm}$64	18$_{\pm}$69	0$_{\pm}$13	493$_{\pm}$30	26$_{\pm}$35	20$_{\pm}$92
	2018	1001	37609$_{\pm}$34	54$_{\pm}$29	18$_{\pm}$77	0$_{\pm}$13	500$_{\pm}$20	26$_{\pm}$67	20$_{\pm}$99
	2015	a 995	a 42778$_{\pm}$12	a 55$_{\pm}$43	18$_{\pm}$31	a 0$_{\pm}$15	524$_{\pm}$63	a 26$_{\pm}$72	21$_{\pm}$96
Thinning from below	2016	b 699	b 30087$_{\pm}$23	b 45$_{\pm}$95	20$_{\pm}$32		458$_{\pm}$45	ab 28$_{\pm}$9	22$_{\pm}$05
	2017	b 676	b 29534$_{\pm}$7	b 45$_{\pm}$32	20$_{\pm}$42	b 0$_{\pm}$28	452$_{\pm}$54	b 29$_{\pm}$30	22$_{\pm}$12
	2018	b 668	b 29518$_{\pm}$57	b 45$_{\pm}$94	20$_{\pm}$53	b 0$_{\pm}$27	459$_{\pm}$44	b 29$_{\pm}$75	22$_{\pm}$22
	2015	a 950	a 48603$_{\pm}$11	a 54$_{\pm}$75	18$_{\pm}$46	a 0$_{\pm}$10	a 503$_{\pm}$91	27$_{\pm}$17	21$_{\pm}$76
Selective thinning	2016	b 631	b 30524$_{\pm}$35	b 38$_{\pm}$8	19$_{\pm}$98		b 373$_{\pm}$36	28$_{\pm}$03	21$_{\pm}$77
	2017	b 614	b 30551$_{\pm}$81	b 38$_{\pm}$80	20$_{\pm}$08	b 0$_{\pm}$39	b 374$_{\pm}$47	28$_{\pm}$46	21$_{\pm}$84
	2018	b 599	b 30697$_{\pm}$18	b 38$_{\pm}$95	20$_{\pm}$20	b 0$_{\pm}$32	b 377$_{\pm}$4	28$_{\pm}$94	21$_{\pm}$93

Table 3. Main dendrometric features in the years of observation (2015-2018) for the different silvicultural treatments. Different letters (ab) indicate significant differences ($P \leq 0.05$).

3.2 Biodiversity

3.2.1 Taxonomic richness

A total of 936 taxa were recorded (Table 4). As for mushrooms, 52,244 fruiting bodies were analysed. The occurrences of Microarthropods and nematodes were 24,766 and 9,506, respectively. The total taxonomic richness (all the groups of organisms) varied between 247 and 316 among the 18 macroplots, over all the monitoring years (2015-2018, and excluding 2014 for mushrooms). Regarding single groups of organisms, mushrooms showed the highest variation and nematodes showed the lowest variation in taxonomic richness between the poorest and the richest macroplot (Table 4).

Group	Total taxonomic richness	Taxonomic richness, $\bar{x}$ ± SD	Range
Bacteria	262	222 ± 6	212-233
Nematodes	15	6 ± 2	3-10
Arthropods	19	9 ± 2	5-14
Mushrooms	391	34 ± 17	5-80
Vascular plants (understorey)	223	36 ± 13	14-62
Vascular plants (overstorey)	26	4 ± 2	0-10
Total	936	277 ± 17	247-316

Table 4. Total number of recorded taxa (taxonomic richness), average taxonomic richness at the macroplot level (average taxonomic richness, $\bar{x}$ ± SD), and range in taxonomic richness for each group of organisms in the 18 macroplots, for all treatment types (control, selective thinning, and thinning from below) and study years.

The results of the permutational univariate analysis of variance, performed on the number of taxa per macroplot, showed that the year of observation was significant for all taxa, whereas the treatment was significant only for mushroom, nematode, and overstorey plant species richness. Overall, the two sites hosted a different number of bacteria, mushrooms, understorey vascular plants and microarthropods taxa (Table 5). The first-order interaction between period of observation (BA) and treatment was significant only for understorey vascular plants, but the *t*-test revealed that there were no significant differences among treatments in the period after treatment application (C *vs* TB, $t = 0.49$, $P = 0.9$; C *vs* ST, $t = 4.19$, $P = 0.11$; TB *vs* ST, $t = 4.02$, $P = 0.09$). However, the increase in species richness in the ST samples is higher, compared to the TB treatment (Figure 4). The interaction 'period of observation (BA) × treatment × site' was never significant, indicating that there was no difference between the two sites in response to treatment application (Table 5).

Source of variation	*df*	**Bacteria**				**Nematodes**				**Microarthropods**			
		Richness		Multivariate community composition		Richness		Multivariate community composition		Richness		Multivariate community composition	
		MS	*F*	*MS*	*F*	*MS*	*F*	*MS*	*F*	*MS*	*F*	*MS*	*F*
Period (*BA*)	1	2660.7	1.37	697.22	0.36	3.13296	0.18	5079.6	0.93	43.56	2.45	2660.7	0.93
Treatment (*Tr*)	2	412.89	3.13	158.93	**4.05****	12.255	**8.07***	696.75	1.88	2.5988	0.35	412.89	1.23
Site (*Si*)	1	3574.1	**21.33***	25585	**183.02****	0.074074	0.01	919.12	0.54	117.04	**20.45***	3574.1	3.62
Year (*BA*)	2	2871.3	**56.82*****	1928	**18.46*****	17.352	**10.16*****	5432.1	**15.20*****	17.80796	**11.97*****	2871.3	**10.53*****
BA × Tr	2	200.12	0.71	17.9878	0.46587	4.5046	2.97	254.58	0.69	0.64352	0.09	200.12	0.60
BA × Si	1	1353.8	0.94	5.97656	0.043	0.302963	0.05	397.84	0.23	2.0417	0.36	1353.8	1.37
Tr × Si	2	263.54	2.16	205.13	**10.86****	0.376574	0.08	508.96	0.64	1.2917	0.47	263.54	1.61
Tr × Year (BA)	4	335.78	0.57	39.193	0.37	1.52185	0.89	371.33	1.04	7.463	**5.02****	335.78	1.23
Si ×x Year (BA)	2	986.55	**3.74***	139.8	1.34	6.3519	**3.72***	1696.3	**4.75*****	5.7222	**3.85***	986.55	**3.62*****
BA × Tr × Si	2	134.64	0.81	13.954	0.74	0.1713	0.04	278.13	0.35	1.125	0.41	134.64	0.82
Tr × Si × Year (BA)	4	164.06	0.65	18.882	0.18	4.6852	**2.74***	788.65	**2.21****	2.7222	1.83	164.06	0.60
Residual	48	272.66		104.46		1.71083		357.3		1.49861		272.66	
Total	71												

* = P≤0.05; ** = P≤0.01; ***= P≤0.001.

Source of variation	*df*	**Mushrooms**				Source of variation	*df*	**Vascular plants (understorey)**				**Vascular plants (overstorey)**			
		Richness		Multivariate community composition				Richness		Multivariate community composition		Richness		Multivariate community composition	
		MS	*F*	*MS*	*F*			*MS*	*F*	*MS*	*F*	*MS*	*F*	*MS*	*F*
Period (*BA*)	1	11904	1.38	12119	1.66	Period (*BA*)	1	563.89	2.97	2914.7	0.59	2.67667	0.15	1011.1	166.25
Treatment (*Tr*)	2	3686.1	**1.54***	2993.8	**2.28****	Treatment (*Tr*)	2	4.12157	0.56	2870.5	**3.78****	32.6767	**7.54***	6340.1	**1025.5*****
Site (*Si*)	1	33598	**4.73***	39436	**6.66***	Site (*Si*)	1	5370	**28.16***	27225	**12.76***	31.13	5.48	31848	**5322.8***
Year (*BA*)	3	8635.9	**3.22*****	7287.6	**3.81*****	Year (*BA*)	2	190.02	**3.03***	4907.4	**4.46*****	18.1767	**4.45***	6.0818	0.004
BA × Tr	2	1971.3	0.82	1408.9	1.07	*BA × Tr*	2	63.6216	**8.59***	1632.7	2.15	2.17667	0.5	259.26	**41.94****
BA × Si	1	10344	1.46	11461	1.93	*BA × Si*	1	15.042	0.08	3446.2	1.61	6.6852	1.18	616.67	**103.07***
Tr × Si	2	3885.4	1.66	2798.3	**2.02***	*Tr × Si*	2	19.181	4.32	2318.2	**2.51***	1.6852	0.40	4112.7	**673.97*****
Tr × Year (BA)	6	2394.5	0.89	1310.2	0.69	*Tr × Year (BA)*	4	7.41074	0.12	758.32	0.69	4.3333	1.06	6.1823	0.004
Si × Year (BA)	3	7105.1	**2.65*****	5925.2	**3.10*****	*Si × Year (BA)*	2	190.72	3.04	2133.5	**1.94****	5.6852	1.39	5.9832	0.004
BA × Tr × Si	2	2232.3	0.95	1520.6	1.10	*BA × Tr × Si*	2	23.3547	5.25	1386.3	1.50	3.91074	0.92	361.91	**59.31*****
Tr × Si × Year (BA)	6	2341.5	0.87	1384.7	0.72	*Tr × Si × Year (BA)*	4	4.4444	0.07	921.88	0.84	4.2407	1.04	6.1022	0.004
Residual	60	2679.7		1910.5		Residual	48	62.7878		1099.8		4.0833		1433	
Total	89					Total	71								

* = P≤0.05; ** = P≤0.01; *** = P≤0.001.

Table 5. Results of permutational analysis of variance (PERMANOVA) for community composition (multivariate) and richness (number of taxa per macroplot) of all the groups.

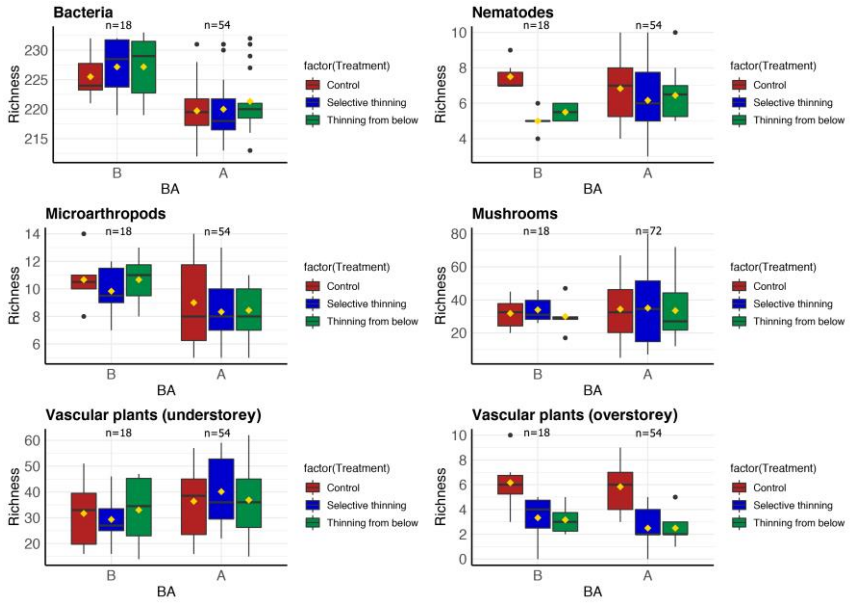

Figure 4. Boxplots of taxonomic richness. Boxplots of number of orders for microarthropods, number of families for nematodes, number of genera for bacteria, and number of species for mushrooms and vascular plants per macroplot, for each treatment in the period before (B) and after (A) silvicultural treatment. Diamonds indicate the mean value per plot.

