

EFFECTS OF THINNINGS ON PLANTS AND FUNGI BIODIVERSITY IN A *PINUS NIGRA* PLANTATION: A CASE STUDY IN CENTRAL ITALY

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Abstract

One of the main consequence of intensive forest exploitation, overgrazing and recurring wildfires over the centuries is the decay of forest cover and soil erosion. In many areas of the Italian Apennines, Black pine (*Pinus nigra* Arn.) plantations were established after the Second World War to improve forest soil quality in marginal and eroded soils. The main aim of these reforestations was to re-establish the pine as a first cover, pioneer species. This was a preparatory step to the reintroduction of broadleaf trees originally living in the same areas, such as oaks and beech trees, and thus to the reestablishment of mixed forests (renaturalisation). Currently in Tuscany, the key functions of these stands are the protection against soil erosion and the hydrological regulation of catchments. In order to guarantee the multifunctional role of these stands, it is necessary to realize silvicultural treatments finalized to the renaturalisation. The present research, carried out in a mountainous area in Tuscany, aims to evaluate the effects of different thinning (selective and traditional) on floristic and mycological diversity. Six experimental plots were established, and different intensity of thinning were applied. Results highlight that the intensity of thinning is directly related to the stability of the stands and also increases the overall biodiversity. In particular, positive effects were evidenced on marketable mushrooms production and on floristic diversity.

Key words: floristic diversity, mycological diversity, pine plantations, renaturalisation, silviculture.

Introduction

Black pine (*Pinus nigra* Arn.) has been one of the most frequently used conifer species for reforestation plantings during the 20th century in Italy, mainly in order to

protect land from erosion (Piermattei et al. 2012). However, this species has been commonly used for conservation purposes not only in Italy, but also in Central Europe, in the Balkans and in the other Mediterranean countries (Bussotti 2002).

Pine forests have been considered among the most suitable stands to favour the natural succession toward mixed forests rich of hardwoods (Cantiani et al. 2011). In this framework, the commercial value of the stands was not a priority.

In Central and Southern Apennine, Black pine stands were established during the post 2nd World War period, both to restore forest ecosystems degraded by over-exploitation and to afforest overgrazed pastures during World War I and II.

Black pine is a pioneer species widely planted across Italy due to its low mortality, rapid juvenile growth and low incidence of health issues. Nowadays in Italy Black pine occurs mostly in pure stands of even-aged forests (mean age: 50 years) and covers an area of 236,467 hectares (23 % of national conifers stands) (INFC 2005).

Black pine pure stands were usually planted at a density of 2,500 trees per hectare with a rotation period of approximately 90 years depending on the site characteristics. The common silvicultural management of Black pine even-aged forests consisted of clear cut with artificial regeneration using species with a higher ecological value (Cantiani and Chiavetta 2015). In pure Black pine stands, canopy closure occurs at an early age, therefore pre-commercial thinning around age 30, followed by additional thinning every 15 years, were considered essential for an appropriate management (Cameron 2002, Brüchert and Gardiner 2006).

It is a fact that thinning was rarely carried out in Black pine planted forests mainly because of the low commercial value of the timber assortments in the pre-commercial thinning, which consequently are a cost in the forest management.

Currently, in the majority of the cases, Black pine stands have reached their main objective and they are approaching

to the next successional phase. Conforming to the evolution of the forestry theories since the early 1990s, new management strategies were evaluated in order to promote the multifunctional and sustainable role of these stands. In this context, the treatments have been planned to favour stand growth and stability and, at the same time, the ecosystem complexity and biodiversity, with the consequent increase of protective, environmental, economic, and recreational values of the forest (Hartley 2002, Kerr 1999). Thus, the current silvicultural approach is aimed to favour a gradual succession by the establishment of natural regeneration (Nocentini 2013, Mercurio et al. 2009).

