

Combined effects of thinning and decline on fine root dynamics in a *Quercus robur* L. forest adjoining the Italian Pre-Alps

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- **Aims** Oak decline is a complex phenomenon, characterized by symptoms of canopy transparency, bark cracks and root biomass reduction. Root health status is one of the first stress indicators, and root turnover is a key process in plant adaptation to unfavourable conditions. In this study, the combined effects of decline and thinning were evaluated on fine root dynamics in an oak forest adjoining the Italian Pre-Alps by comparison of acute declining trees with non-declining trees, both with and without thinning treatment of surrounding trees.
- **Methods** Dynamics of volumetric root length density (RLD_V) and tip density (RTD_V), root tip density per unit length of root (RTD_L), diameter, branching index (BI) and mycorrhizal colonization were monitored by soil coring over 2 years as possible descriptors of decline.
- **Key Results** At the beginning of the experiment, the relationship between canopy transparency and root status was weak, declining trees having slightly lower RLD_V (−20 %) and RTD_V (−11 %). After a 1 year lag, during which the parameters were almost unaffected, BI and RLD_V, together with tip density, tip vitality and mycorrhizal colonization, became the descriptors most representative of both decline class and thinning. Thinning of declining trees increased RLD_V (+12 %) and RTD_V (+32 %), but reduced tip mycorrhizal colonization and vitality over time compared with non-thinned trees, whereas the opposite occurred in healthy trees, together with a marked decrease in branching. After thinning, there was an initial reduction in the structure of the ectomycorrhizal community, although recovery occurred about 10 months later, regardless of decline severity.
- **Conclusions** Decline causes losses of fine root length, and a moderate recovery can be achieved by thinning, allowing better soil exploration by oak roots. The close correlation between root vitality and mycorrhizal colonization and their deterioration after thinning indicates that decline does not benefit from reduced root competition, excluding the hypothesis of limited water and nutrient availability as a possible cause of the syndrome in this forest.

Key words: Fine roots, mycorrhizal colonization, oak decline, *Quercus robur* L., root length and tip density, thinning.

INTRODUCTION

Since the early 20th century, various types of forest in Europe have been stricken by a complex phenomenon called forest decline (Ragazzi *et al.*, 2000), including in pedunculate oak (*Quercus robur* L.) (Falk, 1924). However, only in the 1980s did its severity in this species intensify across the whole of Europe (Delatour, 1983), and the occurrence of dieback has been observed to increase progressively over time (Thomas, 2008; Denman and Webber, 2009). The causes are not completely clear yet, although the aerial symptoms, which include increased crown transparency associated with leaf yellowing and wilting, have been well described in the literature (e.g. Ragazzi *et al.*, 2000). Early leaf fall is often observed, together with the delayed appearance of new, short, small leaves. In the advanced stage of decline, some main branches may dry out, several epicormic twigs may grow and longitudinal cracks may appear along the trunk, resulting in wood colonization by several

organisms (Jakš, 2001; Jonsson, 2004; Montecchio *et al.*, 2004). However, fungal parasites and bark beetles are generally considered secondary contributing factors of decline (Manion and Lanchace, 1992; Capretti and Battisti, 2007). The phenomena are complex, and the presence of a few unmistakable symptoms has recently been confirmed at the acute stage of oak decline in *Q. robur* (Denman *et al.*, 2014; Brown *et al.*, 2016).

In roots, only a few symptoms have been recognized as being associated with decline. A decrease in root biomass and living roots has been observed in affected specimens of pedunculate oak (Causin *et al.*, 1996), associated with significant changes in the structure of the ectomycorrhizal (ECM) community (Wargo *et al.*, 1983). In this *Quercus* species, the number and abundances of ECM fungi have been found to decrease in acute declining trees (Mosca *et al.*, 2007).

In relation to their age, fine roots play an important role in water and nutrient uptake in woody species (De Silva *et al.*, 1999). Fine roots are often defined as roots with

diameters of < 2 mm, and they are mainly responsible for nutrient and water uptake. This definition has recently been debated (Pierret *et al.*, 2016), since this group of roots includes both absorptive and transport fine roots (McCormack *et al.*, 2015). Fine roots are naturally subject to rapid decay and renewal (turnover) over time, thus contributing to carbon and nutrient balances at the ecosystem level (Gill and Jackson, 2000). Fine roots account for almost 50 % of net primary annual below-ground production (Nadelhoffer and Raich, 1992), and several environmental factors and diseases can modify the dynamics of this natural process (Atkinson, 1991; Smucker, 1993; Eissenstat *et al.*, 2000). For instance, in coffee plants, Defrenet *et al.* (2016) found a relatively short fine root turnover ($1\text{--}1.25\text{ year}^{-1}$) in the top 0–0.3 m layer of soil, with a fine root biomass twice as high in a row compared with between rows, but no difference between sunny and shaded sites. Declining oaks are also expected to undergo substantial changes in root dynamics, potentially detectable in the medium term with minirhizotrons (Lopez *et al.*, 2001; Ponti *et al.*, 2004), although soil coring is still regarded as the reference method (Norby and Jackson, 2000; Hertel and Leuschner, 2002; Satomura *et al.*, 2007).