3.2.2 Community composition

The PERMANOVA performed on community composition showed that the factor 'year of observation' was significant for all taxa except overstorey vascular plants (Table 5). The community compositions of bacteria, vascular plants, and mushrooms were significantly affected by the factor 'site', while the factor 'treatment' affected the composition of all the communities except for nematodes (Table 5). The interactions 'period of observation (BA) × treatment' and 'period of observation (BA) × treatment × site' were significant only for the overstorey plant community (Table 5), but the *t*-test revealed that there were no significant differences among treatments, in the period after treatment application, within the two study sites (Amiata: C *vs* TB, $t = 0.69$, P = 1; C *vs* ST, $t = 1.4$, P = 0.12; TB *vs* ST, $t = 0.93$, P = 0.81; Pratomagno: C *vs* TB, $t = 1.09$, P = 0.42; C *vs* ST, $t = 1.11$, P = 0.3; TB *vs* ST, $t = 1.12$, P = 0.31). A significant effect was also showed by the interactions 'treatment × site' (bacteria, mushrooms, and vascular plants) and by 'site × year' (microarthropods, nematodes, mushrooms, and understorey vascular plants). This indicates that there was a difference among the macroplots belonging to the same treatment between the two study areas, and that the pattern in time of the two sites differed, respectively.

Figure 5 shows the first PRC diagrams, representing the temporal dynamics of communities' composition for each experimental treatment.

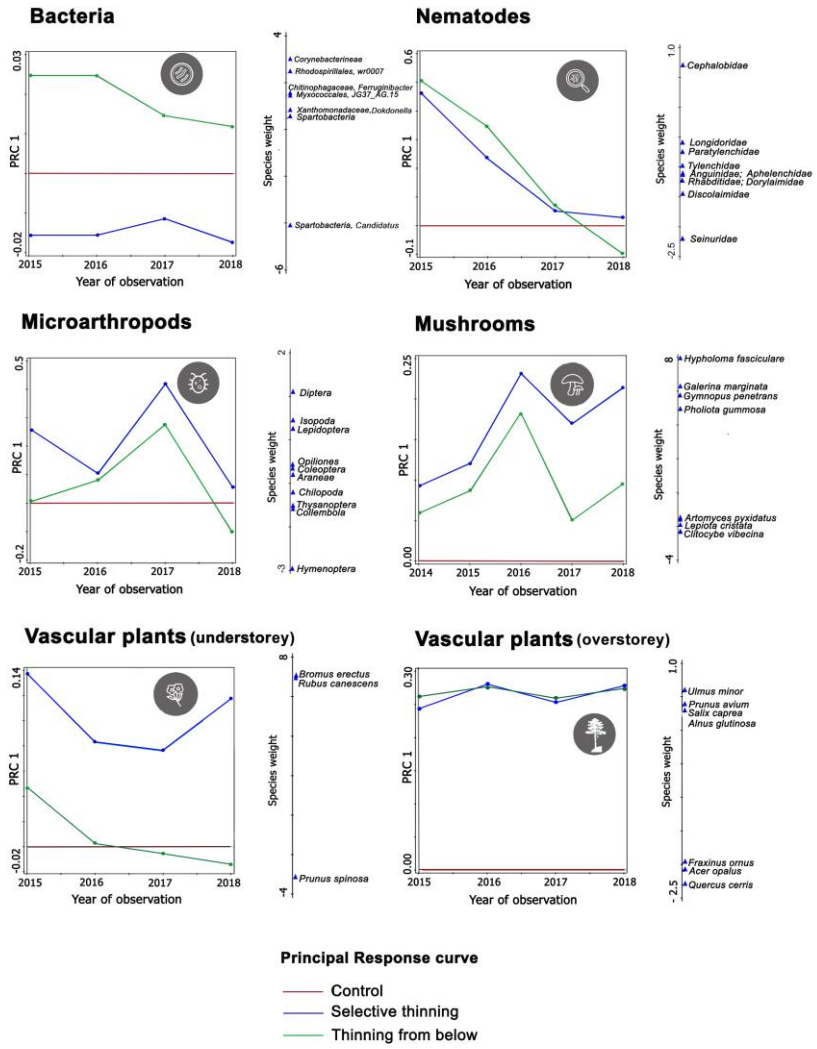

Figure 5. Principal response curves (PRC) for all the taxonomic groups. First principal response curves (PRC) diagram, showing the effects of treatments plus the interaction between year and treatment, on different taxa in *P. nigra* plantations over time. Species weight on the right can be read as the affinity of every taxon with the shown diagram: taxa with high positive values follow the overall community response as indicated by the PRC, while for negative values the community response is the opposite. Zero-lines show the control treatment, the other lines show the course of treated macroplots over time with respect to the control treatment.

The first principal component response was not significant (bacteria: F = 0.4, P = 0.96; mushrooms: F = 1.6, P = 0.94; nematodes: F = 3.3, P = 0.35; overstorey vascular plants: F = 4.1, P = 0.6; understorey vascular plants: F = 2.2, P = 0.73; microarthropods: F = 2, P = 0.79). The percentage of variance explained by treatment regime described by the first PRC was quite high for all the investigated taxa: soil bacteria = 50.9%; understorey vascular plants = 31.8%; overstorey vascular plants = 56.9%; mushrooms = 24,9%, nematodes = 41.6%; microarthropods = 33.5%.

PRC diagrams displayed a similar pattern for bacteria and understorey vascular plants: TB macroplots approached C ones in the year 2018, while the ST treatment diverged (Figure 5). Bacteria such as *Corynebacterineae* and *Rhodospirillales* decreased in TB macroplots in 2017 and 2018 with respect to C macroplots, while *Sparctobacteria* and *Candidatus* showed the opposite pattern (Figure 5). Understorey plant species such as *Bromus erectus* and *Rubus canescens* showed the highest sensitivity to treatment application, decreasing in TB and ST macroplots in the year 2016 with respect to C, but increasing again in ST in the last year of observation. On the other hand, *Prunus spinosa* showed the opposite pattern. Notably, TB macroplots approached C ones after management application. The overstorey plant composition of TB and ST macroplots did not change, in respect to C ones, during the years of observation (Figure 5). Regarding mushrooms, ST macroplots differed from C and TB macroplots in the last year of observation: the number of individuals of *Hypoloma fasciculare*, *Galerina marginata*, *Gymnopilus penetrans*, and *Pholiota gummosa* increased from 2015 to 2016, decreased in 2017, and increased again in 2018 in ST and TB macroplots with respect to C macroplots, while *Artomyces pyxidatus*, *Lepiota cristata*, and *Clitocybe vibecina* showed the opposite pattern. In the same way, ST and BT macroplots for microarthropods showed a fluctuating pattern in the years after management application, with respect to C macroplots: the number of individuals of *Diptera*, *Isopoda*, and *Lepidoptera* increased from 2015 to 2016, decreased in 2017, and increased again in 2018, while *Hymenoptera* showed exactly the opposite pattern (Figure 5). Nematodes showed a progressive increase in similarity to the C macroplots, with the diminishing of the abundance of *Cephalobidae* and the increase of *Seirunidae* in TB and ST macroplots, as compared to C (Figure 5).

4. Discussion

Despite the observed changes in forest parameters such as crown volume and standing biomass generated by both the applied thinning systems, biological communities showed a non-significant shift, with high resistance to forestry practices. In fact, the investigated taxa performed very similarly regardless of silvicultural practices, and consequently a high taxon congruence, as none of the two thinning approaches significantly affected biodiversity, neither regarding taxonomic richness nor the composition of the entire community. Only vascular plants showed a slight tendency regarding species richness and composition. Despite the differences in the environmental and in the structural

characteristics of the two plantations, the applied treatments ~~provoked~~caused a similar response of the different taxonomic groups~~a~~.

The short time between the treatments and post-harvesting surveys does not allow any consideration or inference on the effectiveness of the treatments in increasing the stability of the trees. In fact, evaluations on the effect of thinning on growth trends require at least 15-20 years (Marchi et al., 2018; del Río et al., 2017).

Thinning of *Pinus* plantations increases the structural complexity of forest overstorey, changing the ecological conditions in the lower layers. This may result in an increase of plant species richness (Osorio et al., 2009; Marchi et al., 2018). This evidence is in contrast with some others provided by experiments on the application of clear cutting, where a drastic change in plant species composition was found, with the disappearance in the understorey of *Brachypodium sylvaticum* and a massive encroachment of non-forest species like *Brachypodium rupestre* in short-term monitoring (Maccherini et al., 2019). A big change in forest coverage can also reduce the likelihood of seedling survival and growth, as well as the richness and diversity of shrubs (Deng et al., 2020). From our results, vascular plant richness and composition did not actually change both in the understorey and overstorey, at least three years after management. The establishment of the black pine plantation~~s of black pine~~ deeply changed the environmental conditions of the stand~~s, in~~ compare~~dison with~~ to the ones that would have occurred under native plant communities. F~~f~~rom an~~the~~ edaphic and macroclimatic point of view, the introduction of black pines affect~~eding~~ soil reaction, litter decomposition, moisture, and temperature (Mikulová et al., 2019). The two thinning systems provoked the reduction in soil moisture content and an increase in soil temperature in respect to control sites (Landi et al., 2020) but these changes were ~~have probably not substantially altered the environmental conditions, or at least~~ not enough to cause changes in the vascular plant communities.

Soil bacterial communities are sensitive to any environmental change (Jansson and Hofmockel, 2020). Surprisingly, they can also exhibit an unexpected resilience under biotic/abiotic perturbations, thus maintaining the functioning and stability of natural ecosystems (Awasthi et al., 2014; Mocali et al., 2015). Our results indicated that the overall bacterial richness decreased over time regardless of the treatment, showing that such communities were not significantly affected by thinning practices. However, the PRC diagrams report that the abundance of bacteria in TB macroplots changed over time towards the values of the control C, especially in 2018, whereas the abundances of bacteria in ST macroplots were more stable. *Corynebacteriaceae* and *Rhodospirillales* were the taxa more influenced by management application and those more abundant in TB macroplots. Interestingly, although *Corynebacteriaceae* are commonly detected in both rhizosphere and bulk soils, they have been also found to be strictly associated with plant roots and ectomycorrhizas (Vik et al., 2013). Moreover, despite they have been shown to stimulate basidiospore germination (Ali and Jackson, 1989), they did not show any correlation with mushroom abundance. On the other hand, previous studies indicated that the

family *Rhodospirillaceae* has an important role in fixing molecular nitrogen and a wide variety of photosynthetic bacteria belong to the class *Alphaproteobacteria* and to the order *Rhodospirillales* (Madigan et al., 1984; Wang et al., 2017). Furthermore, the relative abundance of *Rhodospirillaceae* increases with the addition of organic fertilizers and the increase of soil organic matter (Wang et al., 2017). Thus, their lower abundance in ST might also be correlated to the distribution of *Spartobacteria* in TB and ST compared to C. Interestingly, *Spartobacteria*, included within the *Verrucomicrobia* phylum, are characterized by a small genome and a high metabolic versatility, and they prefer soils containing low amounts of labile carbon inputs with a low C/N ratio, and high pH values (Brewer et al., 2016; Shen et al., 2017). We hypothesize that the higher amount of *Spartobacteria* and the opposite distribution of *Rhodospirillaceae* in ST compared to TB and C soils may be linked to the reduced carbon availability in ST macroplots, where higher rainfall and light inputs enhance the microbial mineralization of organic matter, thus depleting the available organic carbon.