Experimental trials recently carried out by Council for Agricultural Research and Economics–Forestry research centre showed an encouraging reaction of Black pine stands to a first delayed heavy thinning (stand age: 30–35 years), which eliminated most of the dominated plants and included also some trees of the dominant layer. During the thinning more than half of the plants were cut in total (intensity >50 %). Black pine is a shade-intolerant pioneer species, and it revealed to be adaptable and reactive to this treatment. Canopy closure occurred very quickly (6–8 year after the thinning) and a strong incremental effect on diameter growth was observed (Cantiani and Piovosi 2009), during the following ten years no mortality resulted in the thinned stands.

On the basis of the positive results obtained by thinning from below of medium-heavy intensity (Bonet et al. 2011, Lindgren et al. 2006, Taki et al. 2010), a new experiment on selective thinning was set up. In selective thinnings, the best trees of the stand are selected according to vigour and stability, and

their growth and development is actively promoted by removing competitors in the dominant layer, whereas plants in the dominated layer are harvested only in case of economic convenience.

In this framework, the objective of this study was to compare the selective thinning, the traditional thinning (thinning from below) and no treatment (control) in Black pine stands in Central Italy (in Tuscany). The stands are included in the pilot areas of the project Life13BIO/IT/000282 SelPiBioLife – Innovative silvicultural treatments to enhance soil biodiversity in artificial Black pine stands.

The study was aimed to provide evidence to support the idea that a multifunctional forest management represents a valid alternative to timber production for pine stands. More specifically, the aim was to highlight that selective thinning can be effective not only on stability and growth to enhance wood production, but also on floristic and mycological biodiversity of the forest. The relationship of the stand structure (directly

related to the applied treatment) with the following aspects was evaluated: i) floristic diversity; ii) mycological diversity; iii) mushrooms marketable production.

Material and Methods

Study area

The study was carried out in Arezzo province (Tuscany, Central Italy), a mountain area where Black pine stands are widely spread (Fig. 1). In Tuscany, the total area of Black pine forests is 20,500 ha and the stands mean age is about 60 years. Pratomagno-Valdarno forest is located in the upper part of pre-Apennine ridge Massiccio del Pratomagno, prevalent South-West and West exposure. It occupies 3,300 ha, 500 of which are covered by Black pine reforestation (86 % are pure Black pine stands). The native geological formation is “Macigno del Chianti”, which is composed of layers of sandstone and

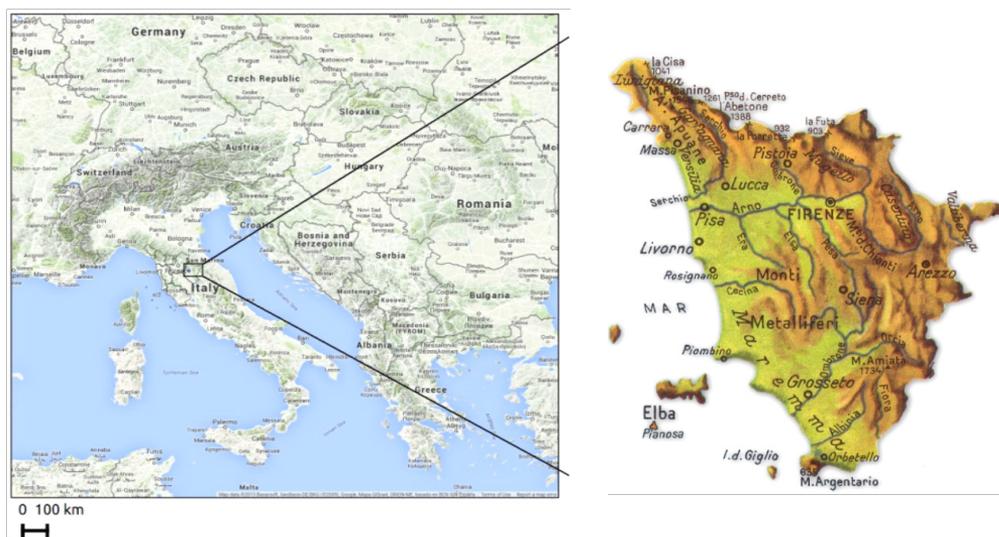


Fig. 1. Study area.

