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Research papers

Assessing seed regeneration in chestnut coppices: a methodological approach

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Abstract - Over the last decades, the abandonment of the traditional management due to many adverse factors caused a general aging of chestnut coppices; this led to an increased mortality of the chestnut stools and a consequent replacement with the entry of other species. Preservation and improvement of the chestnut coppice emphasize the importance of natural regeneration for future forest management: seed regeneration contributes to provide new stools for future coppice generations and promotes a proper development of the stand in terms of specific and structural diversity. In this study, we propose a method for investigating the relationship between density, diversity, development of natural regeneration and possible driving forces in terms of site conditions and stand parameters. For this purpose, a survey based on mixed sampling plots was conducted in different coppice systems (simple coppice, coppice with standards), 4÷8 years after the coppicing: measurements on stools, shoots and standards, as well as seed regeneration were carried out. Chestnut seed regeneration was characterized by taller individuals in simple coppice plots, even though the seedlings were fewer than those in coppice with standards treatment. Canopy cover and amount of standards, density of stools and resprouting shoots negatively influenced the establishment of chestnut seed regeneration: likewise, within the same treatment, plots with greater site index promoted the development of chestnut regeneration.
The proposed methods allowed a characterization of the dynamics related to the natural regeneration of classical chestnut coppice systems, identifying the main controlling factors. Among them, factors modifiable by management, such as stand structure and amount of standards, offer forest managers multiple silvicultural options to control seed regeneration processes.

Keywords - chestnut coppice; seed regeneration; sampling protocol; coppice system; standards

Introduction

In Europe, chestnut coppices for timber production cover 1.5 million hectares, corresponding to 66% of the total chestnut-growing area (Conedera et al. 2004). Traditional chestnut coppices consisted in short-rotation systems (5-25 years according to the targeted specific product) for the production of small- to medium-sized poles (Manetti et al. 2017), thus maximizing the very high resprouting capacity of the stools and the remarkable initial growth-rate of the shoots (Manetti et al. 2001).

Recently, shifts in the socio-economic structure of rural areas and in the timber market caused significant changes in the silvicultural objectives of chestnut coppices towards large size and high quality products. As a consequence, new silvicultural approaches emerged, aiming at high quality wood production by extending the rotation period (50-60 years) and applying silvicultural treatments to chestnut coppices growing on favorable site conditions (Amorini et al. 2000). Silvicultural treatments generally consist in early (starting at about 10 years), frequent (every 7 years) thinning from medium to high intensity (Manetti et al. 2006). At a mature stage, such stands display a high forest-like structure with many good quality stems. Recently, Manetti et al. (2016) proposed a similar but slightly different approach, named single-tree-oriented silviculture, based on the early selection (ca. 8-12 years, that is after the self-thinning phase) of 100-150 evenly distributed, dominant, well-shaped, vigorous and healthy target trees per hectare. Silvicultural management consists here in completely freeing up the crown of the selected candidates by eliminating direct competitors. Such intervention should be repeated two or three times every 4-6 years and should possibly be integrated by a progressive green pruning action of the lower stem part (5-7 m). Its aim is to improve timber quality and to stimulate free growth of the crown and a high and constant diameter increment allowing to reach a commercial size (DBH 30-60 cm according to local market conditions) within a reasonable rotation period from 25 to 45 years.

In less favorable growing conditions, the economic perspectives for coppices are very scarce:

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former coppice stands are often abandoned to a post-cultivation? natural evolution (Conedera et al. 2001; Pividori et al. 2005) or subjected to sporadic and silviculturally undefined harvesting activities by private owners. Abandoned chestnut coppices evolve towards mature and close stands with a reduced number of vigorous and stable stools (Vogt et al. 2006; Conedera et al. 2009) and a temporary loss in diversity, especially for rare species ecologically connected to coppicing or cultivation (Mullerova et al. 2015; Guitian, 2012).

Although coppice management mainly bases on the vegetative resprouting capacity of the stools, in the long run stimulating seed regeneration may contribute to increase stool density and to substitute old, exhausted or dead stools with young trees recruited from seed (vigorous, healthy and morphologically well-shaped candidates).

Unlike the bulk of scientific literature concerning resprouting capacity, productivity and functionality of coppice stands as a function of different rotation periods, thinning regimes and stand structure (Cutini, 2000; Gallardo-Lancho, 2001; Giudici & Zingg, 2005; Covone & Gratani, 2006; Gondard et al. 2006; Manetti et al 2009; Manetti et al. 2010; Zlatanov et al. 2013), very little is known about the potential and the driving factors of seed regeneration in chestnut coppice systems (e.g., Ott et al. 2003; Zlatanov et al. 2015).

The aim of this paper is to propose a methodological approach to assess the potential for seed regeneration in mature chestnut coppice stands. For this purpose, we conducted a preliminary survey in 4- and 8-years old coppices that differ in structure and growing conditions, looking for relationships between density, diversity and development of natural regeneration as well as possible driving forces in terms of site conditions and stand parameters.