Root turnover is considered a key process in plant adaptation to unfavourable conditions. Fine roots adapt to local conditions, and their characteristics are expected to change in order to maximize functionality (Zadworny *et al.*, 2015). In water- and nutrient-rich environments, trees are expected to produce a network of relatively thin roots and a high specific root length (SRL) to enhance resource acquisition (Brassard *et al.*, 2009; Chen and Brassard, 2013), whereas in resource-poor stands, trees may favour roots with a greater diameter and a lower SRL to deliver a longer functional life span and reduced loss of resources through turnover (Yanai and Eissenstat, 2002). Early studies on forest oak decline across Europe have indicated root health status to be one of the first stress indicators, as the root system displays decline symptoms, such as reduction in fine root production and increased mortality, earlier than the canopy (Vogt *et al.*, 1993; Clemenson-Lindell and Persson, 1995).

Forest management may have an important role in attenuating oak decline. Thinning has been found to improve water availability and tree growth as a result of a longer growing period in *Quercus petraea* (Bréda *et al.*, 1995), and to increase evapotranspiration in *Q. robur* (Nardini *et al.*, 2003; Vincke *et al.*, 2005). Although there is scarce literature available on the effects of silvicultural treatments, significant above-ground ameliorations have been recorded in oak species after thinning (Juodvalkis *et al.*, 2005).

Against this background, the present study aimed at identifying the relationship between fine root status and oak decline syndrome, and at ascertaining if any changes in root dynamics occurred in the medium term following silvicultural treatment. The case study was a pedunculate oak forest (*Q. robur* L.) located in the Po plain, close to the Italian Pre-Alps. Seasonal variations in root length and tip densities, and in the number of living and ECM roots were investigated over a 2 year period by sequential soil coring. It was hypothesized that in declining oaks, competition for soil resources among neighbouring trees could be one cause of the syndrome, and that therefore the removal of competition through thinning may increase root production and the number of living tips. Four trees with acute symptoms of decline, referred to here as declining trees, and four trees with

moderate symptoms, referred to as non-declining trees, were considered in this study. Fine root dynamics were investigated following thinning treatment applied to two trees per decline class at the beginning of the study period.

MATERIALS AND METHODS

Experimental site

The trial was carried out in a declining oak wood called the 'Basalghelle Forest', which covers an area of 13 ha in the province of Treviso (NE Italy, 45°49'N, 12°31'E, 12 m a.s.l.). The stand is mainly *Quercus robur* L., representing approx. 42 % of the total area, and *Carpinus betulus* L., covering approx. 36 % of the understorey. In the remaining basal area, there are several hardwood species, such as *Sambucus nigra* L., *Staphylea pinnata* L. and *Corylus avellana* L., with scarce or no herbaceous vegetation. During the Second World War, the forest was clear-cut and the stand regenerated from the coppice. At present, most of the oaks are about 60 years old and show symptoms of decline to various extents. The soil profile is characterized by a dark brown loamy A₁ horizon (50 mm deep), followed by a mottled clayey B horizon of approx. 500 mm. In the 50–150 mm depth interval of the B horizon, the soil had a pH of 6.3, a 1.5 % organic carbon content (TOC), and high cation exchangeable capacity (CEC, 28.4 cmol⁺ kg⁻¹) and nitrogen availability (1.25 g kg⁻¹) (Table 1).

The site has a historical mean (15 years) annual temperature of 12.5 °C and precipitation of 705 mm, winter having a minimal amount of rain (approx. 80 mm) and similar values in the other seasons (approx. 250 mm). Temperature dynamics were similar in the two experimental years, with monthly maxima of approx. 30 °C in August and approx. 7 °C in February. Precipitation was higher than the historical mean, at 958 mm in 2005 and 973 mm in 2006 (Mosca *et al.*, 2007).

Treatments under study

A total of eight trees of similar size (approx. 50 cm in diameter at breast height; about 20 m in height) were chosen as representative of the two stages of decline, i.e. acute and moderate, here referred to as declining and non-declining classes, respectively. Mainly on the basis of canopy transparency (>50 % in declining trees, <20 % in non-declining trees), but also leaf discoloration, abundance of epicormic twigs, longitudinal cracks in the bark and the presence of wood-rot fungi, four trees were assigned to the non-declining class (class 0) and four to the declining class (class 1). Each tree was located quite far from the others (>50 m) and they were all therefore regarded as independent replicates.

In July 2004, silvicultural treatment was carried out around two trees in each decline class, resulting in the following four groups of trees: non-thinned non-declining (CL0-NT); non-thinned declining (CL1-NT); thinned non-declining (CL0-T); and thinned declining (CL1-T). Treatment consisted of removing canopy competition by felling all neighbouring trees within a radius of 8 m from the trunk of the trees under study; the trees were cut with a chainsaw at a height of 10 cm and removed with a tractor.