Table 1. Characteristics of the experimental sites.

| Site | Elevation a.s.l., m | Stand establishment | Geographical coordinates (UTM-WGS84) | Slope, % | Exposition |
|----------------|------------------------|------------------------|---|-------------|------------|
| La Baita (A) | 880 | 1956 | 43°34'53"N 11°42'47"E | 20 | SW |
| Scoiattolo (B) | 900 | 1950 | 43°36'11"N 11°41'33"E | 15 | W |

layers of siltstone and silt argillites. In such conditions soils are acid and rich in Potassium. The forest is spread over the temperate continental sub-Mediterranean bioclimatic zone according to Rivas Martinez classification (2004).

Rainfall follows the Apennine submontane regime, with a maximum peak in fall and a second peak in spring. Minimum precipitation occurs in July with a dry period between the end of July and the beginning of August. The annual rainfall is 897 mm. The mean annual temperature is 11.6 °C.

The experimental studies were carried out in young high-forest stand of Black pine (aged 56–58 years). The stand was a homogeneous and simplified ecosystem in relation to orography, geological formation and silvicultural treatments. Two sites were included in the experiment: La Baita and Lo Scoiattolo (Table 1).

Sampling was conducted in 2011–2012, during two vegetative seasons after harvesting, and the first one was characterized by an unexpected climate regime in comparison with long term observations. Comparing the thermo-pluviometric regime of the studied period (November 2011 – November 2012) with past long term observations (1995–2010), the total precipitation was lower in 2011 (109 mm vs 315 mm) and the mean temperature was higher (12.7 °C vs 11.5 °C).

Experimental design

In both sites three permanent experimental plots were set up, where three treatments were applied (one plot for each treatment): two different thinnings (traditional and selective) and one Control (no treatment, Table 2). The traditional thin-

Table 2. Applied treatment and mensurational parameters per plot.

| Plot | Treatment | Area, m ² | Age, years | Last har- vesting | <i>N</i> , ha ⁻¹ | <i>BA</i> , m ² ·ha ⁻¹ | <i>V</i> , m ³ ·ha ⁻¹ | <i>Dbh</i> , cm | <i>H</i> , m |
|------|---------------------|-------------------------|---------------|----------------------|-----------------------------|---|--|--------------------|-----------------|
| A1 | Thinning from below | 2000 | 56 | 2009 | 455 | 36.7 | 349 | 32.1 | 24.9 |
| A2 | Selective thinning | 2000 | 56 | 2009 | 405 | 37.2 | 361 | 34.2 | 25.7 |
| A3 | Control | 1400 | 56 | - | 1064 | 67.3 | 625 | 28.4 | 24.0 |
| B1 | Thinning from below | 1250 | 58 | 2009 | 866 | 59.1 | 625 | 29.1 | 24.0 |
| B2 | Selective thinning | 1250 | 58 | 2009 | 624 | 44.2 | 468 | 30.3 | 24.4 |
| B3 | Control | 750 | 58 | - | 1065 | 67.5 | 720 | 28.0 | 20.9 |

Note: *N*, ha⁻¹ – number of trees per ha; *BA*, m²·ha⁻¹ – basal area per ha; *V*, m³·ha⁻¹ – volume per ha; *Dbh*, cm – quadratic mean diameter; *H*, m – height of mean diameter tree.

ning consisted of a thinning from below, 45 % of the trees were removed, corresponding to 35 % of stand basal area. In the selective thinning, 100 plants in the dominant layer were identified to be favoured and competitors were removed in order to promote stand growth and stability, in this case thinning intensity was 53 %, corresponding to 40 % of stand basal area.

24 permanent sub-plots were identified inside the plots (4 per plot) to study mycological diversity and mushrooms marketable production. Each sub-plot was selected randomly, and the sub-plot surface varied from 300 m² (minimum threshold) to 364 m².

In each plot, during spring and summer 2011 and 2012, floristic diversity was sampled according to the Braun-Blanquet (1932) method in a central area of each plot, defined "vegetational sub-plot", in order to avoid edge effect. Plant species were identified and their abundance-dominance was assessed.