Materials and Methods

Study sites and sampling design

The chestnut is a warm-temperate species that needs mean yearly temperature ranging between 8° and 15°C, a minimum rainfall between 600 and 800 mm and prefers well-drained soils (Conedera et al. 2016). Edaphic and microclimatic conditions at the growing site play a paramount role among the possible factors influencing seed production and germination as well as seedling survival in chestnut coppices. Whereas chestnut can regenerate in half-shadow conditions, seedlings need light and limited summer water stress to survive and grow (Ott et al. 2003).

Based on these considerations, we selected two different study areas corresponding to important chestnut cultivation regions in Tuscany (Fig. 1) and characterized by very different site conditions: Monte Amiata (Province of Siena) and Colline Metallifere (Province of Grosseto).

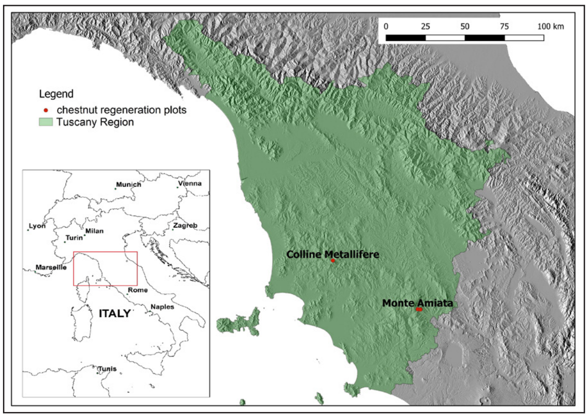

Figure 1 - Map of the two study areas located in Tuscany region (green layer): Colline Metallifere (CM) and Monte Amiata (MA).

Monte Amiata (1738 m a.s.l., hereafter referred to as MA) is an isolated mountain between the Apennines and the Tyrrhenian Sea and represents one of the most important chestnut areas in Tuscany. Chestnut stands devoted to wood production (both as coppices and high forests) cover 3534 ha between 800 and 1200 m a.s.l.. Silvicultural treatments vary according to different land ownership and site fertility: stands are subjected to early, frequent and medium intensity thinnings within a rotation period from 25 to 50 years on public lands (13% of the area), conversely stands managed by private owners denote shorter rotations (16-20 years) and no thinnings.

Colline Metallifere (hereafter referred to as CM) is the most extensive hill and mountain system of the Tuscan pre-Apennines. The total forest area covers 48000 hectares, where 80% (38400 ha) is represented by coppice stands, mainly oak. Chestnut coppices were common in the past but have declined to 1310 hectares at present. Most of them (90%) are private-owned and managed as coppice, frequently on rotation (18-20 years) without any thinning, whereas the remaining part is public and thus subjected to longer rotation periods (30 years).

The geological substrate differs considerably between the two sites: trachyte lava rich in silicates and poor in bases at MA, silty clay with siliceous limestone at CM. Thus, these soils belong to two different taxonomic categories (Tab. 1): GUA 1 - Andic Dystrudepts coarse-loamy, siliceous, mesic at MA e CBO1 - *typic ustorthents loamy-skeletal*, *mixed*, *calcareous*, *mesic*, *shallow* at CM (LaMMA Consortium, n.d.).

The climatic data (Fig. 2) recorded in the two study areas for the period 1993-2010, show a slightly cooler weather in MA (mean annual temperature

Tab. 1.- Soil classification in the two study areas (from Soil Map of Tuscany http://sit.lamma.rete.toscana.it/websuoli/).

Cartgraphic unit	Soil Taxonomy	Description
GUA 1	Andic Dystrudepts coarse-loamy, siliceous, mesic	Deep, Oe1A-Bw-C-R profile, very soft, not gravelly, sandy loam and loam texture, non-calcareous, from moderately to strongly acid, very low saturation, well drained
CBO 1	Typic ustorthents loamy-skeletal, mixed, calcareous, mesic, shallow	Shallow, A-AC-Cr-(R) profile, from gravelly to very gravelly and pebbly, clay loam and loam texture, from limestone to calcareous, from neutral to weakly alkaline, from well drained to moderately well drained

of 12.3 °C and annual rainfall of 1036 mm) than CM (mean annual temperature of 13.4 °C and annual rainfall of 827 mm).