In this experiment, contrarily to many previous studies (Muller et al., 2007; Lin et al., 2011; Baral et al., 2015; Lin et al., 2015; Maghnia et al., 2017; Castaño et al., 2018; Tomao et al., 2020), forest management did not have a negative impact on mushrooms communities.~~,~~ ~~w~~We confirm~~ed~~, as reported by previous studies (Landi et al., 2015 and references therein), ~~–~~ ~~that~~ fungi, in forests, are strongly correlated with woody plant communities. PRC diagrams showed that some species increased their abundance in ST and TB macroplots with respect to C ones, in accordance with the findings by Muller et al. (2007) and Parisi et al. (2018). The latter stated that an improvement of forest structural heterogeneity and an accurate assessment of deadwood patterns across spatial and temporal scales are beneficial to wood-inhabiting fungi, that increase in frequency and abundance. By contrast, some species (*Artomyces pyxidatus*, *Lepiota cristata*, and *Clitocybe vibecina*) were negatively affected by thinning, reducing the number of individuals in treated macroplots. This confirms their preference for closed and moist habitats (Bon, 1983; Julich, 1989; Candusso and Lanzoni, 1990). The greatest changes in mushroom abundances in treated plots with respect to control plots occurred in 2017, in correspondence with the driest spring-summer season. Mushrooms are highly influenced by climatic conditions (Boddy et al., 2013) especially in autumn, but also in spring. In fact, spring is the period of mycelium growth, which influences the production of fruiting bodies in autumn (Salerni et al., 2002; Boddy et al., 2013).

Microarthropod communities were weakly affected by thinning, although time (years under evaluation) and the interaction 'year × treatment' were significant. In accordance with the findings of Landi et al. (2020) for the same dataset (years under evaluation: 2016 to 2018), a great variability in microarthropod abundance over time is a consequence of changes in soil temperature and moisture. The highest total abundance of individuals was detected in a drought year (2017), especially when plots were thinned (Landi et al., 2020). Changes in soil conditions are the main factor influencing microarthropod communities (Parisi et al., 2005; Mocali et al., 2015). In contrast with the findings of

several authors (Huhta et al., 1967; Bird et al., 2004), mites and springtails were dominant in terms of number of individuals and were little affected by thinning. On the contrary, less represented taxa were more sensitive to the treatments. Hymenoptera, that usually live on the soil surface, were negatively affected by thinning. This behaviour was also observed for other epi-edaphic microarthropods, as previously described by Landi et al. (2020). By contrast, Diptera were positively influenced by the forest condition created after thinning. As reported by Huhta et al. (1967) and Landi et al. (2020), most of hemi-edaphic species tend to decline because of thinning practice, but some increase in abundance, since they find optimal ecological conditions.

Nematode richness was poorly affected by thinning practices, in accordance with previous findings (Landi et al., 2020). Landi et al. (2020) detected changes in the composition of nematode communities, which however revealed to be temporary, in accordance with previous findings and comparably to the effects of clear cutting (Forge and Simar, 2001; Sohlenius, 2002). Our results showed that the changes in community composition after the treatment were not significant. From a perspective of trophic level, bacterial and fungal feeders were little affected by thinning, except for *Cephalobidae*, which increased constantly in ST and TB macroplots over the years of observation. Instead, omnivores and predators (especially *Seinuridae*) decreased in treated macroplots compared to C ones, due to their low colonization ability and to their high sensitivity to disturbance (Bongers, 1990).

5. Conclusion

The main aim of silvicultural treatments is to modify the structure of forest stands, possibly fostering positive effects, operating on trees within the forest. The two tested treatments represent the main current management options for *P. nigra* reforestations in Italy, and both are allowed by the Italian law. Our continuous monitoring of forest structural features and of biological data, the first of its kind using a BACI approach, revealed that both the silvicultural treatments can be safely applied without relevant impacts on biodiversity in a short-term period. The vascular plant, soil bacteria, mushroom, microarthropod, and nematode communities showed a high resistance to forestry treatments and thus a similar response, regarding either species richness or the entire community's composition. Nevertheless, some taxa within the four kingdoms showed a higher sensitivity to the silvicultural management when combined with the influence of climatic patterns, and in particular with the spring and summer drought of 2017. ~~In~~With respect to ~~this regard we~~this, our results ~~emphasised~~highlighted the importance of multi-taxo~~na~~ studies and of long-term monitoring to confirm or not the~~is~~ observed patterns, also in the light of the evidence that unexpected climatic events~~, that~~ interact with management in ~~a~~ different ways, as already noted in the analysis of a longer time-series data by Chiarucci et al.~~,~~ (2007) and Maccherini et al. (2018).

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~~Given the high congruence in the response to the treatments between the several groups of organisms, our results suggest a possible use of only one of them as a surrogate of the total biodiversity and of its dynamics.~~

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Table captions

Table 1. Descriptive statistics on the main dendrometric variables measured in 2015, before and immediately after the application of silvicultural treatments.

Table 2. Results of permutational analyses of variance (PERMANOVA) for dendrometric parameters.

Table 3. Main dendrometric features in the years of observation (2015-2018) for the different silvicultural treatments. Different letters (ab) indicate significant differences ($P \leq 0.05$).

Table 4. Total number of recorded taxa (taxonomic richness), average taxonomic richness at the macroplot level (average taxonomic richness, $\bar{x} \pm$ SD), and range in taxonomic richness for each group of organisms in the 18 macroplots, for all treatment types (control, selective thinning, and thinning from below) and study years.

Table 5. Results of permutational analysis of variance (PERMANOVA) for the community composition (multivariate) and richness (number of taxa per macroplot) of all the groups.

Figure captions

Figure 1. Study area. Location of Amiata and Pratomagno study sites in Tuscany and Italy.

Figure 2a,b. Climatic data. Total seasonal rainfall (p) and seasonal mean temperature (T med) for the two sites (Fig. 2a, Castiglion d'Orcia – Amiata; Fig. 2b ~~and~~ Pratomagno) in the years of observation (2014-2018).

Figure 3. Density distributions and social ranking of the analysed stands, measured in the year 2015, before (black line) and immediately after (grey line) the application of silvicultural treatments.

Figure 4. Boxplot of taxonomic richness. Boxplots of number of orders for microarthropods, number of families for nematodes, number of genera for bacteria, and number of species for mushrooms and vascular plants per macroplot, for each treatment in the period before (B) and after (A) silvicultural treatment. Diamonds indicate the mean value per plot.

Figure 5. Principal response curves (PRC) for all the taxonomic groups. First principal response curves (PRC) diagram, showing the effects of treatments plus the interaction between year and treatment, on different taxa in *P. nigra* plantations over time. Species weight on the right can be read as the affinity of every taxon with the shown diagram: taxa with high positive values follow the overall community response as indicated by the PRC, while for negative values the community response is the opposite. Zero-lines show the control treatment, the other lines show the course of treated macroplots over time with respect to the control treatment.

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Silvicultural management does not affect biotic communities in conifer plantations in the short-term: A multi-taxon assessment using a BACI approach

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Abstract

Biodiversity maintenance is a key strategy for sustainable forestry in both above-ground and below-ground biotic communities. However, few studies applied continuous monitoring to analyse the responses of different taxonomic groups to silvicultural treatments. We studied the short-term effects of three silvicultural treatments (no thinning, thinning from below, and selective thinning) on taxonomic richness and composition in two *Pinus nigra* J.F. Arnold plantations in Tuscany (Italy). We conducted a 1 year before–3 years after control-impact (BACI) experiment with a complete randomized design and analysed the responses of five different taxonomic groups (bacteria, nematodes, microarthropods, mushrooms and vascular plants (overstorey and understorey), along with the patterns of different structural variables. The silvicultural treatments induced a sudden decrease of many parameters such as number of trees per hectare, basal area, and standing volume, with a direct impact on the Photosynthetic Active Radiation on the ground (PAR). Despite this, biological communities showed a high resistance to the tree thinning intensities. Indeed, none of the thinning treatments significantly affected the entire community in the short-term, neither regarding taxonomic richness nor composition. The different taxonomic groups showed a similar, low or null, sensitivity to forest management, and thus a high congruence in their responses.

Keywords: biodiversity monitoring, black pine forests, rehabilitation, stand structure, surrogate, thinning

1. Introduction

Planted forests are about 7% of world's forests (FAO 2020). These are mainly represented by pure and even-aged coniferous plantations, thanks to the low ecological requirements and high profitability of many conifer species (Kenk and Guehne, 2001). Such extensive plantations have a relatively low ecological value, compared to uneven-aged and mixed stands (Felton et al., 2010; Diaci et al., 2019; Deng et al., 2020). Thus, there were several attempts to convert non-native coniferous monocultures to mixed forests dominated by native species, with contrasting results (Knoke et al., 2008; Felton et al., 2010; Deng et al., 2020; Li et al., 2020; Kremer et al., 2021). The European black pine (*Pinus nigra* J.F. Arnold) is a long-lived, fast-growing and pioneer conifer (Kenk and Guenhe, 2001; Vallauri et al., 2002; Enescu et al., 2016; Olsson et al., 2020). It is native to the Mediterranean area, and it is widely used in reforestation practices both in Europe and worldwide (Enescu et al., 2016; Mikulová et al., 2019). The species has been extensively planted across European mountains in the XIX and XX centuries. In many European countries, such as Italy, the species was also used to cover bare soils that were overexploited by agricultural practices and later abandoned, when people moved from rural areas to cities (Marchi et al., 2020). According to the latest Italian National Forest Inventory, black pine forests cover almost 235,000 hectares in the country (2.7% of the whole national forest extension), mainly as pure and even-aged stands (Gasparini and Tabacchi, 2011). Despite the common use of the species across different ecological regions and administrative boundaries, its silviculture has often been neglected over the last decades. In this context, different studies showed that black pine plantations outside the species' native range are more prone to fire and biological invasions (Mikulová et al., 2019). Furthermore, they are affected by several biotic and abiotic stressors such as insect infestations and, more recently, to diseases fostered by climate change (Corona et al., 2009). Consequently, the rehabilitation of stands with a low compositional and structural heterogeneity is crucial to achieve a sustainable management of *P. nigra* planted forests (Muscolo et al., 2017; Diaci et al., 2019).

The rotation period of a planted forest can range between 40 and 90 years, depending on which timber product is needed. Most of Italian black pine plantations are currently too young to have experienced a full-rotation period. Silvicultural management is uncommon in black pine plantations, since the harvested wood generally has a low economic value (Cantiani and Chiavetta, 2015; Marchi et al., 2017). In Italy and elsewhere, the most frequently used silvicultural treatment in *P. nigra* plantations is thinning from below, which is applied to 45-65 year old stands. Such a treatment induces an almost homogeneous increase of the light radiation on the ground, affects the dominant layer by determining discontinuity in the crowns and thus affects the microclimate to a great extent and in a non-spatially homogeneous way (Landi et al., 2020). Recently, an alternative thinning system has been tested in Italy. This system uses the principles of selective thinning to remove the competitors of selected trees, freeing the crowns of "candidate" plants from competition for light (i.e., a positive selection). It recently proved to be effective in increasing stability and

biodiversity in middle-aged (45-50 years old) black pine planted forests, thanks to the reactivity and pioneer character of black pine itself (Cantiani et al., 2010; Cantiani, 2016). In addition, selective thinning was showed to enhance most of the main ecosystem services provided by planted forests in the Italian Apennines (Marchi et al., 2018).