Data collection

In each plot the main dendrometric parameters of all the trees were measured, and each tree was assigned to a social crown class (3 classes: dominant, co-dominant and dominated) (Pretzsch 2009). On all trees the following variables were also measured: Diameter at breast height (*DBH*), Height (*H*), crown length, single crown cover (according to 4–8 crown radii), and plant coordinates (topographic position). Topographic position (azimuth and horizontal distance from plot centre) was determined with Field Map forest mapping software.

The variables (Table 3) were used to calculate two stand structural indexes (Neumann and Starlinger

2011, Pommerening 2002 and 2006): aggregation index *CE* (Clark and Evans 1954) and Vertical Evenness (Staudhammer and LeMay 2001). Furthermore all gaps inside the plots were identified and measured using software Q Gis. Gap was defined as proportion of openings more than 11 m² in an area, and is expressed as a percentage (Bottero et al. 2011).

Concerning mycological diversity, measurements on carpophores were taken during fall in 2011 and 2012 and the protocol included:

- weekly harvest of carpophores per sub-plot;
- carpophores species determination;
- α -diversity by Shannon index;
- trophic group distinction (mycorrhiza and saprophytes);
- fresh weight of soil and carpophore (per species);
- drying: soil – 24 hours at 100 °C, carpophores – 24 hours at 50 °C;
- dry weight of carpophores.

Vegetation cover (in total) and, separately, of tree layer and of herbaceous layer were also assessed. Moreover, the phytosociological classes of the species inside the vegetation sub-plots were recorded in order to define the different coenosis from an ecological point of view. Dichotomous-analytical key reported in Flora d'Italia (Pignatti 1982), in Nuova Flora Analitica d'Italia (Fiori 1923–1929), in Flore forestale française (Rameau et al. 1989), and in Flora Europaea (Tutin et al. 1964) were used to help in species identification.

To analyse the data, Braun-Blanquet values were transformed according to the Van der Maarel scale (1979). The Shannon index ($-\sum p_i \ln p_i$) was calculated to assess α -diversity of each vegetation sub-plot (p_i = relative frequency of the

Table 3. List of the stand structure diversity indices.

| Index | Equation | Variables | Range values |
|--|--|--|--|
| Aggregation index (CE) (Clark and Evans 1954) | $CE = \frac{r_A}{r_E}$ | $r_A = \frac{\sum_{i=1}^n HDist_j}{n}$ $r_E = \frac{1}{2} \sqrt{\frac{A}{N}}$ <i>HDist_{ij}</i> = Euclidean distance between <i>i</i> -th tree and its nearest neighbor <i>A</i> = plot area <i>N</i> = plot tree number | min value = 0 max value = 2.1491 CE = 1 → completely spatially random distribution CE < 1 → spatially clustered distribution CE > 1 → spatially regular distribution |
| Vertical Evenness index (VE) (Neumann and Starlinger 2001) | $VE = \sum_i^3 (-\log_2 \pi_i) \frac{\pi_i}{\log_2 3}$ | π_i = is the relative crown area of all trees in the <i>i</i> -th height layer | VE < 1 monostoried stands VE > 1 multistoried stands VE = 1 the vertical distribution of the plants belonging to different classes is homogeneous |

Note: CE – Index of aggregation; VE – Vertical Evenness index.

species, which in our study corresponded to relative abundance).

and mycological biodiversity were performed using the Spearman correlation test.

Statistical analysis

The data did not show a normal distribution, consequently non-parametric test were used (U-Mann-Whitney) to highlight statistical differences of the variables related to the mycological study, and the three different silvicultural treatments were considered as source of variation.

Correlation between stand structural indices and parameters related to floristic

Results

Results showed that the last thinning, operated in 2009, influenced stand structure features, as illustrated by the values of the structural indices reported in Table 4 (CE, VE, Gaps area, and Number of gaps). The plots horizontal structure was random, CE is >1 in four plots and the thinning sim-

Table 4. Values of stand structure diversity indexes after the thinning.