Overall, 13 circular sample plots of 10 m in radius (9 at MA and 4 at CM) have been selected among coppice stands originated from former orchards and converted into coppice management in the early 1950s. At CM, all stands were managed as coppice with standards, whereas at MA 4 plots corresponded to coppice with standards and 5 to

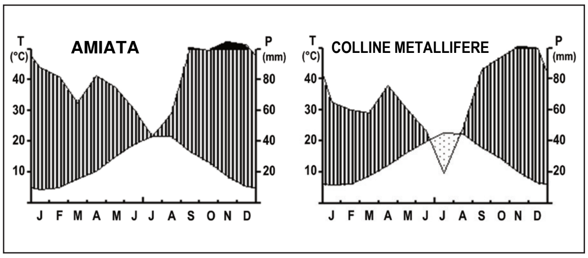

Figure 2 - Climate diagrams for the meteorological station Abbadia S. Salvatore (Monte Amiata - 829 m a.s.l.), Chiusdino (Colline Metallifere - 450 m a.s.l.), the closest meteorological stations to the study sites (period 1993-2010, Settore Idrologico Regione Toscana).

simple coppicing, falling thus into three different categories of plots: coppice with standards at CM (4 plots, hereafter referred to as CMM, where M stays for “matricinato” that is the Italian word for standards), coppice with standards at MA (4 plots, hereafter referred to as MAM), and simple coppice at AM (5 plots, hereafter referred to as MAS). At the coppicing time, the stands presented ages varying between 30 and 50 years; when the field survey was conducted (in 2015), shoot age rangd between 4 and 8 years (Tab. 2).

Stand characterization

Among stand-related factors influencing seed regeneration in coppice stands, we first considered those light conditions that influence both the probability of germination and the growth performances of seedlings, but also regulate water availability as well as vigor and composition of the competing shrub layer. We also assessed number and characteristics of the standards and the vegetative reproduction on stools that represent key factors in the competition for nutrients available in the soil.

The following elements related to stools and standards have been recorded for each 10 m circular sample plot: species, stools and standards social position (dominant, intermediate, dominated), number of living and dead shoots, stools and standards crown area, total height and crown height of the dominant shoot per stool and of all standards, diameter at breast height of standards (Tab. 3).

Furthermore, the following stand parameters were calculated through data pre-processing: tree species composition, canopy cover (stool crown area-StCA, standard canopy cover-StdCA), percentage of dominant stools (Dom), number of shoots per stools (Sh/St), density and height growth of stools (StN, StH) and standards (StdN, StdH), density of shoots (ShN) and standards basal area (StdBA).

Gamic regeneration assessment

For each 10 m circular plot, eight rectangular transects (5 m x 2 m) were placed in order to cover all main and intermediate cardinal directions (Fig. 3). In each transect, the presence, development, and vitality of seed regeneration has been assessed by collecting species, height and vitality of all seed-originated individuals.

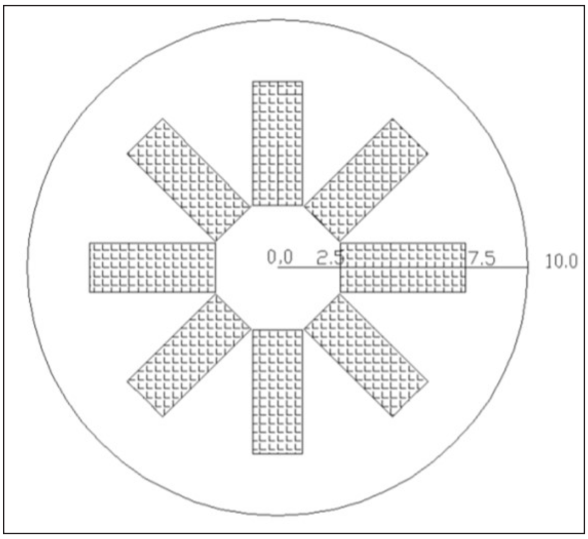

Figure 3 - Scheme of sampling protocol used for the collection of seed regeneration.

Tab. 2.- Site characteristics and basic information on past and present management recorded in the selected research plots. T in plot description = years from last coppicing; Elev. = altitude of sampling plot; Age in past management = age of shoots before the last coppicing. Rotation t = rotation time in present management (years between two successive coppicing).

ID	Site	T (yrs)	Elev. (m asl)	Slope (%)	Aspect	Past management	Age (yrs)	Present managment	Rotation t (yrs)
CMM1		7	823	30	NE		35		30
CMM2	Colline Metallifere	4	740	25	SW	Coppice with standards	30	Coppice With standards	30
CMM3		8	768	10	NW		30		30
CMM4		8	768	58	NW		30		30
MAM1		5	850	9	S		50		30
MAM2	Monte Amiata	5	850	9	S	Coppice with standards	50	Coppice with standards	30
MAM3		4	850	9	S		50		50
MAM4		6	1000	5	E		30		30
MAS1		5	850	9	S		50		50
MAS2		5	850	9	S		50		50
MAS3	Monte Amiata	5	850	9	S	Coppice with standards	50	Simple coppice	30
MAS4		5	850	9	S		50		30
MAS5		6	1000	5	E		30		30

Tab. 3.- Stand characteristics and related variables considered.