Thinning of *Pinus* plantations, or coniferous plantations in general, may increase plant species richness (Osorio et al., 2009; Marchi et al., 2018). Nevertheless, an excessive thinning may induce relevant changes in plant communities, with results that are not always environmentally positive (Maccherini et al., 2019; Deng et al., 2020). Similarly, recent studies highlighted how the structure of microbial communities and their functional role in forest soils might be sensitive to land-use change and forest management practices (Amoo and Babalola, 2019; Isobe et al., 2020). Fungal communities as well may respond in a negative way to thinning interventions (Muller et al., 2007; Lin et al., 2011; Baral et al., 2015; Lin et al., 2015; Maghnia et al., 2017; Tomao et al., 2020), even though to different extents depending on the intensity of cutting (Bonet et al., 2012). By contrast, several authors reported that thinning did not determine relevant losses in richness and abundance of both nematodes and microarthropods (Huhta et al. 1967; Peck and Niwa, 2005).

The analysis of the responses of different taxonomic groups to forest management is essential to understand the effects of the latter on biodiversity, and to develop species-based surrogates useful for a sustainable management of forest ecosystems (Lindenmayer et al., 2014; Sabatini et al., 2016; Zara et al., 2021). Nevertheless, most of the recent studies on monitoring rehabilitation or restoration of pine stands are based on the response of a single taxonomic group, usually vascular plants (Vrška et al., 2016; Mikulová et al., 2019; Deng et al., 2020).

In this work, we carried out a multi-taxon analysis to quantify the effects of different thinning practices on biodiversity in two black pine plantations of Tuscany (Central Italy), where black pine was widely introduced for silvicultural purposes outside its native range (Pignatti et al., 2017-2019). We used data coming from the SelpiBioLife (LIFE13 BIO/IT/000282) project, started in 2014 and ended in May 2019. The aim of the project was to assess the effects of different silvicultural practices on structural features, soil microclimate, and soil biodiversity and functioning in *P. nigra* planted forests (Cantiani, 2016). In a previous paper about cross-taxon relationship in the year before management application (Barbato et al., 2019), the analysis of recorded SelpiBioLife biotic data revealed a robust covariation in species diversity among different groups of organisms (bacteria, vascular plants, mushrooms, ectomycorrhizae, mycelium, carabids, microarthropods), except for nematodes, which were highly mediated by environmental variables (Barbato et al., 2019). Here, we considered a wide range of organisms as well, belonging to four kingdoms: Bacteria, Fungi (mushrooms), Animalia (nematodes and microarthropods), and Plantae (vascular plants, both understorey and overstorey). Three different treatments (no thinning, thinning from below, and selective thinning)

were applied to black pine stands. Then, we conducted a before-after control-impact (BACI) experiment with continuous monitoring over 4 years (5 years for fungi), to assess the effects of management on biodiversity.

To the best of our knowledge, this is the first multi-taxon, continuous monitoring study assessing the effects of forest management practices on biodiversity using a BACI approach. Our main aim was to test whether species richness and composition of different biotic communities are affected by the treatments, and to highlight if silvicultural practices may cause significant biodiversity changes (either positive or negative) in the investigated communities.

2. Material and methods

2.1 Study areas

The study was carried out in two black pine plantations in Tuscany (central Italy) (Figure 1).

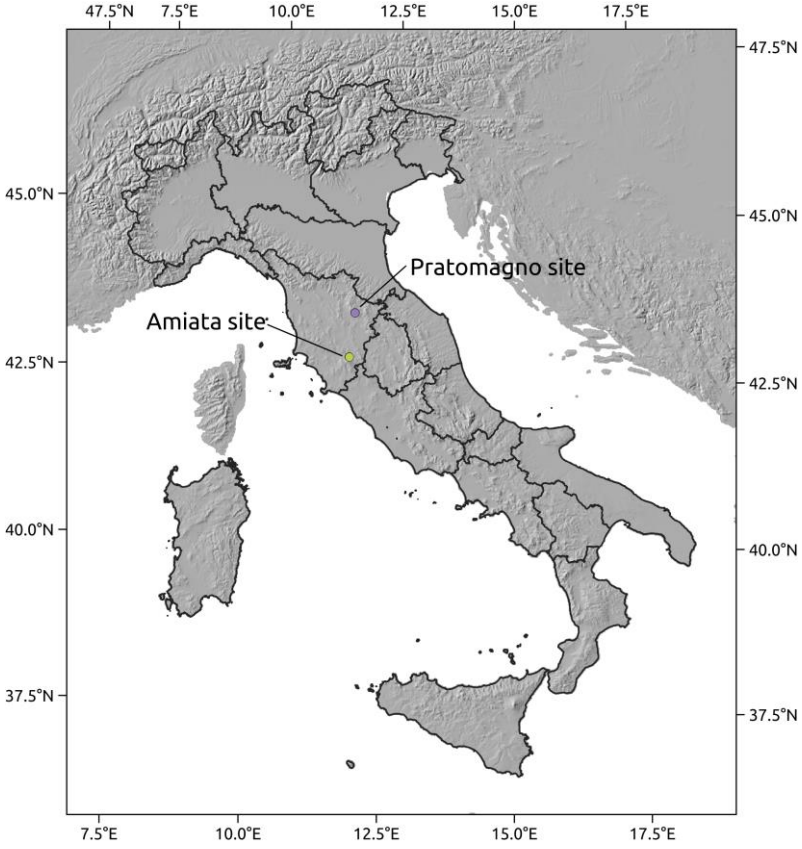

Figure 1. Study area. Location of Amiata and Pratomagno study sites in Tuscany and Italy.

The first plantation (Amiata) is in southern Tuscany (Castiglion d'Orcia, Siena; 42°56'8''N, 11°38'13''E, mean elevation 780 m a.s.l.). It was dominated by 44-year-old black pines (91.3% and 97.3% of total trees/hectare and of basal area/hectare, respectively), accompanied by >10% of *Quercus pubescens* Willd. and *Quercus cerris* L. (Cantiani, 2016). The second plantation (Pratomagno) is in north-eastern Tuscany (Arezzo; 43°35'55.3"N, 11°42'33.9"E, mean elevation 960 m a.s.l. It was dominated by 57 years-old black pines (83.4% of total trees/hectare and 86% of total basal

area/hectare), with scattered *Abies alba* (especially at higher elevations), and occasionally broad-leaved species such as *Fagus sylvatica*, *Fraxinus ornus*, and *Quercus cerris* (Cantiani, 2016).

2.2 Climatic, edaphic, and geological features

We retrieved data on precipitation and temperature for the study years, relatively to the closest climatic stations: Castiglione d'Orcia (Amiata), 544 m a.s.l., and Pratomagno, 695 m a.s.l. (Regione Toscana, 2021) (Figure 2a,b). Seasonal mean temperatures were comparable across the years of the experiment in both the study sites. On the contrary, the total seasonal rainfall had a fluctuating pattern and was particularly low in spring and summer 2017 at both sites (Figure 2a,b). In the Amiata site (Fig. 2a), the average annual temperature was 12.5°C (max: 21.7 °C in July; min: 4.5 °C in January) and the mean annual rainfall was 687 mm, with November being the rainiest month. In the Pratomagno site (Fig. 2b), the mean annual temperature was 10.5°C (max: 19°C in July; min: 1.5°C in January) and the mean annual rainfall was 997 mm, with an absolute peak in autumn and a relative peak in spring.

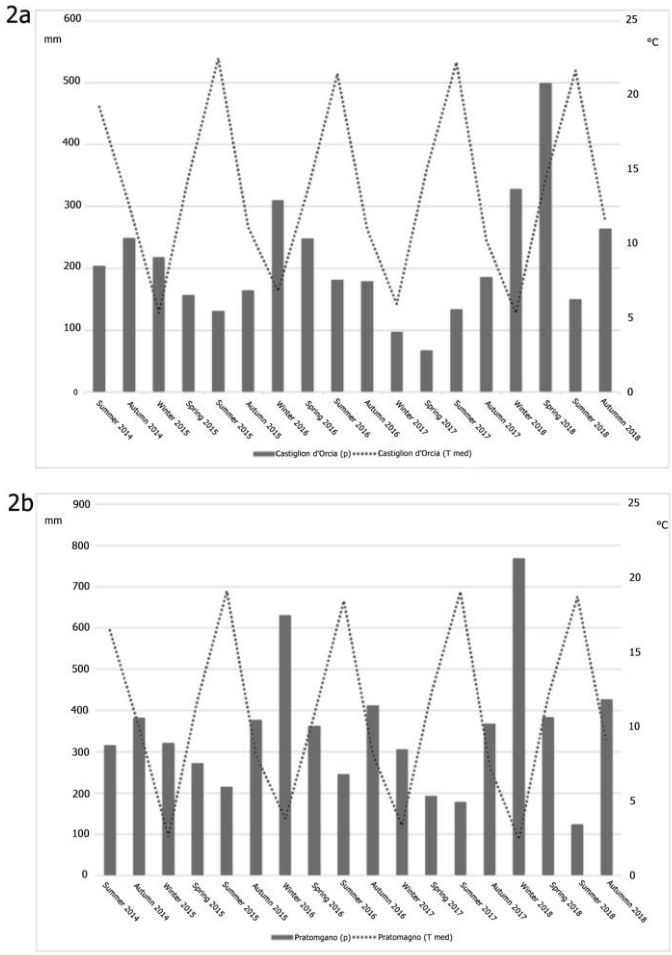

Figure 2a,b. Climatic data. Total seasonal rainfall (p) and seasonal mean temperature (T med) for the two sites (Fig. 2a, Castiglion d'Orcia – Amiata; Fig. 2b Pratomagno) in the years of observation (2014-2018).

147 In the Amiata site, geological substrates are characterized by clay, calcareous, and marly lithofacies. Soils are deep and
148 rich in organic matter (average content: 4.7%) in the topsoil "A" horizons (Barbato et al., 2019).
149 In the Pratomagno site, lithotypes are represented by quartz-feldspar sandstones, alternating with siltstones and argillite.
150 The average organic matter in the topsoil "A" horizons is 6.8% (Barbato et al., 2019). Soils are generally moderately
151 deep, and locally deeper due to strong erosion.
152
153 *2.3 Experimental design and silvicultural treatments*
154 To test the effects of thinning on biodiversity in *P. nigra* plantations, we carried out a before-after control impact
155 (BACI) experiment over 4 years of monitoring (5 years for fungi – 1 year before, 3-4 years after). The monitoring
156 activity started in 2015 (2014 for fungi) and ended in 2018. In such studies, the BACI approach is especially useful
157 because it allows attributing changes in community diversity to an impact, rather than to natural variability (Stewart-
158 Oaten et al., 1986).
159 A complete randomized design was established in 2014 in the two plantations. In each site, we selected a stand of 20 ha
160 dominated by black pine, being as homogeneous as possible in terms of features like basal area, tree density, and gaps
161 in the canopy cover (Barbato et al., 2019). In each stand, nine squared macroplots of 1 ha were marked on the ground.
162 Three circular plots were randomly selected in each macroplot, with a total of 27 plots per site, within which we
163 recorded biological (10 m radius plots or 1 ha macroplot) and structural (15 m radius plots) data (Cantiani and Marchi,
164 2017). Three different treatments were applied in July 2015 (Marchi et al., 2018):
165 a) control, no thinning (C).
166 b) Thinning from below (TB), which removes only dominated, small, or standing dead trees below the main canopy
167 layer, aiming at concentrating the growth on the remaining trees. This is the silvicultural treatment most adopted in
168 Apennine pine forests.
169 c) Selective thinning (ST), aiming at favouring a certain number of trees with good phenotypic features (target trees or
170 crop trees), to guarantee a high degree of mechanical stability already in the juvenile phase (Klädtke, 2002). Thus, crop
171 trees are those that are wished to reach the end of the rotation cycle, favoured by the removal of their competitors. We
172 selected on average 100 crop trees/hectare.
173
174 *2.4 Forest features*
175 In each site, the following variables were measured (Landi et al., 2020):
176 a) Diameter at Breast Height (DBH), i.e., the diameter at a height of 1.30 m, recorded using a diameter measurement
177 tape.

b) Total height, i.e., the height of the first living whorl and the height at maximum crown width, using Vertex III.

c) Mean crown radius, assessed using the vertical sighting method, as the quadratic mean of eight crown radius (Pretzsch et al., 2015).

d) Total Photosynthetically Active Radiation on the ground (PAR, i.e., the total amount of light available for photosynthesis), evaluated using ceptometers (AccuPAR model PAR-80 e LP-80 - Decagon Devices Inc., Pullman, WA, USA).

e) Crown radius at crown base, used to calculate the total crown volume, i.e., the space occupied by the canopy (Pretzsch, 2009).