| Plot | Treatment | CE | VE | Gaps area, m ² | | | Number of gaps |
|------|---------------------|------|------|---------------------------|-----|-----|----------------|
| | | | | Mean | min | max | |
| A1 | Thinning from below | 1.46 | 0.37 | 28.6±14 | 12 | 64 | 17 |
| A2 | Selective thinning | 1.33 | 0.39 | 44.1±16 | 24 | 75 | 15 |
| A3 | Control | 1.22 | 0.62 | 35.4±15 | 14 | 61 | 14 |
| B1 | Thinning from below | 0.66 | 0.18 | 23.6±9 | 15 | 47 | 22 |
| B2 | Selective thinning | 1.40 | 0.63 | 28.8±12 | 13 | 53 | 15 |
| B3 | Control | 0.97 | 0.72 | 23.2±15 | 7 | 43 | 12 |

Note: CE – Index of aggregation; VE – Vertical Evenness index; Gaps area and number of gaps.

plified the vertical structure ($VE < 1$ in all plots). In the selective thinning plots a higher area of gaps was observed in comparison to below and control plots.

Stand structure is a factor strictly linked to eco-physiological parameters, which influence vegetation and carpophores development (light, temperature and soil humidity).

The Shannon index values recorded during 2001 and 2012 in all plots are shown in Figure 2. Both thinning treatments, from below and selective, caused a biodiversity increase in comparison with control plots from a floristic and mycological point of view. The Shannon index mean values were equal to 1.7 and to 1.6, respectively, for thinning from below plots and selective thinning plots,

while 1.5 was recorded in control plots. Concerning mycological diversity, higher values of the Shannon index occurred in thinned plots (0.92 for thinning from below plots and 0.87 for selective thinning plots) than in control (0.81).

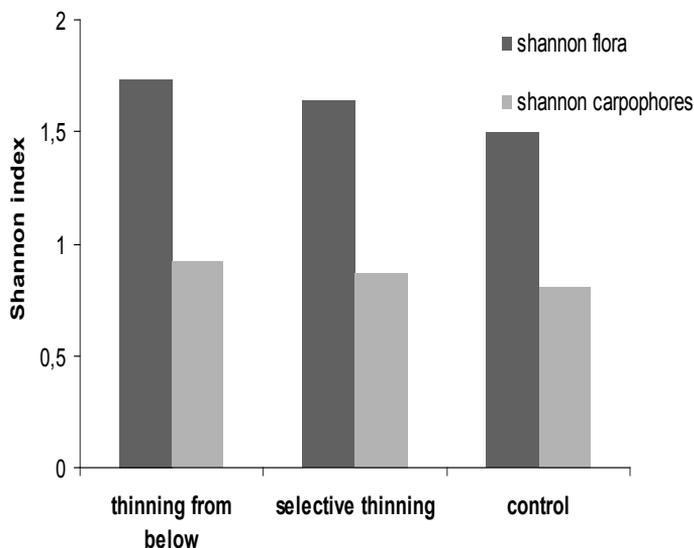


Fig. 2. The mean Shannon index values for flora and carpophores.

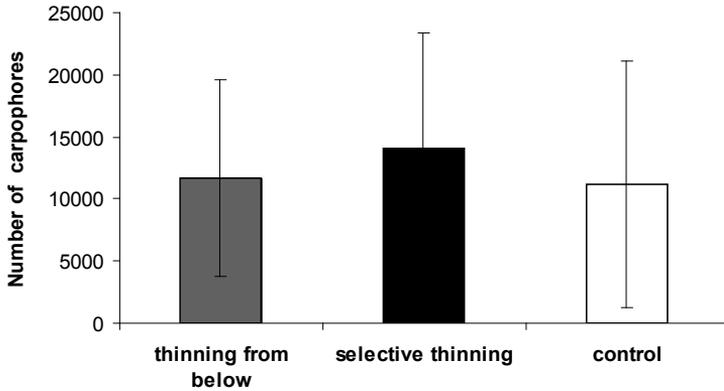


Fig. 3. Number of carpophores per treatment.

Both thinning treatments influenced positively fungal production as revealed by carpophores number. During the two studied years (2011 and 2012) carpophores mean number was 14,040 in selective thinning plots, 11,684 in thinning from below plots and 11,218 in control (Fig. 3).