Key factor	Parameter	Details
Stand density (n•ha-1)	Number of standards; Number of stools; Number of living and dead shoots* per stool;	(*) All the shoots of at least 1.3 m in height;
Canopy cover (m2)	Crown area of standards; Crown area of stools;	Ground area covered by the vertical projection of crown perimeters;
Stand structure	Social position, total height and crown length of standards; Social position, total height* and crown length of stools;	Social classes: Dominant, Intermediate, Dominated (*) Height of the dominant shoot within stool

Data were then pre-processed in order to calculate the following indicators:

$$SH = -\sum_{i=1}^{N_s} p_i \cdot log\, p_i \qquad [1]$$

a) Diversity – expressed as number of species (Ns) and Shannon index (SH, Shannon-Weaver 1948):

N_s = number of species; $p_i = n_i/N$;
n_i = number of seedlings for the i species;
N = total number of seedlings.

b) Density - amount of seedlings per m^2 and per species (N_i) as well as overall total number (N_{tot});

c) Growth - mean height (H), height distribution (in three H classes: < 50 cm, 50-130 cm, > 130 cm), and vertical evenness (VE, Neumann and Starlinger 2001):

$$VE = -\sum_{i=1}^{3} p_i \cdot \log p_i \Big/ \log(3) \qquad [2]$$

$p_i = n_i/N$; n_i = number of seedlings in each height class;
N = total number of seedlings.

VE is a diversity index ranging from 0 to 1: values closer to 1 point out a similar number of seedlings in each class, values close to 0 describe a distribution where one class is totally prevailing. The third height class (H > 130 cm) corresponds to the saplings;

d) Regeneration Index (IR) - calculated for each transect and per species as the combination of seedlings density (N as n/m^2) and their mean height (H in cm): IR = N x H (Magini 1967).

Statistical analysis

Differences among treatments (CMM, MAM and MAS) on the measured variables were assessed applying t-test analysis and chi-square tests; correlation matrices were computed in order to investigate the relationships among the variables of the entire dataset. The previous analyses were performed by mean of the STAT 7.1 software.

A redundancy analysis (RDA) was performed using the R statistical software (version 3.4.3) and the Vegan library in order to discuss the influence of site conditions, standards and stand density on gamic regeneration. The RDA can be described as a constrained principal component analysis (PCA), where the ordination axes of the response variables are also constrained to be a linear combination of the predictor variables, so that the RDA axes represent the percentage of variance of the response variables explained by the predictors (Legendre and Legendre, 1998). In our case, the predictor variables selected were elevation, mean precipitation of July, standards rate (percent of shoots that were not harvested), as well as density of standards, stools, and shoots, respectively. Regeneration response variables were density and mean height of the seedlings (<50 cm) and the saplings (>=50 cm).

Differences in the distribution of the response variables according to the plot categories CMM,

Tab. 4.- Main parameters of stools (StN = number per hectare, Dom = percentage of dominant ones, StH±se = mean total height ± standard error, CA±se = mean crown area ± standard error) and shoots (ShN = number per hectare, Sh/St = number of shoots per stool) recorded in the selected plots in Colline Metallifere (CMM = Colline Metallifere, coppices with standards) and Monte Amiata (MAM = Monte Amiata, coppices with standards; MAS = Monte Amiata, simple coppices).

				Stools			Shoots
ID	Age (yrs)	StN (n ha-¹)	Dom (%)	StH±se (m)	CA±se (m²)	ShN (n ha-¹)	Sh/St
CMM1	7	446	50	5.1±0.3	12.65±2.22	11682	26.2
CMM2	4	2196	33	4.0±0.2	3.96±0.41	23969	10.9
CMM3	8	1273	35	7.4±0.2	9.24±0.81	9231	7.3
CMM4	8	882	54	8.1±0.4	18.15±2.73	9422	11.0
MAM1	5	796	28	7.6±0.4	16.42±2.72	10218	12.8
MAM2	5	1019	37	7.7±0.4	10.59±1.29	10568	10.4
MAM3	4	477	53	4.9±0.5	8.64±1.98	7639	16.0
MAM4	6	725	45	7.4±0.3	11.20±1.75	8350	11.5
MAS1	5	828	35	7.5±0.4	11.91±1.62	10568	12.8
MAS2	5	637	40	7.1±0.4	17.61±2.37	11943	18.7
MAS3	5	477	60	5.8±0.6	21.11±3.98	7862	16.5
MAS4	5	535	57	6.0±0.4	19.23±2.48	7690	14.4
MAS5	6	650	44	7.1±0.4	16.50±2.54	8738	13.4

AMM, AMS were tested for statistical significance with a non-parametric Wilcoxon rank-sum test.

Results

Coppice stands

At Colline Metallifere (CM), the number of stools ranges from 446 to 2196 per hectare, whereas at Monte Amiata (MA) stool density and its variability are lower (from 477 to 1019 stools per hectare) without differences between simple coppice (MAS) and coppice with standards (MAM) (Tab. 4).

The stools in MA are taller than those in CM. The average increment in height is 0.9 m per year at CM and 1.3 m per year at MA, without differences between MAS and MAM treatments.