TABLE 1. Main chemical soil characteristics of the site in Basalghelle (Treviso, Italy) at the beginning of the experiment

	Soil layers A ₁ (0–50 mm)	B (50–150 mm)	B (150–250 mm)
pH (in H ₂ O)	6.25	6.25	5.8
CEC* (cmol ⁺ kg ⁻¹)	43.5	28.4	27.7
Exchangeable Ca ²⁺ (cmol kg ⁻¹)	1.9	1.05	1.1
Exchangeable Mg ²⁺ (cmol kg ⁻¹)	1.15	0.75	0.85
Exchangeable K ⁺ (cmol kg ⁻¹)	0.15	<0.1	0.1
Exchangeable Na ⁺ (cmol kg ⁻¹)	<0.1	<0.1	<0.1
TOC [†] (g kg ⁻¹)	41	15	13
Total N (g kg ⁻¹)	3.15	1.25	1.1
C/N	13	12	12

*Cation exchangeable capacity.

[†] Total organic carbon.

Soil sampling and root analysis

Around each tree (replicate), 12 soil cores (22 mm diameter, 10 cm deep) were collected at three distances from the trunk base (0.5, 1 and 1.5 m) in the four cardinal directions (N, S, E and W). Samples were taken every 3 months for 2 years, from July 2004 until January 2006, in order to investigate seasonal variations in root characteristics. Soil samples were placed in plastic bags and stored at –10 °C until processing.

For root analysis, each frozen soil sample was kept at room temperature for 6 h then washed in a beaker containing 1 % (w/v) sodium hexametaphosphate under horizontal shaking (1 h, 40 cycles min⁻¹). All roots in each sample, collected on 2 mm mesh sieves, were arranged in a Petri dish with enough water to allow the root tips to be observed and dissected under a stereomicroscope (magnification ×10–50). Root vitality and mycorrhizal colonization were assessed on ten root fragments randomly chosen as representative of each sample. To take into account the different sizes of root samples, indices of tip vitality (LRT, living root tips) and mycorrhizal colonization (MRT, mycorrhized root tips) revealed on the ten root fragments were standardized on the total root length of the same sample, so that they refer to the number of tips per centimetre of root length, i.e. S-LRT and S-MRT, respectively.

Roots of plant species other than *Q. robur*, identified by their morphological traits, occasionally recovered with soil sampling were discarded. Among the neighbouring species of the shrub layer, only *C. avellana* is affected by ECM colonization, but its roots are different in colour and morphology from those of the pedunculate oak, which are reddish-brown.

The entire root sample was then stored in ethanol solution (50 %, v/v) at 4 °C for analysis of root parameters. Root images were taken through a flatbed scanner (Epson Expression 10000 XL, Canada) and analysed using WinRHIZOTM software (Regent Instruments Inc., Quebec, Canada), which allows the following root parameters to be automatically assessed: length, number of tips, average diameter and number of forks (ramifications). Root length and number of tips were standardized on soil sampling volume, yielding volumetric root length density (RLD_V, cm cm⁻³) and root tip density (RTD_V, number cm⁻³), respectively. Average diameter (AD) was expressed in micrometres, and the branching index (BI) was calculated as the number of forks per unit length of roots (number cm⁻¹). A further index of tip density was determined by dividing the number of tips in a sample by root length (RTD_L, number cm⁻¹).

Data analysis

Before statistical analysis, data normality was tested with the Kolmogorov–Smirnov test. As the data did not satisfy this assumption, RLD_V, RTD_V, RTD_L, AD and BI were log transformed, and S-LRT and S-MRT were arc-sine transformed before data analysis with the SAS program (SAS Systems, SAS Institute, Cary, NC, USA).

The significances of sampling distance (from the trunk) and direction (along the cardinals) were tested by analysis of variance (ANOVA), and differences among means were checked with post-hoc analyses by Bonferroni's test.

To test the effects of decline class and thinning on root parameters, a model-fitting approach was used in both multivariate and univariate analyses. The experimental set-up indicated that a nested model should be used and the effects of the two fixed factors (i.e. decline class and thinning) were tested during analysis, considering individual sampling data as replicates (experimental units) and the factor tree – nested in decline class and thinning – as the error term.

A univariate ANOVA was carried out on data collected before thinning (July 2004) to test for differences between decline classes.

As the same variables were measured repeatedly over time (sampling dates), the effects of factors were subjected to a repeated-measures ANOVA [General Linear Model (GLM) procedure] to test the following three hypotheses: (1) no factor effect; (2) no time effect; and (3) no decline class or thinning × time interactions.

Univariate analyses were carried out for each sampling date; Bonferroni's test and a partial correlation analysis were used to check statistical differences among the four treatments and among sampling dates.