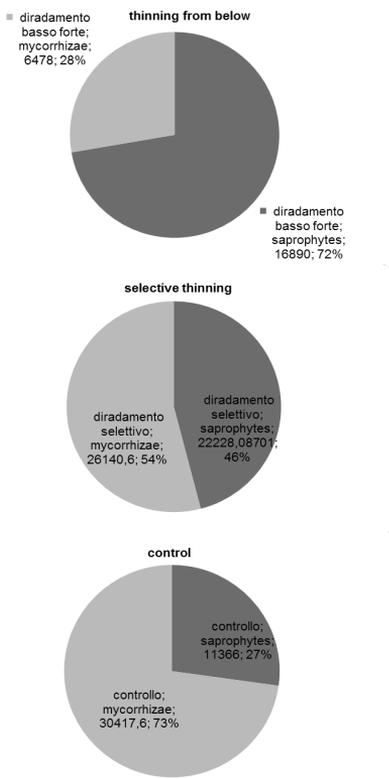


Fig. 4. Percentage of mycorrhizae and saprophytes per treatment.

Percentage of fungi species classified in trophic groups can be used as parameter to assess the health status of forest ecosystems. In more detail, the fraction of mycorrhizae species of total macromycetes is considered an effective index of external disturbance factors on a forest ecosystem: the higher is the disturbance level, the lower is the frequency of mycorrhizae. According to our results, the higher disturbance level was observed where thinning from below was applied. However, the studied Black pine stands, which were harvested more recently, are still affected by anthropic disturbance (Fig. 4).

The U-Mann-Whitney test results (Fig. 5a and 5b) showed that the difference in carpophores number between thinned stands (both treatments) and their respective control was significant. Statistical differences were also observed for this variable between selective thinning and control in both experimental sites (Fig. 5b).

The Spearman correlation test (Table 5) highlighted that floristic biodiversity

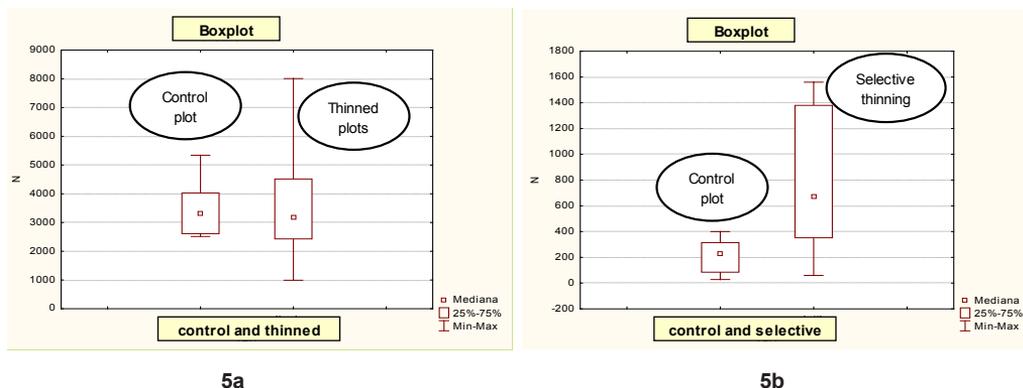


Fig. 5. The U-Mann-Whitney test results between thinned plots and control and between selective thinning and control (p level < 0.05).

and species number were significantly related ($p < 0.05$) to horizontal structure diversity index (CE), gap area, basal area (BA), and stand density (N). Concerning fungi, significant correlation ($p < 0.05$) resulted for total production (fungi dry weight) and: mean height (H), vertical stand structure index (VE), basal area (BA) and stand density (N).

Total 86 floristic taxa were totally recorded. More than 40 % of the species are typical for forest ecosystems, while the rest are characteristic to open habitats with plenty of sunlight.

A higher number of species (57) occurred where thinning from below was applied, whereas lower species richness was observed in the plots with the other two treatments (selective thinning 44, control 47).

In control plots higher number of species characteristic to *Quercus-Fagetea* and *Quercetea pubescentis* classes was observed. These classes include nemoral species, which are related to mesophyllous conditions of *Quercus-Fagetea* and to more

thermophyllous conditions of *Quercetea pubescentis*. On the other hand, in thinned plots the present species were characteristic of: sub-thermophile and sunny edges, open environments (perennial herbs), arid meadows (annual herbs) and mesophile meadows. Plots thinned from below resulted to be rich of these groups of species (35), while in the selective thinning plots and in control the number was lower, respectively 21 and 26.