Similarly, shoots density (ShN) and number of shoots per stools (Sh/St) show higher variability at CM (ShN = 9231÷23969; Sh/St = 7.3÷26.2) with respect to the MA (ShN = 7639÷11943; Sh/St = 10.4÷18.7), without any statistical difference between the two coppice management systems (MAM, MAS). Generally, stool crown area (StCA) is significantly lower ($p < 0.05$) with more variability (from 3.96 to 18.15 m²) at CM than in MA (from 8.64 to 21.11 m²); significant differences ($p < 0.05$) have been detected between MAS and MAM systems (mean stool crown area of 11.7 vs. 17.3 m², respectively).

The standards in both areas (Fig. 4), exceed in most cases the 90 units per hectare. Although the number of standards in both areas are greater at MA

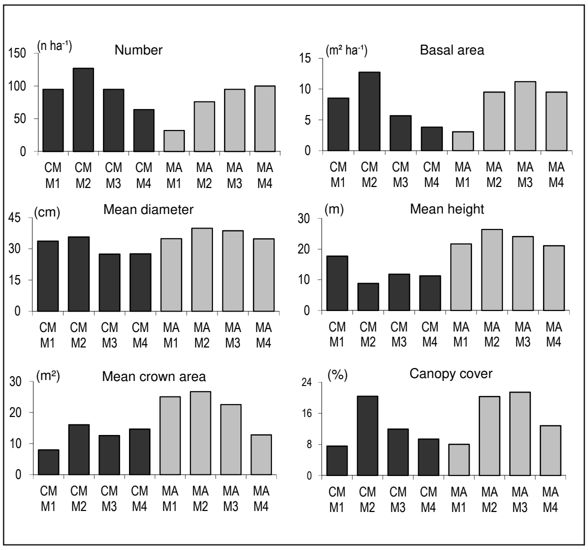

Figure 4 - Main mensurational parameters related to the standards released in Colline Metallifere (CMM sampling plots) and Monte Amiata (MAM sampling plots).

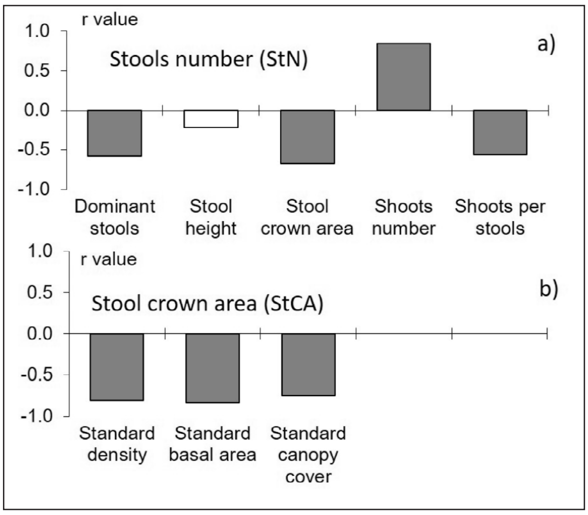

Figure 5 - Pearson correlations between: a) stools number (StN), b) stool crown area and the other explanatory variables related to the standards released. The significant correlations ($p < 0.05$) are marked in grey.

Tab. 5.- Descriptive statistics of the main regeneration variables (Ns = number of species; SH = Shannon index; Cs = number of chestnut seedlings per square meter; t = t-test with $p < 0.01$; Oth = number of seedlings of other species per square meter; All = number of total seedlings per square meter) in the three areas (CMM = Colline Metallifere, coppices with standards; MAM = Monte Amiata, coppices with standards; MAS = Monte Amiata, simple coppices).

	Diversity		Density (n m^{-2})					Height (cm)					Regeneration index		
	Ns	SH	Cs	t	Oth	All	t	Cs	t	Oth	All	t	Cs	Oth	All
CMM															
Mean	6.8	1.80	0.81		0.91	1.72		46.02		35.76	40,58		37.13	32.50	69.63
SD	----	0.93	0.32	A	0.60	0.54	A	32.83	A	47.59	41.60	A	19.82	37.42	55.94
Min	2	0.31	0.36		0.03	1.04		10		2	2		14.97	0.51	25.50
Max	11	2.47	1.06		1.30	2.36		260		230	260		62.27	84.40	146.60
MAM															
Mean	1	0.00	0.56		----	0.56		92.44		----	92.44		51.33	----	51.33
SD	----	----	0.13	B	----	0.13	B	60.45	B	----	60.45	B	21.60	----	21.60
Min	----	----	0.46		----	0.46		20		----	20		33.35	----	33.35
Max	----	----	0.75		----	0.75		380		----	380		82.65	----	82.65
MAS															
Mean	1	0.00	0.43		----	0.43		116.09		----	116.09		49.73	----	49.73
SD	----	----	0.08	B	----	0.08	B	96.19	C	----	96.19	C	15.37	----	15.37
Min	----	----	0.33		----	0.33		14		----	14		31.20	----	31.20
Max	----	----	0.54		----	0.54		470		----	470		71.64	----	71.64

than in CM, the only significant differences ($p < 0.05$) concerned total height values and crown area.