To highlight possible variations in root parameters over time and to take pre-thinning differences into account, data were normalized on initial values (July 2004) and interpolated with linear regressions for each treatment. After checking the significance of the regression coefficients, data autocorrelation was verified with the Durban–Watson test.

Principal component and discriminant analyses

In order to facilitate interpretation of root responses, the data set was subject to multivariate statistical analyses, by principal

TABLE 2. Mean values of root descriptors, RLD_V (volumetric root length density, $cm\ cm^{-3}$), RTD_V (volumetric root tip density, $no.\ cm^{-3}$), AD (average diameter, μm), BI (branching index, $no.\ cm^{-1}$ of root) and RTD_L (root tip density standardized on root length, $no.\ cm^{-1}$) in pedunculate oak trees over a 2 year period from pre-thinning date (July 2004), and coefficients of variation (CV)

Descriptor	RLD_V				RTD_V				AD				BI				RTD_L			
	CL0		CL1		CL0		CL1		CL0		CL1		CL0		CL1		CL0		CL1	
Treatment	NT	T	NT	T	NT	T	NT	T	NT	T	NT	T	NT	T	NT	T	NT	T	NT	T
Jul 04	1.51 ^a	1.35 ^a	1.18 ^a	1.10 ^a	6.94 ^a	7.05 ^a	6.66 ^a	5.73 ^b	640 ^b	808 ^a	599 ^b	736 ^a	6.57 ^a	5.93 ^a	6.32 ^a	6.33 ^a	4.79 ^b	4.69 ^b	5.04 ^a	4.48 ^b
Oct 04	1.51 ^a	1.50 ^a	1.24 ^a	1.07 ^a	7.20 ^a	7.17 ^a	6.63 ^a	6.48 ^a	658 ^a	656 ^a	615 ^a	694 ^a	6.60 ^a	6.01 ^a	6.18 ^a	6.58 ^a	4.81 ^b	4.88 ^b	5.31 ^b	6.01 ^a
Jan 05	1.28 ^a	1.41 ^a	1.06 ^b	1.06 ^b	5.76 ^a	6.35 ^a	5.91 ^a	4.39 ^b	705 ^a	681 ^b	741 ^a	716 ^a	4.73 ^b	6.10 ^a	6.06 ^a	6.69 ^a	4.19 ^b	4.45 ^b	5.27 ^a	4.16 ^b
Apr 05	1.71 ^a	1.68 ^a	1.61 ^a	1.22 ^b	7.42 ^a	6.93 ^b	6.88 ^b	5.65 ^b	667 ^a	685 ^a	635 ^a	663 ^a	7.20 ^a	5.95 ^b	6.59 ^a	6.22 ^a	4.42 ^a	3.77 ^b	4.30 ^b	4.60 ^a
Jul 05	1.52 ^a	1.37 ^a	0.85 ^b	1.12 ^b	7.24 ^a	5.09 ^b	4.86 ^b	5.12 ^b	659 ^b	816 ^a	634 ^b	737 ^a	6.88 ^a	4.43 ^b	6.07 ^a	6.13 ^a	4.83 ^a	3.46 ^b	4.47 ^a	4.33 ^a
Oct 05	1.54 ^a	1.05 ^b	0.96 ^b	0.91 ^b	7.04 ^a	4.16 ^b	4.15 ^b	4.32 ^b	653 ^a	682 ^a	584 ^b	668 ^a	7.08 ^a	5.08 ^b	6.15 ^a	6.46 ^a	4.64 ^a	3.48 ^b	4.44 ^a	4.90 ^a
Jan 06	1.53 ^a	0.88 ^b	1.18 ^b	1.08 ^b	5.94 ^a	3.49 ^a	4.89 ^a	4.62 ^a	640 ^b	808 ^a	599 ^b	736 ^a	6.60 ^a	6.01 ^a	6.18 ^a	6.58 ^a	3.95 ^a	4.19 ^a	4.37 ^a	4.28 ^a
CV (%)	8.3	21.3	21.7	9.9	9.8	26.1	18.9	15.5	12.1	19.9	15.6	15.5	12.7	15.8	4.2	6.1	7.6	13.96	9.4	13.59

Within the same date, different letters indicate statistically significant differences for each descriptor (Bonferroni's test, $P \leq 0.05$).

component analysis (PCA) to reduce the number of variables, and by discriminant analysis (DA) to identify common data distribution patterns. The second year data set, from July 2005 to January 2006, was analysed in order to better highlight the effect of tree thinning and decline. Before analysis, data were standardized by subtracting the mean and dividing by the standard deviation within each variable.

Factorial DA [multigroup discriminant analysis (MDA), with Wilks' lambda and Pillai's trace tests] and PCA allowed the root characteristics of the two decline classes and their responses to thinning in terms of variations in RLD_V , RTD_V , RTD_L , BI , $S-LRT$ and $S-MRT$ to be described. Multivariate data normality was preliminarily verified by the Shapiro test. All analyses were performed with R software (2013) and MS Excel XLSTAT (Addinsoft, Paris, France).