Control plots included characteristic species of forest environments, as trees in juvenile stage and shrubs, and herbs such as *Brachypodium rupestre* (Host) R. et S.

Table 5. Pair-wise Spearman Rank Correlation Coefficient matrix among calculated indices (only significant correlation at $p < 0.05$ after Bonferroni's correction for multiple comparisons).

| Index | CE | gap | BA | N |
|----------------------|-------|-------|-------|-------|
| Flora species number | 0.005 | 0.003 | 0.004 | 0.002 |
| Shannon on flora | 0.004 | 0.003 | 0.003 | 0.003 |

Note: CE – index of aggregation; gap – proportion of total gap area on the total; BA – basal area; N – number of trees; VE – Vertical Evenness index; H – height of mean diameter tree.

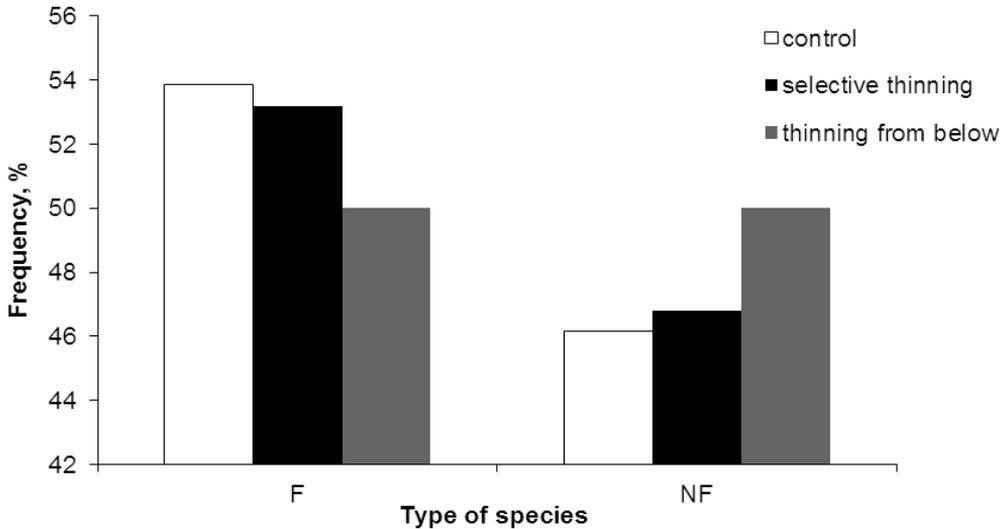


Fig. 6. Frequency of forest species (F) and non-forest species (NF) separated per treatment.

In thinned plots (both treatments), species like *Rubus canescens* DC., *Pteridium aquilinum* (L.) Kuhn and *Rumex acetosella* (L.), which are related to plenty of light and disturbance, were mostly found. Moreover high frequency of woody species was observed, as well as Gramineae *Agrostis tenuis* Sibth., which belongs to the *Molinio-Arrhenatheretea* class, characteristic of humid meadows.

Figure 6 shows the frequency (in percentage) in terms of presence/absence of recorded species, “forest” or “non-forest” mean a shrub or a herb characteristic respectively of forest or meadows, in terms both of frequency and abundance. Such kind of categorization is based on both literature (Pignatti 1982, Rameau et al. 1989) and field experience. The graph provides also information about the increase of non-forest species in thinned stands in comparison with control, especially for thinning from below plots. In selective thinning plots, such increase was not equally evident.

Discussion and Conclusions

According to our results, experimental trials on different kind of thinning in Black pine stands aged 60 provided useful information.

First, results showed that the silvicultural treatments were effective in influencing horizontal and vertical structure of Black pine stands.

It is a fact that thinning influences competition relationships among plants, changing not only stand stability but also ecosystem complexity. Thus it is important to support experiments on different thinning regimes where the objective is to implement the forest multi-functionality (biodiversity, wood production, soil protection) (Jonard et. al 2006, Skovsgaard 2009).