Significant ($p < 0.05$) positive correlations have been found between the StN and ShN ($r = 0.84$) whereas negative Pearson correlations were highlighted between StN and Dom ($r = -0.57$), StN and StCA ($r = -0.67$), StN and Sh/St ($r = -0.56$), as well as StCA and StdN ($r = -0.80$), StdBA ($r = -0.83$,) and STdCA ($r = -0.75$) (Fig. 5).

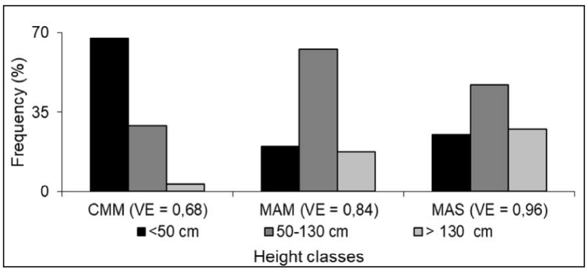

Figure 6 - Distribution of chestnut seedlings among the height classes in the study areas (CMM = Colline Metallifere, coppice with standards; MAM = Monte Amiata, coppice with standards; MAS = Monte Amiata, simple coppice). The resulting vertical evenness (VE) is reported in bracket in the legend of x-axis.

Seed regeneration

Whereas seed regeneration at MA is based mainly on chestnut seedlings, a great number of other species (i.e., *Quercus cerris* L., *Acer campestre* L., *Acer pseudoplatanus* L., *Fraxinus ornus* L., *Ostrya carpinifolia* Scop., *Populus tremula* L., *Pyrus pyraster* (L.) Burgsd., *Prunus avium* L., *Ilex aquifolium* L., *Pseudotsuga menziesii* (Mirb.) Franco, *Corylus avellana* L., *Abies alba* Mill., *Pinus nigra* Arnold) has been observed at CM. The Shannon index (null value at MA) ranges from 0.31 (two species) to 2.47 (11 species) at CM (Tab. 5).

Density of chestnut seed regeneration is significantly higher at CMM (0.81 seedlings·m^{-2}) than at MA (MAM: 0.56 seedlings·m^{-2}; MAS: 0.43 seedlings·m^{-2}), but no significant differences have been detected between the two coppice systems at MA (Tab. 5).

The average height of the chestnut regeneration increases from CMM (46.02±32.8 cm) through MAM (92.44±60.5 cm), to MAS (116.09±96.2 cm), with significant differences ($p < 0.01$) between CM and MA, as well as between the two management systems (MAM coppice with standard and MAS simple coppice). The Regeneration Index displays a similar trend without significant differences ($p > 0.05$) among areas and between coppice systems (Tab. 5).

The occurrence of seedlings in the three height classes (Fig. 6) shows unevenness in distribution in CMM (VE = 0.68) with a relevant percentage (67%) of seedlings lower than 50 cm and a small amount of saplings (3% of regeneration taller than 130 cm). In MAM (VE = 0.84) and in MAS (VE = 0.96) the distribution in height classes of regeneration is more even, although seedlings in the 50-130 cm class are dominant (63% in MAM e 47% in MAS, respectively). According to chi-square test, there are significant differences in height distributions