RESULTS

Root status at the beginning of the experiment and its evolution

Within each group of trees (treatment), no differences were found among the 12 sampling positions (four cardinal directions \times three distances from trunks) for any of the root parameters; therefore, sampling positions could be used as replicates.

Mean values of the root descriptors were calculated for treatments on each date over the whole study period (see Tables 2 and 3). Before thinning (July 2004), there were no significant differences between decline classes with respect to RLD_V or BI . Lower RTD_V was found in declining trees having undergone thinning treatment (CL1-T), while thinned trees of both decline classes had higher diameters (ADs) (Table 2). This initial examination could not clearly identify significant differences between the roots of the two decline classes, although declining trees (CL1) had slightly lower RLD_V (mean \pm s.e. $1.14 \pm 0.12\ cm\ cm^{-3}$ vs. 1.43 ± 0.35 , -20%) and RTD_V (6.2 ± 0.7 vs. $7 \pm 1.3\ tips\ cm^{-3}$, -11%).

Three months after thinning (October 2004), no differences were found among the four groups of trees for any of the root descriptors, with the exception of RTD_L and BI , which increased in declining trees (CL1). Some significant differences among treatments emerged on subsequent sampling dates. At

the end of the experiment (January 2006), although non-thinned non-declining trees had the highest RLD_V , thinned trees of both decline classes had higher root diameters than non-thinned controls ($P < 0.05$).

Over the 2 year study period, the most representative (significant) root traits indicative of declining status were RLD_V (three times), $S-LRT$ (three times), particularly in the second part of the experiment, $S-MRT$ (twice) and AD (once; October 2005) (Table 3). The parameters most sensitive to thinning were RLD_V , RTD_V and BI , which each turned out to be significant five times (Table 3). Among sampling times, the maximum number of significant root descriptors highlighting differences in declining intensity and silvicultural treatment were observed in October 2005, almost at the end of the study period. However, the importance of multiple root descriptors became evident about 1 year after thinning. On all sampling dates, the 'tree effect' was significant for RLD_V and RTD_V ; the least affected parameter was root diameter (AD).

Modelling the dynamics of root parameters

After data normalization on initial values (July 2004), non-thinned non-declining trees (CL0-NT) had stable RLD_V values, whereas all the other treatments displayed a negative trend (Fig. 1A). Only in the first part of the study period did thinning have a positive influence on RLD_V in the non-declining trees followed by a marked reduction, whereas there was a slight recovery in the declining trees (CL1) on the last sampling date of the experiment.

The dynamics of RTD_V and RLD_V (Fig. 1B) were similar, both having stable values for almost 1 year followed by a marked decrease under almost all treatments, with the exception of non-thinned non-declining trees. Removal of competition had a clear effect on RTD_V in the second year, with benefits for the declining trees and a progressive reduction in non-declining trees. When the number of tips was standardized on root length (RTD_L), variations from initial values were less marked but similar to RTD_V , with thinning reducing this index (i.e. leading to longer roots) in non-declining trees and vice versa in acute declining trees (Fig. 1C). A similar trend was also recorded for root branching (BI), highlighting a reduction

TABLE 3. Univariate analyses calculated for each root parameter in pedunculate oak trees at sampling dates across 2 years

Period	Between factors	RLD _V	RTD _V	AD	BI	RTD _L	S-LRT	S-MRT
July 2004	Class							
	Treatment			***			**	
	Class × Treatment						*	
	Tree × Class × Treatment	**	**				*	***
October 2004	Class					***		
	Treatment	*		*				
	Class × Treatment							
	Tree × Class × Treatment	***	***		***	**	**	***
April 2005	Class							
	Treatment	**	*		**			
	Class × Treatment	*	**			*		
	Tree × Class × Treatment	***	***		***	**		
February 2005	Class					*	**	
	Treatment	**	**		**			*
	Class × Treatment					**	*	
	Tree × Class × Treatment	***	***		***	***	**	***
July 2005	Class	*					*	*
	Treatment		**	***	***	***		
	Class × Treatment	*	***		***	***		*
	Tree × Class × Treatment	***	***	***	***	**		***
October 2005	Class	***	*	**		*	*	***
	Treatment	**	***	***	**		***	
	Class × Treatment	**	***		***	**		
	Tree × Class × Treatment	***	***	***	***	*	***	
January 2006	Class					**		
	Treatment	***	***		**			
	Class × Treatment	*	*	*	**			**
	Tree × Class × Treatment	***	***		**	***		***

Significance at * $P \leq 0.05$, ** $P \leq 0.01$ and *** $P \leq 0.001$.

in the number of forks in thinned non-declining trees, especially after 1 year of study (Fig. 1E).