Then, the main dendrometric variables were calculated, such as the total basal area per plot and the total standing volume per plot. The volumes of single trees were calculated using the most recent equations from the Italian national forest inventory (Tabacchi et al., 2011). Each tree was progressively numbered and geo-referenced using a polar coordinate system (horizontal distance from the centre of the circular plot and degrees from the north) by means of FieldMap® technology, and then converted into metric planar coordinates (Cantiani and Marchi, 2017). Descriptive statistics about the main structural variables measured in the year 2015, before and immediately after silvicultural treatments, are shown in Table 1.

		Before treatment					***After treatment***					***Percent removal***		
Site	**Treatment**	**N trees** *n ha^{-1}*	**Basal area** *m^2 ha^{-1}*	**Standing Volume** *m^3 ha^{-1}*	**Quadratic mean** *cm*	**Mean height** *m*	**N trees** *n ha^{-1}*	**Basal area** *m^2 ha^{-1}*	**Standing** *m^3 ha^{-1}*	**Quadratic mean** *cm*	**Mean height** *m*	**N trees** *%*	**Basal area** *%*	**Standing Volume** *%*
AMIATA	*Thinning from below*	971	42.3	357.6	23.7	17.9	675	34	290.8	25.3	18.3	30.4	19.7	18.7
	Selective thinning	971	47.4	446.4	24.9	18.2	638	32.3	309.2	25.4	18.4	34.3	31.9	30.7
PRATOMAGNO	*Thinning from below*	1085	72.6	722.3	29.3	19.1	695	56.1	582.9	32.1	19.9	35.9	22.6	19.3
	Selective thinning	1056	66.6	586.6	28.6	18.9	731	47	412.6	28.6	19	30.8	29.4	29.7

Table 1. Descriptive statistics on the main dendrometric variables measured in 2015, before and immediately after the application of silvicultural treatments.

The density distributions and the social ranking of the analysed stands, before and immediately after the application of silvicultural treatments, are shown in Figure 3. Although the proportion of harvested trees in the two study areas was similar for TB and ST treatments (30.4% to 35.9% - Table 1), the two thinning systems had a different impact on the

social classes. Namely, the TB treatment had a more intense effect on the dominant trees (overtopped layer) without any influence on the dominant layers (the tallest trees), while the ST treatment had a more extensive effect, with an influence on all the social classes (Figure 3).

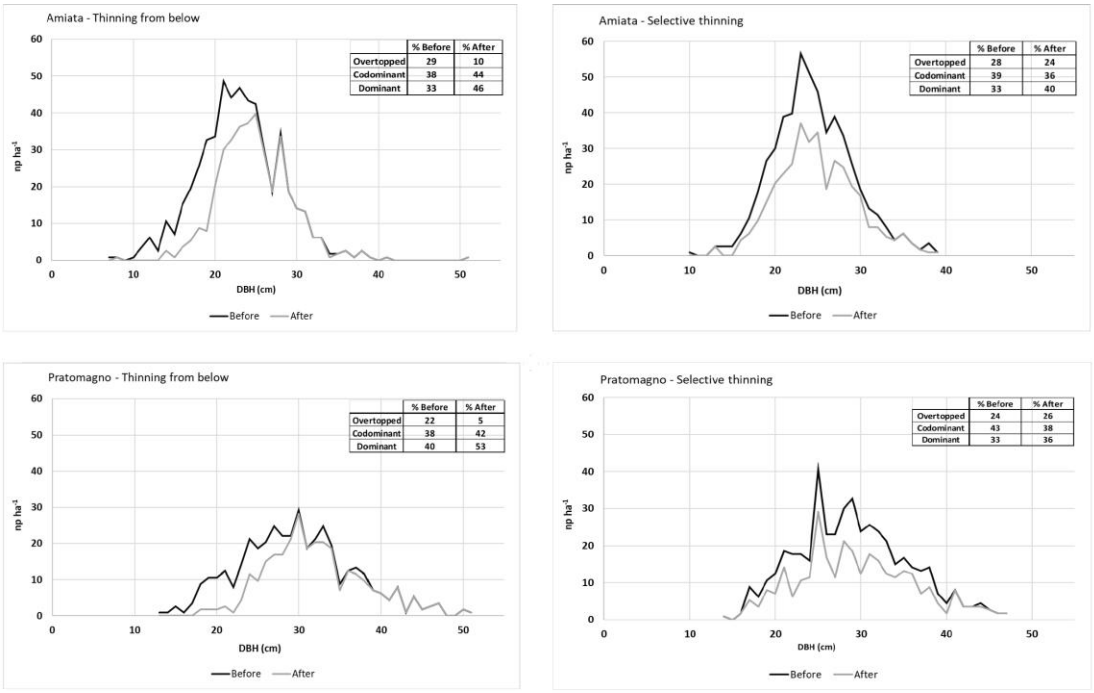

Figure 3. Density distributions and social ranking of the analysed stands, measured in the year 2015, before (black line) and immediately after (grey line) the application of silvicultural treatments.

2.5 Biodiversity sampling

Five different taxonomic groups (bacteria, nematodes, microarthropods, mushrooms and vascular plants) were sampled at plot or macroplot level in the years from 2015 to 2018 (2014 to 2018 for fungi). The organisms were identified to the species (vascular plants and fungi), genus (bacteria), family (nematodes), or order level (microarthropods).

Bacteria were sampled from the soil at the end of May. Five different soil sub-samples were randomly collected from the topsoil (0-20 cm) of each macroplot and homogenized for laboratory analyses. After sieving at 2 mm, total DNA was extracted from 0.5 g of soil using the commercial kit FastDNA^{TM} SPIN Kit for Soil (MP Biomedicals, Santa Ana, California, USA). The structural characterization of the bacterial community was carried out by means of a high-throughput sequencing approach with Miseq Illumina technology (IGA Technology Services s.r.l., Italy), targeting the 16S DNA ribosomal genes with primers 515F and 806R (Caporaso et al., 2012). The structure of the bacterial community was reported as the relative abundance of each taxon, expressed as percentage/0.5 g of soil. Data were processed by using the standard pipeline of QIIME2 (Bolyen et al., 2019).

221 Nematodes were sampled at the end of May, through three soil samples randomly collected in each macroplot with a
222 hand auger, at a depth of 15 cm in the top layer of bulk soil, after removing surface residues. Samples were then pooled
223 to form a composite sample. Individuals were extracted from 100 ml of soil using the cotton-wood filter method for 48
224 hours at room temperature (≃ 20°C), counted, and identified.
225 Microarthropods were sampled at the end of May in each plot, through three soil samples randomly collected using a
226 special 10 cm^3 corer for mesofauna sampling. Soil samples were extracted using modified Berlese-Tullgren funnels and
227 the standard methodology (Parisi et al., 2005). For each sample, the number of individuals was recorded.
228 Mushrooms were sampled in each plot, where all the above-ground epigeous fruit bodies larger than 1 mm were
229 counted (Arnolds, 1981). The sampling was performed every two weeks during the period of highest fungal production
230 (autumn), and once in spring. Species identification was performed with the usual morphological techniques, general
231 analytical keys, and monographs.
232 Understorey and overstorey vascular plants were recorded in each plot yearly, in late spring-early summer. The
233 percentage cover of each understorey vascular plant species was visually estimated in the field. The proportion of
234 ground coverage of overstorey plant species was calculated in each plot by processing in QGIS the eight crown radii
235 measured for each tree.
236
237 *2.6 Statistical analyses*
238 To standardize the observation scale of different taxa, we aggregated all the data collected at the plot level to the
239 macroplot level. For this purpose, we calculated the mean percentage covers as regards vascular plants (separately for
240 overstorey and understorey), whereas we used the sum method for the remnant groups. We obtained a total of 18
241 sampling units (macroplots), 9 for each site. *P. nigra* occurrence and abundance data were excluded from the analyses.
242 First, we tested the effect of time (year of observation - fixed, four levels), site (fixed, two levels), and their interactions
243 on macroplot's forest structural features (total height, basal area, mean height, crown volume, standing volume,
244 Quadratic Mean Diameter -QMD-, dominant height, and PAR), separately for each treatment. Then, we tested the effect
245 of the period of observation (fixed, two levels: before (B) and after (A) management), treatment (fixed, three levels: C,
246 ST, and TB), time (year of observation - random nested in period, five levels for fungi or four levels for the other taxa),
247 and site (fixed, two levels) on taxonomic richness and community composition (occurrence and abundance values for
248 each taxon) of the different groups of organisms. Following, we performed uni/multivariate permutational analyses of
249 variance (PERMANOVA - Anderson, 2001), checking for significant interactions between the period of observation
250 (BA) and the treatment (C, ST, TB), which would imply a press impact of the treatment application (Underwood, 1994).
251 PERMANOVA was based on a Bray–Curtis dissimilarity matrix, calculated from the untransformed bacteria data and

ln(x+1)- the transformed abundance data for the other taxa. Regarding the univariate analyses, we calculated a Euclidean distance matrix derived from untransformed data. The analyses were performed using the PERMANOVA routine in the PRIMER v6 computer program (Clarke and Gorley, 2006), including the add-on package PERMANOVA + (Anderson et al., 2008). All tests were performed with 999 permutations of residuals under a reduced model (Anderson and Ter Braak, 2003), considering an alpha level of 0.05. Significant terms relevant to the hypotheses were investigated through *post hoc* pair-wise comparisons using PERMANOVA *t*-test and 999 permutations.

The effects of different treatments on species composition were also checked through the Principal Response Curve technique (PRC, van den Brink and ter Braak, 1998). This technique, derived from the redundancy analysis (RDA) constrained ordination method, plots the principal components of the effects of TB and ST treatments along the period of observation, expressed as deviations from the control treatment (van den Brink and ter Braak, 1998). The significance of the PRC diagrams was tested by means of Monte Carlo permutation tests (999 permutations), permuting whole time series in the partial RDA from which the PRCs were obtained. The PRC analyses were performed in Canoco 5 (ter Braak and Šmilauer, 2012; Šmilauer and Lepš, 2014).

3. Results

3.1 Forest structural features

None of the variables measured in the control macroplots significantly changed over the years of observation (Tables 2, 3). Tree density, crown volume and basal area significantly decreased in both TB and ST macroplots after management application (Table 2). The PAR increased in all the managed macroplots, while the standing volume decreased only in the ST treatment. The QMD increased in TB macroplots from 2015 to 2017 and stayed constant between 2017 and 2018 (Table 3). The interaction site × year was significant only for mean height values, thus indicating a similar pattern of change over the observation period for the two sites (Table 2).