More specifically, further trials should be carried out in Italian Black pine stands, where the traditional routine operations were not applied because they were not financially sound convenient. For

these stands, the thinning regime should have included an early pre-commercial thinning and further pre-commercial thinning every 10–15 years. Actually, the common approach consisted of delaying the first thinning (mean age 30 years) and of extending the period between two consecutive operations (Cantiani and Chiavetta 2015).

This experiment was carried out in stands where the previous thinning from below, with intensity 30 % (common standard intensity in Italy), resulted to be not efficient to affect crown competition in the dominant layer and, consequently, it did not favour tree growth.

Our results showed that selective thinning can be efficient in stimulating tree growth and stability. Furthermore, this thinning type influences the crown cover, causing a different light regime (more gaps) and changes water and temperature conditions at soil level. Such reactions have positive effects on general ecosystem biodiversity and complexity, which show an increase, with direct consequences on fungi and flora biodiversity. The positive change of the soil ecological conditions favours the natural process of regeneration, and thus the ecological succession of the Black pine stands. The new stand structure after selective thinning offers a higher management flexibility. The selected individuals, which take advantage of eliminating their competitors, provide more stability to the whole stand even at an older age than that in a usual rotation. Concerning flora, both thinnings caused a rise of the non-forest species in comparison with control during the years after the disturbance operations: shade-intolerance species and, more in general, species characteristic of meadows increased the most. This

reaction has been observed where ecological conditions, especially in terms of light and temperature, change and favour annual and non-forest species at the expense of the species characteristic of forest environments (Mattioli et al. 2008, Gondard and Romane 2005). Biodiversity and floristic abundance is mainly related to stand horizontal structure and to the presence of the gaps. A higher area of gaps was observed in selective thinning plots.

Egli et al. (2010) observed a recovery during the fourth year after thinning, with low production during the first 3 years after thinning. These authors suggested that the effect of the temporary site disturbance caused by the intervention could be a possible explanation for these dynamics.

More time is necessary to evaluate whether the thinning effect on biodiversity will last long and to observe whether non-forest species are able to regenerate when the crown cover tends to close (Lust et al. 1998, Götmark et al. 2005). The future different effects on biodiversity of each treatment will also require more time to be assessed. Considering the multi-functionality of the studied stands, it will be possible to plan suitable thinnings aimed to ensure a constant level of biodiversity.

Structure changes have influenced positively fungi presence, in terms of carpophores number and biodiversity, and this is more evident in the thinning which affected more the micro-climate conditions at soil level (Egli et al. 2010). Biodiversity rise and fungi production are actually linked with the parameters of the stands vertical structure which were altered by the thinnings.

Mycorrhizae species percentage on total macromycetes is an effective indicator of external disturbance factors

on forest ecosystem: a lower percentage is associated with a higher disturbance level.

Results concerning mycorrhizae species percentage in relation to treatments showed that where no disturbance treatment occurred (control), a higher percentage of mycorrhizae was observed (73 % of carpophores number).

Comparing the two type of thinning, a higher frequency of mycorrhizae resulted where selective thinning was applied (54 % of carpophores number), in relation to the fact that this treatment localizes the disturbance only around the selected plants, while in thinning from below all surface is affected by the harvesting, with a consequent higher impact on the whole stand. A plausible hypothesis for future studies is that the temporal pattern of thinning effect on mushroom yield depends on the thinning intensity, light thinnings leading to immediate positive response, whereas intensive thinning may lead to short-term negative effects after which the mushroom yield may recover and increase.

A limit of this study is that we could observe the treatment effects during two vegetative seasons after harvesting, and the first one was characterized by an unexpected climate regime in comparison with long term observations. More extensive results will be provided thanks to the project Life13BIO/IT/000282 SelPiBioLife – Innovative silvicultural treatments to enhance soilbiodiversity in artificial Black pine stands, which will finance the future development of this study. Thus it will be possible to carry on the monitoring for a longer period post-thinning (4 years), in order to assess more in detail the response in terms of soil biodiversity in Black pine stands in two different zones of Tuscany.

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