Root diameter dynamics (AD; Fig. 1D) were sensitive to both declining level and thinning. Although roots were subject to a general marked reduction in diameter on the last sampling date (January 2006), in the first year there was an increase in the root diameter of non-thinned trees, regardless of the intensity of decline, but a gradual reduction in the root diameter of thinned plants.

Linear regression of the above trends in root parameters yielded regression coefficients that were never significant for CL0-NT, whereas CL0-T had significant negative trends for RLD_V, RTD_V and RTD_L, suggesting a negative effect of thinning in healthy trees. For severe decline class CL1, the trend was negative for RTD_V and RTD_L in non-thinned trees, and was negative for RTD_V, AD and BI in thinned trees (Table 4). However, in declining trees, thinning exerted a positive effect on RLD_V and RTD_V, the regression coefficients of their trends being increased by 12 and 32 %, respectively.

Root vitality and mycorrhizal colonization

Two indirect parameters, S-LRT and S-MRT, were used to evaluate the health status of trees and the effects of thinning on root tip vitality and mycorrhizal colonization (Fig. 2). As regards living root tips (S-LRT), there were no differences among the groups of trees until April 2005, whereas there was an evident increasing trend in non-thinned trees compared with thinned trees, regardless of the class of decline (Fig. 2A, C).

S-MRT followed an increasing trend over time in all treatments in the second observation year, especially in declining trees. Thinning had contrasting effects, increasing root tip mycorrhizal colonization over time in healthy trees but reducing it in declining trees (Fig. 2B, D).

A repeated-measures ANOVA for between-factor effects (Table 5) showed that decline class was significant ($P < 0.05$) for RLD_V, S-LRT and S-MRT. The thinning effect was marked on RLD_V, RTD_V and AD ($P < 0.0009$), and moderate on BI ($P < 0.019$). The interaction between decline class and thinning was significant for RTD_V and BI ($P < 0.05$). In addition, trees nested in decline class and thinning significantly affected all root descriptors. A repeated-measures ANOVA for within-factor effects and interactions confirmed this significance. There was a highly significant effect of sampling time on all root descriptors, with the exception of S-LRT. The interaction time × decline class was also significant ($P < 0.05$) for AD and S-MRT, and highly significant for BI. The time × thinning interaction also affected all root descriptors, except S-MRT, whereas AD and S-LRT were the only descriptors not influenced by interactions with time. All root descriptors were affected by the time × plant factor interaction.

Results from PCA and DA in the second year

The PCA of the root parameters of the last three sampling times together (July 2005–January 2006) identified two significant dummy variables, which explained >86 % of variability