Source of variation	*df*	**Trees density**						**Basal area**						**Mean height**					
		Thinning from below		Selective thinning		Control		Thinning from below		Selective thinning		Control		Thinning from below		Selective thinning		Control	
		MS	*F*	*MS*	*F*	*MS*	*F*	*MS*	*F*	*MS*	*F*	*MS*	*F*	*MS*	*F*	*MS*	*F*	*MS*	*F*
Site (*Si*)	1	31031	1.81	53372	4.91	29041	0.53	2340.70	**72.23*** **	410.93	**12.63** **	2636.6	**26.77*** **	76.96	**368.86*** **	0.11	0.93	4.98	**6.18***
Year	3	148570	**8.68*** **	169200	**15.55*** **	1364.2	0.02	141.52	**4.37** **	379.38	**11.66** **	1.96	0.02	6.74	**32.33*** **	4.02	**33.86*** **	0.66	0.82
Si× *Year*	3	1073.3	0.06	728.92	0.07	373.59	0.01	21.29	0.66	22.69	0.70	2.50	0.02	3.05	**14.60*** **	0.42	**3.53***	0.03	0.04
Residual	16	17107		10879		54351		32.40		32.54		98.49		0.21		0.12		0.80	
Total	23																		

275 * = P≤0.05; ** = P≤0.01; *** = P≤0.001.

Source of variation	*df*	**Crown Volume**						**Standing volume**						**Quadratic mean diameter (QMD)**					
		Thinning from below		Selective thinning		Control		Thinning from below		Selective thinning		Control		Thinning from below		Selective thinning		Control	
		MS	*F*	*MS*	*F*	*MS*	*F*	*MS*	*F*	*MS*	*F*	*MS*	*F*	*MS*	*F*	*MS*	*F*	*MS*	*F*
Site (*Si*)	1	619700000	**12.94** **	630780000	**12.61** **	288450000	1.95	466810	**144.90*** **	32572	**9.79** **	265840	**22.03*** **	325.22	**136.12*** **	122.42	**66.63*** **	183.48	**20.11** **
Year	3	256450000	**5.35** **	486680000	**9.73** **	82321000	0.56	6955.6	2.16	24913	**7.49** **	1403.4	0.12	10.80	**4.52** **	3.39	1.85	0.83	0.09
Si × *Year*	3	4019800	0.08	54279000	1.08	3824600	0.03	2019.5	0.63	1506.8	0.45	190.76	0.02	0.23	0.10	0.10	0.06	0.01	0.00
Residual	16	47903000		50010000		147600000		3221.6		3325.8		12065		2.39		1.84		9.12	
Total	23																		

276 * = P≤0.05; ** = P≤0.01; *** = P≤0.001.

Source of variation	*df*	**Dominant height**						*df*	**PAR**					
		Thinning from below		Selective thinning		Control			Thinning from below		Selective thinning		Control	
		MS	*F*	*MS*	*F*	*MS*	*F*		*MS*	*F*	*MS*	*F*	*MS*	*F*
Site (*Si*)	1	112.42	**1089.2*** **	0.16	1.01	5.17	**9.74** **	1	0.0046	0.26	0.03	2.87	0.01	2.27
Year	3	0.07	0.70	0.04	0.24	0.04	0.08	2	0	**3.63***	0.13	**10.48** **	0	0
Si × *Year*	3	0.01	0.05	0	0.03	0	0.01	2	0.01	0.81	0.01	1.10	0	0.79
Residual	16	0.10		0.16		0.53		30	0.02		0.01		0	
Total	23					5.17		35						

277 * = P≤0.05; ** = P≤0.01; *** = P≤0.001.

278 **Table 2.** Results of permutational analyses of variance (PERMANOVA) for dendrometric parameters.

Treatment	Year	N trees per ha (Tree density)	Crown volume	Basal area	Mean height	Photosintetically Active Radiation (PAR)	Standing volume	Quadratic mean diameter (QMD)	Dominant height
		nr ha-1	*m^3 ha^{-1}*	*m^2 ha^{-1}*	*m*	*µmol/(m^2s)*	*m^3 ha^{-1}*	*cm*	*m*
	2015	1034	45006.40	52.90	18.04	0.13	466.07	25.78	20.80
Control	2016	1025	37781.92	53.79	18.63		494.72	26.12	20.87
	2017	1008	37421.11	53.64	18.69	0.13	493.30	26.35	20.92
	2018	1001	37609.34	54.29	18.77	0.13	500.20	26.67	20.99
	2015	a 995	a 42778.12	a 55.43	18.31	a 0.15	524.63	a 26.72	21.96
Thinning from below	2016	b 699	b 30087.23	b 45.95	20.32		458.45	ab 28.9	22.05
	2017	b 676	b 29534.7	b 45.32	20.42	b 0.28	452.54	b 29.30	22.12
	2018	b 668	b 29518.57	b 45.94	20.53	b 0.27	459.44	b 29.75	22.22
	2015	a 950	a 48603.11	a 54.75	18.46	a 0.10	a 503.91	27.17	21.76
Selective thinning	2016	b 631	b 30524.35	b 38.8	19.98		b 373.36	28.03	21.77
	2017	b 614	b 30551.81	b 38.80	20.08	b 0.39	b 374.47	28.46	21.84
	2018	b 599	b 30697.18	b 38.95	20.20	b 0.32	b 377.4	28.94	21.93

Table 3. Main dendrometric features in the years of observation (2015-2018) for the different silvicultural treatments. Different letters (ab) indicate significant differences ($P \leq 0.05$).

3.2 Biodiversity

3.2.1 Taxonomic richness

A total of 936 taxa were recorded (Table 4). As for mushrooms, 52,244 fruiting bodies were analysed. The occurrences of Microarthropods and nematodes were 24,766 and 9,506, respectively. The total taxonomic richness (all the groups of organisms) varied between 247 and 316 among the 18 macroplots, over all the monitoring years (2015-2018, and excluding 2014 for mushrooms). Regarding single groups of organisms, mushrooms showed the highest variation and nematodes showed the lowest variation in taxonomic richness between the poorest and the richest macroplot (Table 4).

Group	Total taxonomic richness	Taxonomic richness, $\bar{x}$ ± SD	Range
Bacteria	262	222 ± 6	212-233
Nematodes	15	6 ± 2	3-10
Arthropods	19	9 ± 2	5-14
Mushrooms	391	34 ± 17	5-80
Vascular plants (understorey)	223	36 ± 13	14-62
Vascular plants (overstorey)	26	4 ± 2	0-10
Total	936	277 ± 17	247-316

Table 4. Total number of recorded taxa (taxonomic richness), average taxonomic richness at the macroplot level (average taxonomic richness, $\bar{x}$ ± SD), and range in taxonomic richness for each group of organisms in the 18 macroplots, for all treatment types (control, selective thinning, and thinning from below) and study years.

295 The results of the permutational univariate analysis of variance, performed on the number of taxa per macroplot,
296 showed that the year of observation was significant for all taxa, whereas the treatment was significant only for
297 mushroom, nematode, and overstorey plant species richness. Overall, the two sites hosted a different number of
298 bacteria, mushrooms, understorey vascular plants and microarthropods taxa (Table 5). The first-order interaction
299 between period of observation (BA) and treatment was significant only for understorey vascular plants, but the *t*-test
300 revealed that there were no significant differences among treatments in the period after treatment application (C *vs* TB, *t*
301 = 0.49, P = 0.9; C *vs* ST, *t* = 4.19, P = 0.11; TB *vs* ST, *t* = 4.02, P = 0.09). However, the increase in species richness in
302 the ST samples is higher, compared to the TB treatment (Figure 4). The interaction 'period of observation (BA) ×
303 treatment × site' was never significant, indicating that there was no difference between the two sites in response to
304 treatment application (Table 5).
305
306

Source of variation	*df*	**Bacteria**				**Nematodes**				**Microarthropods**			
		Richness		Multivariate community composition		Richness		Multivariate community composition		Richness		Multivariate community composition	
		MS	*F*	*MS*	*F*	*MS*	*F*	*MS*	*F*	*MS*	*F*	*MS*	*F*
Period (*BA*)	1	2660.7	1.37	697.22	0.36	3.13	0.18	5079.6	0.93	43.56	2.45	2660.7	0.93
Treatment (*Tr*)	2	412.89	3.13	158.93	**4.05****	12.25	**8.07***	696.75	1.88	2.59	0.35	412.89	1.23
Site (*Si*)	1	3574.1	**21.33***	25585	**183.02****	0.07	0.01	919.12	0.54	117.04	**20.45***	3574.1	3.62
Year (*BA*)	2	2871.3	**56.82*****	1928	**18.46*****	17.35	**10.16*****	5432.1	**15.20*****	17.80	**11.97*****	2871.3	**10.53*****
BA × *Tr*	2	200.12	0.71	17.98	0.46	4.50	2.97	254.58	0.69	0.64	0.09	200.12	0.60
BA × *Si*	1	1353.8	0.94	5.97	0.04	0.30	0.05	397.84	0.23	2.04	0.36	1353.8	1.37
Tr × *Si*	2	263.54	2.16	205.13	**10.86****	0.37	0.08	508.96	0.64	1.29	0.47	263.54	1.61
Tr × *Year (BA)*	4	335.78	0.57	39.19	0.37	1.52	0.89	371.33	1.04	7.46	**5.02****	335.78	1.23
Si ×*x Year (BA)*	2	986.55	**3.74***	139.8	1.34	6.35	**3.72***	1696.3	**4.75*****	5.72	**3.85***	986.55	**3.62*****
BA × *Tr* × *Si*	2	134.64	0.81	13.95	0.74	0.17	0.04	278.13	0.35	1.12	0.41	134.64	0.82
Tr × *Si* × *Year (BA)*	4	164.06	0.65	18.88	0.18	4.68	**2.74***	788.65	**2.21****	2.72	1.83	164.06	0.60
Residual	48	272.66		104.46		1.71		357.3		1.49		272.66	
Total	71												

* = P≤0.05; ** = P≤0.01; ***= P≤0.001.

Source of variation	*df*	**Mushrooms**				Source of variation	*df*	**Vascular plants (understorey)**				**Vascular plants (overstorey)**			
		Richness		Multivariate community composition				Richness		Multivariate community composition		Richness		Multivariate community composition	
		MS	*F*	*MS*	*F*			*MS*	*F*	*MS*	*F*	*MS*	*F*	*MS*	*F*
Period (*BA*)	1	11904	1.38	12119	1.66	Period (*BA*)	1	563.89	2.97	2914.7	0.59	2.67	0.15	1011.1	166.25
Treatment (*Tr*)	2	3686.1	**1.54***	2993.8	**2.28****	Treatment (*Tr*)	2	4.12	0.56	2870.5	**3.78****	32.67	**7.54***	6340.1	**1025.5*****
Site (*Si*)	1	33598	**4.73***	39436	**6.66***	Site (*Si*)	1	5370	**28.16***	27225	**12.76***	31.13	5.48	31848	**5322.8***
Year (*BA*)	3	8635.9	**3.22*****	7287.6	**3.81*****	Year (*BA*)	2	190.02	**3.03***	4907.4	**4.46*****	18.17	**4.45***	6.08	0.004
BA × *Tr*	2	1971.3	0.82	1408.9	1.07	*BA* × *Tr*	2	63.62	**8.59***	1632.7	2.15	2.17	0.5	259.26	**41.94****
BA × *Si*	1	10344	1.46	11461	1.93	*BA* × *Si*	1	15.04	0.08	3446.2	1.61	6.68	1.18	616.67	**103.07***
Tr × *Si*	2	3885.4	1.66	2798.3	**2.02***	*Tr* × *Si*	2	19.18	4.32	2318.2	**2.51***	1.68	0.40	4112.7	**673.97*****
Tr × *Year (BA)*	6	2394.5	0.89	1310.2	0.69	*Tr* × *Year (BA)*	4	7.41	0.12	758.32	0.69	4.33	1.06	6.18	0.004
Si × *Year (BA)*	3	7105.1	**2.65*****	5925.2	**3.10*****	*Si* × *Year (BA)*	2	190.72	3.04	2133.5	**1.94****	5.68	1.39	5.98	0.004
BA × *Tr* × *Si*	2	2232.3	0.95	1520.6	1.10	*BA* × *Tr* × *Si*	2	23.35	5.25	1386.3	1.50	3.91	0.92	361.91	**59.31*****
Tr × *Si* × *Year (BA)*	6	2341.5	0.87	1384.7	0.72	*Tr* × *Si* × *Year (BA)*	4	4.44	0.07	921.88	0.84	4.24	1.04	6.10	0.004
Residual	60	2679.7		1910.5		Residual	48	62.78		1099.8		4.08		1433	
Total	89					Total	71								

* = P≤0.05; ** = P≤0.01; *** = P≤0.001.