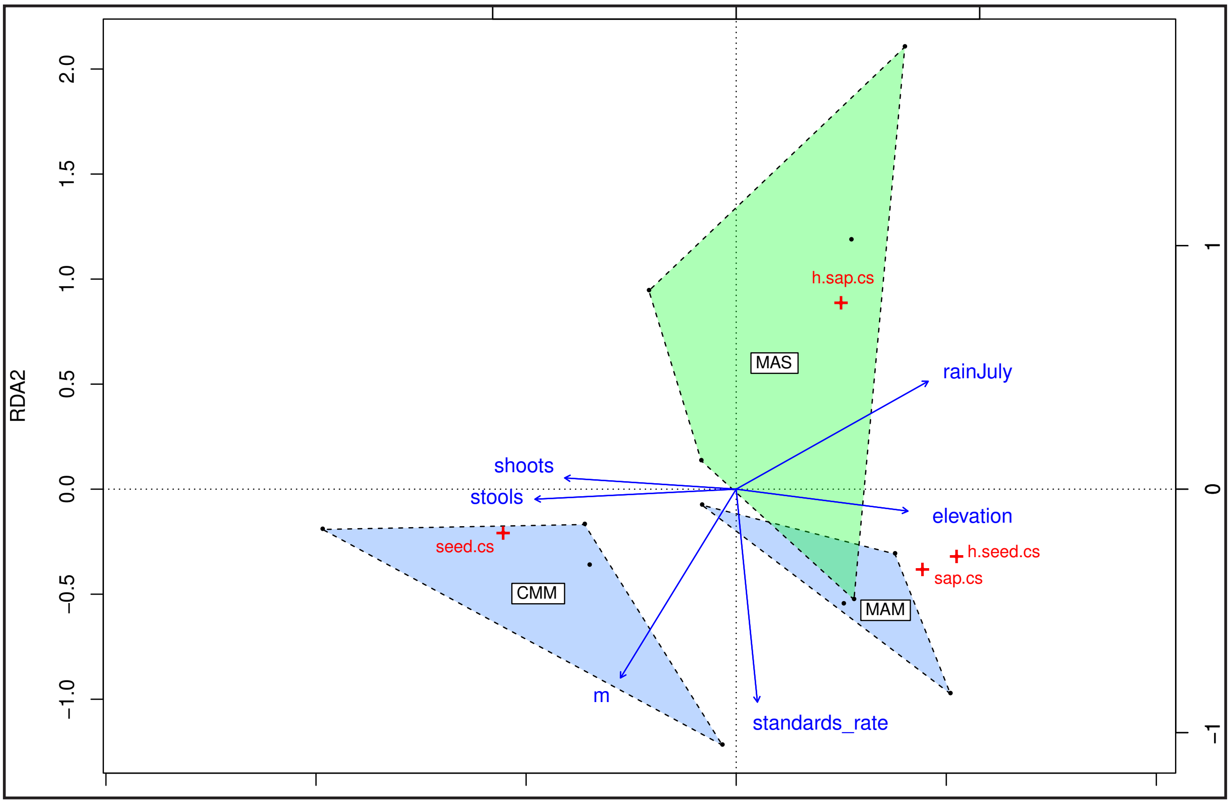

Figure 7 - Results of redundancy analysis (RDA). Black dots represent single sampling plots; shaded areas represent the convex hulls of the plot categories in ordination space (CMM = Colline Metallifere, coppice with standards; MAM = Monte Amiata, coppice with standards; MAS = Monte Amiata, simple coppice). Red dots represent response variables: density of chestnut seedlings (seed.cs) and saplings (sap. cs), and their respective mean height (h.seed.cs, h.sap.cs). Blue arrows represent predictor variables: elevation, mean rainfall in July (rainJuly), standards density (m), standards rate (percent of stools that were not harvested), stool density (stools) and shoot density (shoots) (i.e., potential fire drivers considered in this study). The length and direction of the arrows indicate the strength and the sign of correlation between predictor variables and the first two axes of the ordination space (RDA1 and RDA2), respectively.

among CMM, MAM and MAS categories. (chi^2 = 143; $p < 0.01$)

The first two canonical axes of the RDA analysis explain 64.1 % of the total variance of the response variables (RDA1 = 48.5 % and RDA2 = 15.6 %). The RDA plot shows a quite clear separation among the convex hulls of the different plot categories (Fig. 7). The first RDA axis is indeed mainly related to site conditions and stand density, while the second RDA axis reflects the characteristics of the standards (standards density and standards rate). Accordingly, RDA1 reflects different site conditions between the two study areas MA and CM, while RDA2 shows the transition from simple coppice to coppice with standards. While seedling characteristics and sapling density seem to be aligned on the first axis, height growth of chestnut sapling relates to the second axi

Seedling and sapling densities display two different trends: seedlings density is lower at MA with respect to CM, without any differences between the two silvicultural systems at MA. Conversely, the density of saplings shows significant differences only between CMM and MAM. The height of the saplings is significantly different between CM and MA, whereas seedlings measured in MAM treatment are significantly taller ($p < 0.05$) with respect to CMM and MAS coppicing systems (Fig. 8).

Finally, no random factors affecting seed regeneration, such as browsing damages, windthrow, heavy snowfalls or forest fires have been registered in our study areas.

Discussion

Factors driving chestnut gamic regeneration

The significant presence of seedlings beneath canopy generally indicates a potential for success in restoring a degraded stand by promoting natural regeneration (Kerr 2000; Mattioli et al. 2008, Johnson et al. 2009). After coppicing, the regeneration cover provides soil protection, reducing raindrops impact and mitigating excessive water erosion (Beasley & Granillo 1985). Furthermore, seed regeneration enhances the specific and structural diversity of the stand (Zlatanov et al. 2013), increasing its resilience to diseases and environmental stresses (Zlatanov et al. 2015).