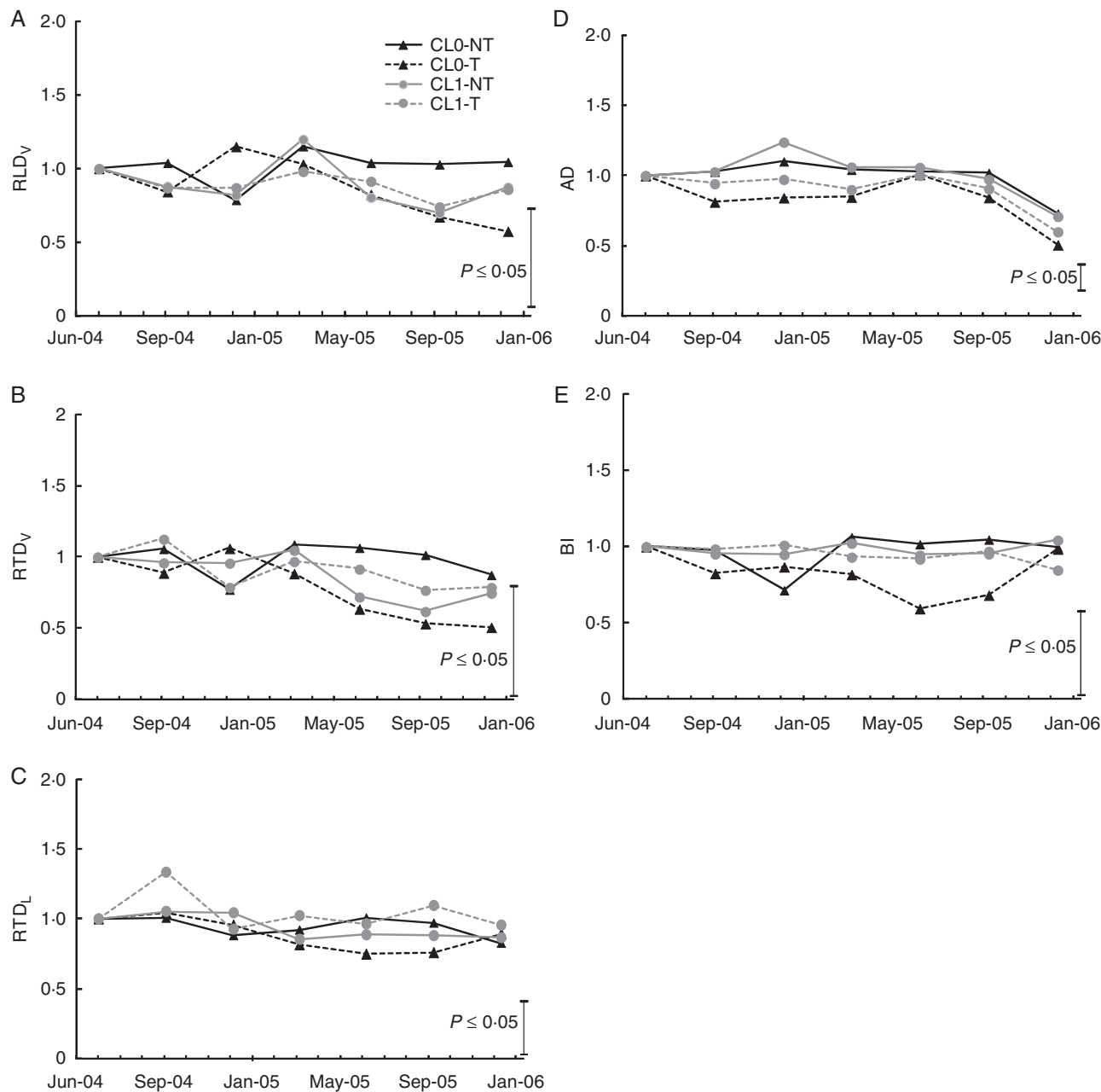


FIG. 1. Dynamics of volumetric root length density (RLD_v; A), volumetric root tip density (RTD_v; B), standardized root tip density (RTD_L; C), average diameter (AD; D) and branching index (BI; E) in pedunculate oak trees. Data are normalized by pre-thinning values (July 2004). Vertical bars: LSD ($P \leq 0.05$) for 'treatment × sampling date' interaction ($P \leq 0.05$).

TABLE 4. Regression coefficients and their significances in linear regression (over time) for root parameters normalized by pre-thinning values (July 2004) in the four groups of trees

	RLD _v		RTD _v		AD		RTD _L		BI	S-LRT		S-MRT	
CL0-NT	1.3×10^{-4}	n.s.	-7.6×10^{-5}	n.s.	-3.3×10^{-4}	n.s.	-1.9×10^{-4}	n.s.	1.5×10^{-4}	n.s.	1.1×10^{-3}	n.s.	5.6×10^{-4} *
CL0-T	-7.3×10^{-4}	*	-9.8×10^{-4}	**	-5.1×10^{-4}	n.s.	-4.0×10^{-4}	*	-2.1×10^{-4}	n.s.	-2.6×10^{-4}	n.s.	1.7×10^{-3} *
CL1-NT	-2.8×10^{-4}	n.s.	-6.2×10^{-4}	*	-4.3×10^{-4}	n.s.	-3.4×10^{-4}	*	4.9×10^{-5}	n.s.	1.8×10^{-3}	*	2.6×10^{-3} **
CL1-T	-2.5×10^{-4}	n.s.	-4.7×10^{-4}	*	-4.8×10^{-4}	*	-1.9×10^{-4}	n.s.	-2.2×10^{-4}	*	5.6×10^{-5}	n.s.	9.1×10^{-4} n.s.

Values in bold show significance for data autocorrelation (Durbin–Watson test, $P \leq 0.05$).

*Significant at $P \leq 0.05$; n.s., non-significant.