Table 5. Results of permutational analysis of variance (PERMANOVA) for community composition (multivariate) and richness (number of taxa per macroplot) of all the groups.

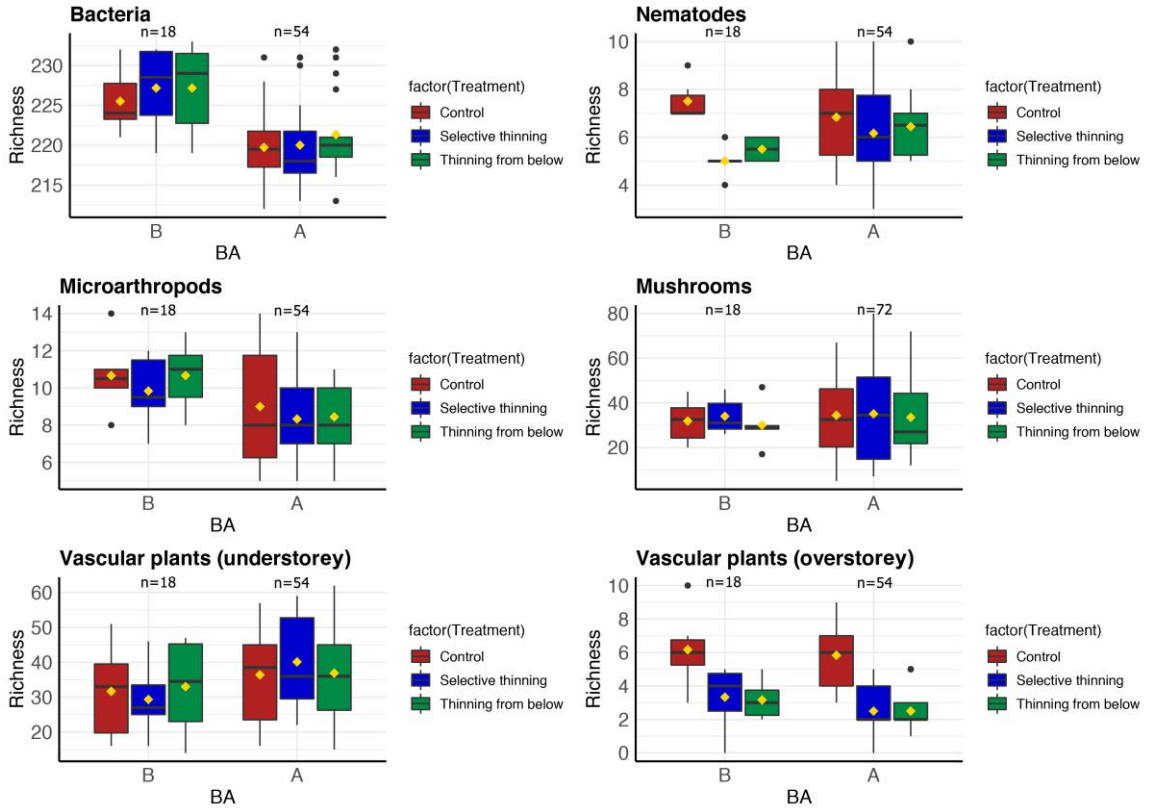

Figure 4. Boxplots of taxonomic richness. Boxplots of number of orders for microarthropods, number of families for nematodes, number of genera for bacteria, and number of species for mushrooms and vascular plants per macroplot, for each treatment in the period before (B) and after (A) silvicultural treatment. Diamonds indicate the mean value per plot.

3.2.2 Community composition

The PERMANOVA performed on community composition showed that the factor 'year of observation' was significant for all taxa except overstorey vascular plants (Table 5). The community compositions of bacteria, vascular plants, and mushrooms were significantly affected by the factor 'site', while the factor 'treatment' affected the composition of all the communities except for nematodes (Table 5). The interactions 'period of observation (BA) × treatment' and 'period of observation (BA) × treatment × site' were significant only for the overstorey plant community (Table 5), but the *t*-test revealed that there were no significant differences among treatments, in the period after treatment application, within the two study sites (Amiata: C *vs* TB, $t = 0.69$, P = 1; C *vs* ST, $t = 1.4$, P = 0.12; TB *vs* ST, $t = 0.93$, P = 0.81; Pratomagno: C *vs* TB, $t = 1.09$, P = 0.42; C *vs* ST, $t = 1.11$, P = 0.3; TB *vs* ST, $t = 1.12$, P = 0.31). A significant effect was also showed by the interactions 'treatment × site' (bacteria, mushrooms, and vascular plants) and by 'site × year' (microarthropods, nematodes, mushrooms, and understorey vascular plants). This indicates that there was a difference among the macroplots belonging to the same treatment between the two study areas, and that the pattern in time of the two sites differed, respectively.

Figure 5 shows the first PRC diagrams, representing the temporal dynamics of communities' composition for each experimental treatment.

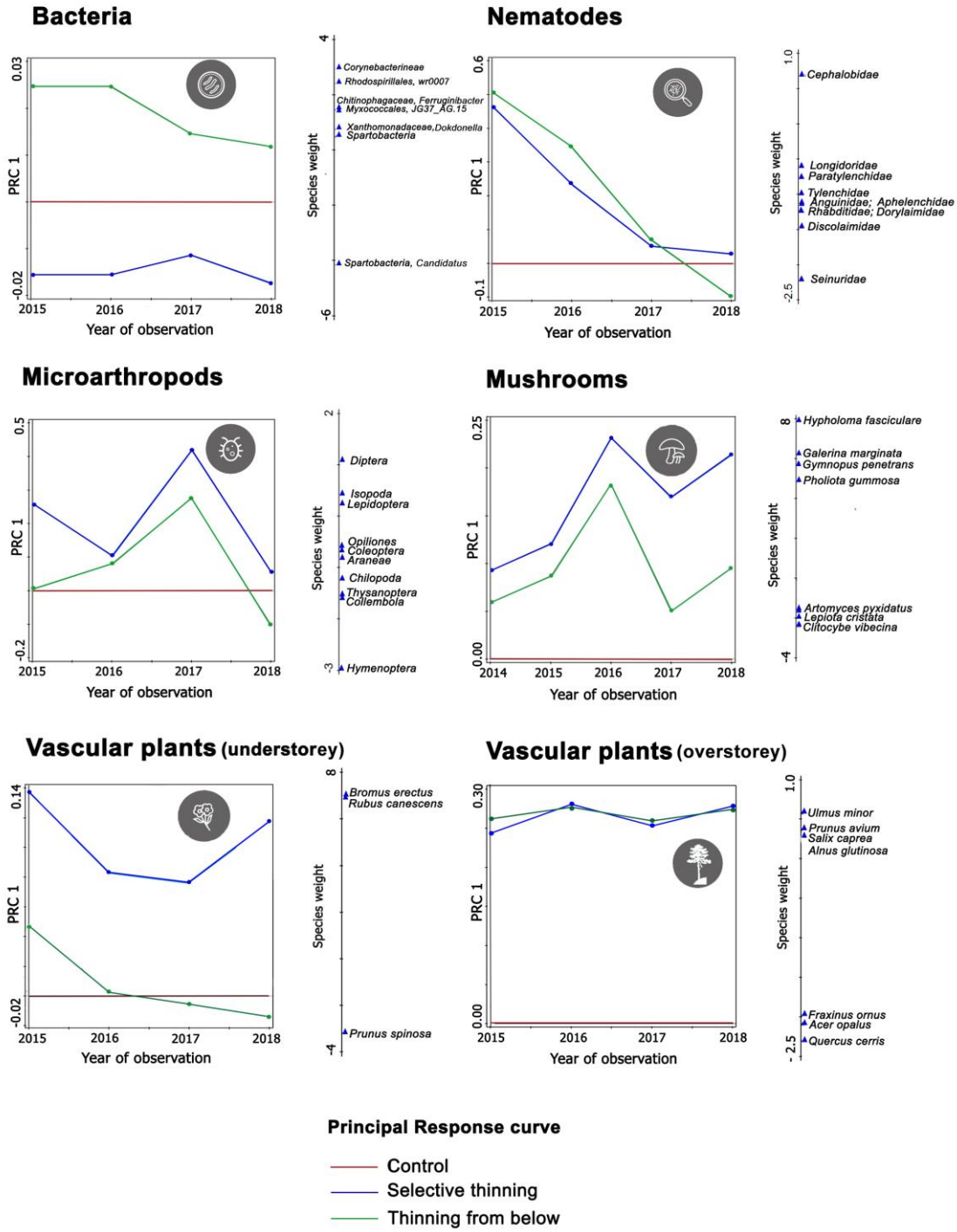

Figure 5. Principal response curves (PRC) for all the taxonomic groups. First principal response curves (PRC) diagram, showing the effects of treatments plus the interaction between year and treatment, on different taxa in *P. nigra* plantations over time. Species weight on the right can be read as the affinity of every taxon with the shown diagram: taxa with high positive values follow the overall community response as indicated by the PRC, while for negative values the community response is the opposite. Zero-lines show the control treatment, the other lines show the course of treated macroplots over time with respect to the control treatment.

The first principal component response was not significant (bacteria: F = 0.4, P = 0.96; mushrooms: F = 1.6, P = 0.94; nematodes: F = 3.3, P = 0.35; overstorey vascular plants: F = 4.1, P = 0.6; understorey vascular plants: F = 2.2, P = 0.73; microarthropods: F = 2, P = 0.79). The percentage of variance explained by treatment regime described by the first PRC was quite high for all the investigated taxa: soil bacteria = 50.9%; understorey vascular plants = 31.8%; overstorey vascular plants = 56.9%; mushrooms = 24,9%, nematodes = 41.6%; microarthropods = 33.5%.