The natural regeneration dynamic includes different processes, such as seed production, seed germination and seedling survival (Borghetti e Giannini 2003). These latter are influenced by different factors that may be grouped into fixed factors (i.e., not or

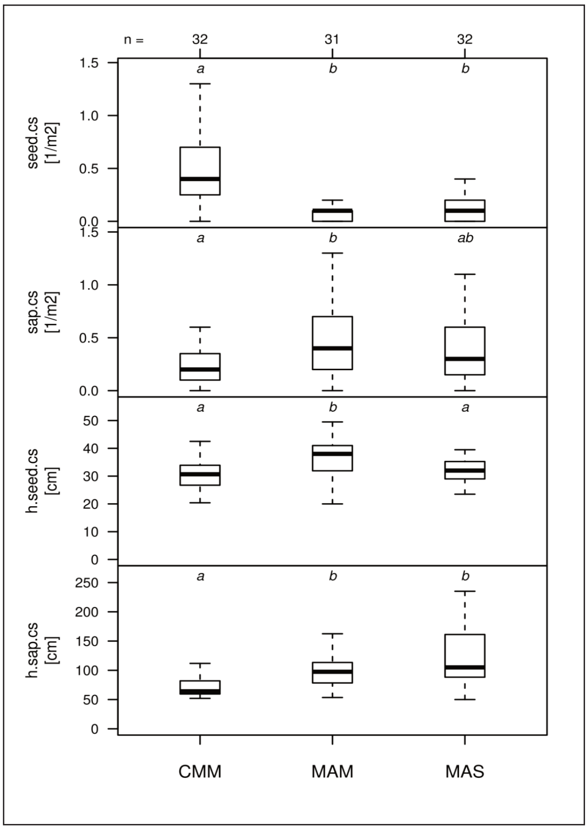

Figure 8 - Box plot of distribution of the response variables retained in the RDA with respect to the defined plot categories. Lines in bold represent the median. Boxes extend from the first to the third quartile, whereas whiskers include the smallest and the largest non-outlier points, namely, points within 1.5 times the interquartile range from the box. Different letters indicate significantly different distributions ($p < 0.05$, non-parametric Wilcoxon rank-sum test).

only hardly modifiable, such as site conditions and climate change - Blanco et al. 2009, Anderson-Teixeira et al. 2013, Petrie et al. 2016), modifiable factors (i.e., through management-induced amendments, such as stand structure and growing space availability - Sheffer 2012, Muscolo et al. 2014, Zhu et al. 2014) and random factors (i.e., natural disturbances and other abiotic and biotic stresses – Lahaire et al 2014, Wrobel 2014, Beguin et al 2016).

In this study case, fixed factors like site conditions, affected the two areas discriminating in terms of growth rate and seedlings survival of chestnut regeneration: this latter resulted clearly greater at MA (MA exhibited a lower seedling density but a higher number of sapling than CM), probably due to the better soil conditions and most suitable summer precipitation (July precipitation in particular).

In our case, modifiable factors were mostly represented by density and size of the standards (Fig. 4) that inhibit the height growth (Fig. 8) of regeneration at the sapling stage. A similar effect on density of chestnut saplings is exerted by stools and shoots density, which represents a gradient of increasing competition and decreasing availability of growing space for seed regeneration. At this purpose, it should be noted that in the study areas, standards always exceeded minimum number prescribed by Tuscany regional forest law which indicates 30 standards per hectare as the minimum number of stems to be released. However this requirement has been met only in one area (in MAM1 with 32 standards per hectare), whereas in most cases the number of standards reaches 90 units per hectare. High numbers of standards and wide crown areas (from 8 to 27 m^2) negatively affect stand development, reducing (even up to 80%) the space available for growth of both shoots and seedlings, while not providing significant additional seed inputs (Conedera et al. 2006; Manetti & Amorini 2012).

Other dependent outcomes such as species richness and overall regeneration density may result from multiple and interacting driving factors. In the past, management practices usually focused on chestnut species, whose growth was enhanced by favorable site conditions (as at MA site). As a result, seed regeneration consists now mostly of chestnut trees, because of both the suitable germination and growing conditions and the lack of tree seeders of other species. Conversely, species richness in regeneration is greater at CM, where the unsuitable growing conditions for chestnut trees induced an abandonment in the past decades that favored the colonization of the area by other tree species (Becagli et al 2010).

Methodological aspects

This methodological approach represents a compromise between field survey and stand inventory, useful to detect site conditions that drive chestnut seed regeneration in coppice stands as well as the characteristics of the resulting regeneration.

Despite the reduced number of plots considered, multivariate analysis allowed us to clearly identify the fixed (July precipitation, elevation) and modifiable factors (stool and standard densities) and their interactions affecting the dynamics of chestnut seed regeneration.

Conclusions and outlook

This study presents a methodological approach to characterize the existing regeneration in chestnut coppice stands and identify the role of possible driving factors. Among them, modifiable factors such as structure and density of standards provide multiple silvicultural options to forest managers to impact on seed regeneration processes.

The role of site fertility (i.e. chestnut growth indices) and water availability (soil water content), as well as a more detailed characterization of summer conditions at site-level (setting up local meteorological stations), remain unexplored.

Using the present field protocol, future research should focus on the interaction between seed generation and the competing and shadowing effect provided by the standards and the sprouts from stools, as well as the competition within chestnut seed regeneration and seedlings of other woody species.

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