PRC diagrams displayed a similar pattern for bacteria and understorey vascular plants: TB macroplots approached C ones in the year 2018, while the ST treatment diverged (Figure 5). Bacteria such as *Corynebacterineae* and *Rhodospirillales* decreased in TB macroplots in 2017 and 2018 with respect to C macroplots, while *Sparctobacteria* and *Candidatus* showed the opposite pattern (Figure 5). Understorey plant species such as *Bromus erectus* and *Rubus canescens* showed the highest sensitivity to treatment application, decreasing in TB and ST macroplots in the year 2016 with respect to C, but increasing again in ST in the last year of observation. On the other hand, *Prunus spinosa* showed the opposite pattern. Notably, TB macroplots approached C ones after management application. The overstorey plant composition of TB and ST macroplots did not change, in respect to C ones, during the years of observation (Figure 5). Regarding mushrooms, ST macroplots differed from C and TB macroplots in the last year of observation: the number of individuals of *Hypoloma fasciculare*, *Galerina marginata*, *Gymnopilus penetrans*, and *Pholiota gummosa* increased from 2015 to 2016, decreased in 2017, and increased again in 2018 in ST and TB macroplots with respect to C macroplots, while *Artomyces pyxidatus*, *Lepiota cristata*, and *Clitocybe vibecina* showed the opposite pattern. In the same way, ST and BT macroplots for microarthropods showed a fluctuating pattern in the years after management application, with respect to C macroplots: the number of individuals of *Diptera*, *Isopoda*, and *Lepidoptera* increased from 2015 to 2016, decreased in 2017, and increased again in 2018, while *Hymenoptera* showed exactly the opposite pattern (Figure 5). Nematodes showed a progressive increase in similarity to the C macroplots, with the diminishing of the abundance of *Cephalobidae* and the increase of *Seirunidae* in TB and ST macroplots, as compared to C (Figure 5).

4. Discussion

Despite the observed changes in forest parameters such as crown volume and standing biomass generated by both the applied thinning systems, biological communities showed a non-significant shift, with high resistance to forestry practices. In fact, the investigated taxa performed very similarly regardless of silvicultural practices, and consequently a high taxon congruence, as none of the two thinning approaches significantly affected biodiversity, neither regarding taxonomic richness nor the composition of the entire community. Only vascular plants showed a slight tendency regarding species richness and composition. Despite the differences in the environmental and in the structural

characteristics of the two plantations, the applied treatments caused a similar response of the different taxonomic groups.

The short time between the treatments and post-harvesting surveys does not allow any consideration or inference on the effectiveness of the treatments in increasing the stability of the trees. In fact, evaluations on the effect of thinning on growth trends require at least 15-20 years (Marchi et al., 2018; del Río et al., 2017).

Thinning of *Pinus* plantations increases the structural complexity of forest overstorey, changing the ecological conditions in the lower layers. This may result in an increase of plant species richness (Osorio et al., 2009; Marchi et al., 2018). This evidence is in contrast with some others provided by experiments on the application of clear cutting, where a drastic change in plant species composition was found, with the disappearance in the understorey of *Brachypodium sylvaticum* and a massive encroachment of non-forest species like *Brachypodium rupestre* in short-term monitoring (Maccherini et al., 2019). A big change in forest coverage can also reduce the likelihood of seedling survival and growth, as well as the richness and diversity of shrubs (Deng et al., 2020). From our results, vascular plant richness and composition did not actually change both in the understorey and overstorey, at least three years after management. The establishment of the black pine plantations deeply changed the environmental conditions of the stands, compared to the ones that would have occurred under native plant communities. From an edaphic and macroclimatic point of view, the introduction of black pines affected soil reaction, litter decomposition, moisture, and temperature (Mikulová et al., 2019). The two thinning systems provoked the reduction in soil moisture content and an increase in soil temperature in respect to control sites (Landi et al., 2020) but these changes were not enough to cause changes in the vascular plant communities.

Soil bacterial communities are sensitive to any environmental change (Jansson and Hofmockel, 2020). Surprisingly, they can also exhibit an unexpected resilience under biotic/abiotic perturbations, thus maintaining the functioning and stability of natural ecosystems (Awasthi et al., 2014; Mocali et al., 2015). Our results indicated that the overall bacterial richness decreased over time regardless of the treatment, showing that such communities were not significantly affected by thinning practices. However, the PRC diagrams report that the abundance of bacteria in TB macroplots changed over time towards the values of the control C, especially in 2018, whereas the abundances of bacteria in ST macroplots were more stable. *Corynebacteriaceae* and *Rhodospirillales* were the taxa more influenced by management application and those more abundant in TB macroplots. Interestingly, although *Corynebacteriaceae* are commonly detected in both rhizosphere and bulk soils, they have been also found to be strictly associated with plant roots and ectomycorrhizas (Vik et al., 2013). Moreover, despite they have been shown to stimulate basidiospore germination (Ali and Jackson, 1989), they did not show any correlation with mushroom abundance. On the other hand, previous studies indicated that the family *Rhodospirillaceae* has an important role in fixing molecular nitrogen and a wide variety of photosynthetic

bacteria belong to the class *Alphaproteobacteria* and to the order *Rhodospirillales* (Madigan et al., 1984; Wang et al., 2017). Furthermore, the relative abundance of *Rhodospirillaceae* increases with the addition of organic fertilizers and the increase of soil organic matter (Wang et al., 2017). Thus, their lower abundance in ST might also be correlated to the distribution of *Spartobacteria* in TB and ST compared to C. Interestingly, *Spartobacteria*, included within the *Verrucomicrobia* phylum, are characterized by a small genome and a high metabolic versatility, and they prefer soils containing low amounts of labile carbon inputs with a low C/N ratio, and high pH values (Brewer et al., 2016; Shen et al., 2017). We hypothesize that the higher amount of *Spartobacteria* and the opposite distribution of *Rhodospirillaceae* in ST compared to TB and C soils may be linked to the reduced carbon availability in ST macroplots, where higher rainfall and light inputs enhance the microbial mineralization of organic matter, thus depleting the available organic carbon.

In this experiment, contrarily to many previous studies (Muller et al., 2007; Lin et al., 2011; Baral et al., 2015; Lin et al., 2015; Maghnia et al., 2017; Castaño et al., 2018; Tomao et al., 2020), forest management did not have a negative impact on mushrooms communities. We confirm, as reported by previous studies (Landi et al., 2015 and references therein), that fungi, in forests, are strongly correlated with woody plant communities. PRC diagrams showed that some species increased their abundance in ST and TB macroplots with respect to C ones, in accordance with the findings by Muller et al. (2007) and Parisi et al. (2018). The latter stated that an improvement of forest structural heterogeneity and an accurate assessment of deadwood patterns across spatial and temporal scales are beneficial to wood-inhabiting fungi, that increase in frequency and abundance. By contrast, some species (*Artomyces pyxidatus*, *Lepiota cristata*, and *Clitocybe vibecina*) were negatively affected by thinning, reducing the number of individuals in treated macroplots. This confirms their preference for closed and moist habitats (Bon, 1983; Julich, 1989; Candusso and Lanzoni, 1990). The greatest changes in mushroom abundances in treated plots with respect to control plots occurred in 2017, in correspondence with the driest spring-summer season. Mushrooms are highly influenced by climatic conditions (Boddy et al., 2013) especially in autumn, but also in spring. In fact, spring is the period of mycelium growth, which influences the production of fruiting bodies in autumn (Salerni et al., 2002; Boddy et al., 2013).

Microarthropod communities were weakly affected by thinning, although time (years under evaluation) and the interaction 'year × treatment' were significant. In accordance with the findings of Landi et al. (2020) for the same dataset (years under evaluation: 2016 to 2018), a great variability in microarthropod abundance over time is a consequence of changes in soil temperature and moisture. The highest total abundance of individuals was detected in a drought year (2017), especially when plots were thinned (Landi et al., 2020). Changes in soil conditions are the main factor influencing microarthropod communities (Parisi et al., 2005; Mocali et al., 2015). In contrast with the findings of several authors (Huhta et al., 1967; Bird et al., 2004), mites and springtails were dominant in terms of number of

individuals and were little affected by thinning. On the contrary, less represented taxa were more sensitive to the treatments. Hymenoptera, that usually live on the soil surface, were negatively affected by thinning. This behaviour was also observed for other epi-edaphic microarthropods, as previously described by Landi et al. (2020). By contrast, Diptera were positively influenced by the forest condition created after thinning. As reported by Huhta et al. (1967) and Landi et al. (2020), most of hemi-edaphic species tend to decline because of thinning practice, but some increase in abundance, since they find optimal ecological conditions.

Nematode richness was poorly affected by thinning practices, in accordance with previous findings (Landi et al., 2020). Landi et al. (2020) detected changes in the composition of nematode communities, which however revealed to be temporary, in accordance with previous findings and comparably to the effects of clear cutting (Forge and Simar, 2001; Sohlenius, 2002). Our results showed that the changes in community composition after the treatment were not significant. From a perspective of trophic level, bacterial and fungal feeders were little affected by thinning, except for *Cephalobidae*, which increased constantly in ST and TB macroplots over the years of observation. Instead, omnivores and predators (especially *Seinuridae*) decreased in treated macroplots compared to C ones, due to their low colonization ability and to their high sensitivity to disturbance (Bongers, 1990).

5. Conclusion

The main aim of silvicultural treatments is to modify the structure of forest stands, possibly fostering positive effects, operating on trees within the forest. The two tested treatments represent the main current management options for *P. nigra* reforestations in Italy, and both are allowed by the Italian law. Our continuous monitoring of forest structural features and of biological data, the first of its kind using a BACI approach, revealed that both the silvicultural treatments can be safely applied without relevant impacts on biodiversity in a short-term period. The vascular plant, soil bacteria, mushroom, microarthropod, and nematode communities showed a high resistance to forestry treatments and thus a similar response, regarding either species richness or the entire community's composition. Nevertheless, some taxa within the four kingdoms showed a higher sensitivity to the silvicultural management when combined with the influence of climatic patterns, and in particular with the spring and summer drought of 2017. With respect to this, our results highlighted the importance of multi-taxon studies and of long-term monitoring to confirm or not the observed patterns, also in the light of the evidence that unexpected climatic events interact with management in different ways, as already noted in the analysis of a longer time-series data by Chiarucci et al. (2007) and Maccherini et al. (2018).

Acknowledgements

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Table captions

Table 1. Descriptive statistics on the main dendrometric variables measured in 2015, before and immediately after the application of silvicultural treatments.

Table 2. Results of permutational analyses of variance (PERMANOVA) for dendrometric parameters.

Table 3. Main dendrometric features in the years of observation (2015-2018) for the different silvicultural treatments. Different letters (ab) indicate significant differences ($P \leq 0.05$).

Table 4. Total number of recorded taxa (taxonomic richness), average taxonomic richness at the macroplot level (average taxonomic richness, $\bar{x} \pm$ SD), and range in taxonomic richness for each group of organisms in the 18 macroplots, for all treatment types (control, selective thinning, and thinning from below) and study years.

Table 5. Results of permutational analysis of variance (PERMANOVA) for the community composition (multivariate) and richness (number of taxa per macroplot) of all the groups.

Figure captions

Figure 1. Study area. Location of Amiata and Pratomagno study sites in Tuscany and Italy.

Figure 2a,b. Climatic data. Total seasonal rainfall (p) and seasonal mean temperature (T med) for the two sites (Fig. 2a, Castiglion d'Orcia – Amiata; Fig. 2b Pratomagno) in the years of observation (2014-2018).

Figure 3. Density distributions and social ranking of the analysed stands, measured in the year 2015, before (black line) and immediately after (grey line) the application of silvicultural treatments.

Figure 4. Boxplot of taxonomic richness. Boxplots of number of orders for microarthropods, number of families for nematodes, number of genera for bacteria, and number of species for mushrooms and vascular plants per macroplot, for each treatment in the period before (B) and after (A) silvicultural treatment. Diamonds indicate the mean value per plot.

Figure 5. Principal response curves (PRC) for all the taxonomic groups. First principal response curves (PRC) diagram, showing the effects of treatments plus the interaction between year and treatment, on different taxa in *P. nigra* plantations over time. Species weight on the right can be read as the affinity of every taxon with the shown diagram: taxa with high positive values follow the overall community response as indicated by the PRC, while for negative values the community response is the opposite. Zero-lines show the control treatment, the other lines show the course of treated macroplots over time with respect to the control treatment.

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Conflict of interest

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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