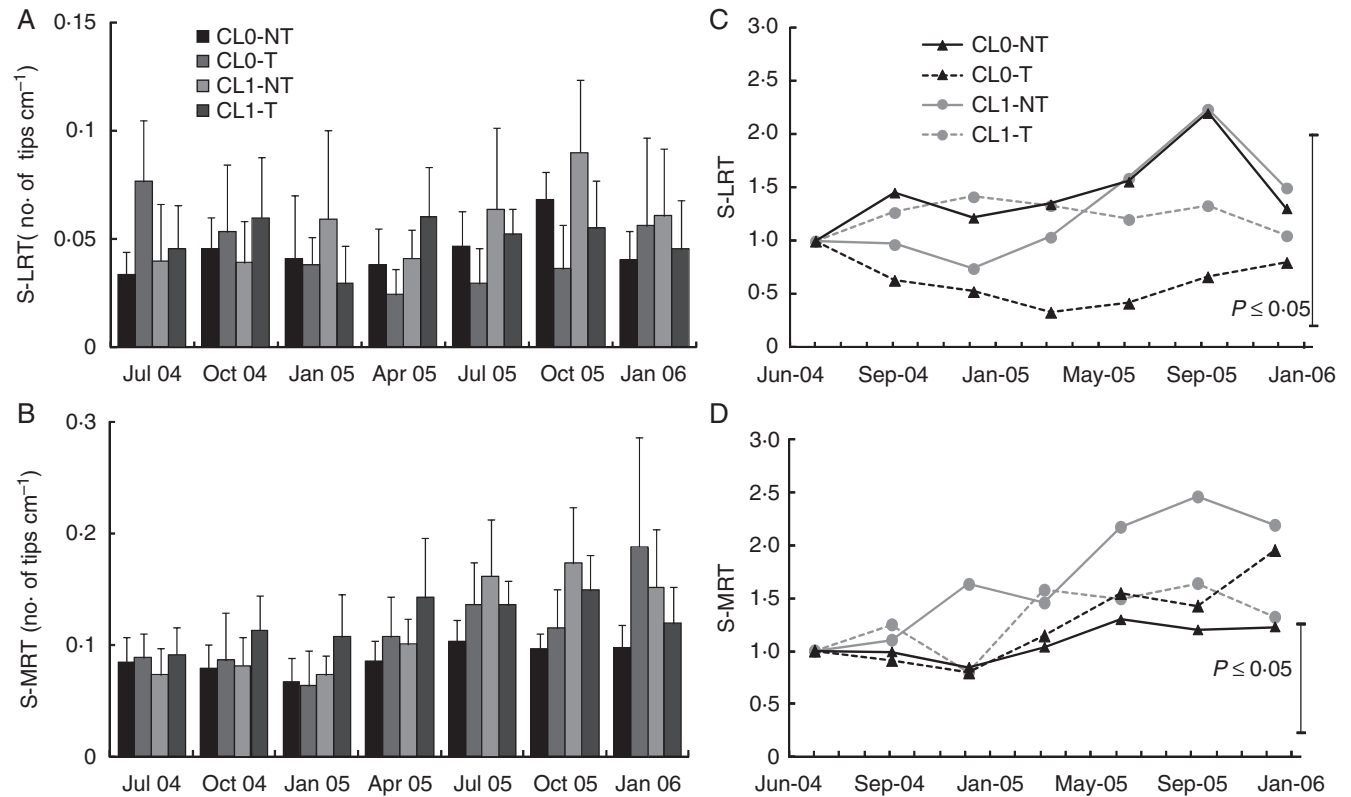


FIG. 2. Left (A, B): dynamics of standardized living root tips (S-LRT) and standardized mycorrhizal root tips (S-MRT) (standardization based on root length in the samples). Vertical bars: s.e. Right (C, D): dynamics of S-LRT and S-MRT normalized by pre-thinning values (July 2004). Vertical bars: LSD ($P \leq 0.05$) for treatment \times sampling date ($P \leq 0.05$).

TABLE 5. Repeated-measures analysis of variance, with P-values for significance between and within factors (time, decline class, silvicultural treatment, tree) for all root descriptors in pedunculate oak trees

	RLD _v	RTD _v	AD	BI	S-RTN	S-LRT	S-MRT
Between factors							
Class	0.0032	0.2069	0.0695	0.5850	<0.0001	0.0241	0.0030
Treatment	0.0009	<0.0001	<0.0001	0.0188	0.0062	0.3438	0.0189
Class \times Treatment	0.0704	0.0139	0.0688	0.0140	0.0111	0.3254	0.1571
Tree \times Class \times Treatment	<0.0001	<0.0001	0.0112	<0.0001	<0.0001	<0.0001	<0.0001
Within factors							
Time	<0.0001	<0.0001	<0.0001	0.0002	<0.0001	0.0474	<0.0001
Time \times Class	0.4629	0.8157	0.0447	0.0046	0.0430	0.1187	0.0367
Time \times Treatment	<0.0001	<0.0001	<0.0001	<0.0001	0.0054	0.0004	0.4717
Time \times Class \times Treatment	0.0012	<0.0001	0.3649	<0.0001	<0.0001	0.0818	0.0006
Time \times Tree \times Class \times Treatment	<0.0001	<0.0001	0.0132	<0.0001	<0.0001	0.0057	0.0030

Class, decline class; Treatment, silvicultural treatment; Time, sampling time.

(Fig. 3). The first factor (F1) explained 64 % of variance and was correlated (loadings $> |0.5|$) with BI and RTD_L; the second (F2) accounted for 22 % of variance and was correlated with RLD_v, RTD_v, S-LRT and S-MRT. Opposite vector directions revealed more evident negative correlations for RLD_v, RTD_v and BI vs. S-MRT, while S-LRT was positively correlated with S-MRT.

Circles (including 25 % of cases of each group) and centroid positions in the DA highlighted the fact that after 1 year of

observation thinning tended to increase root length and tip densities but to reduce tip mycorrhizal colonization in declining trees, whereas the opposite occurred in healthy trees together with a marked decrease in branching. Removal of tree competition also had a negative impact on living root tips in both declining classes. Without any silvicultural treatments, acute declining trees (CL1-NT) had a high number of living and mycorrhizal root tips, while non-declining trees (CL0-NT) had high root length and tip densities.

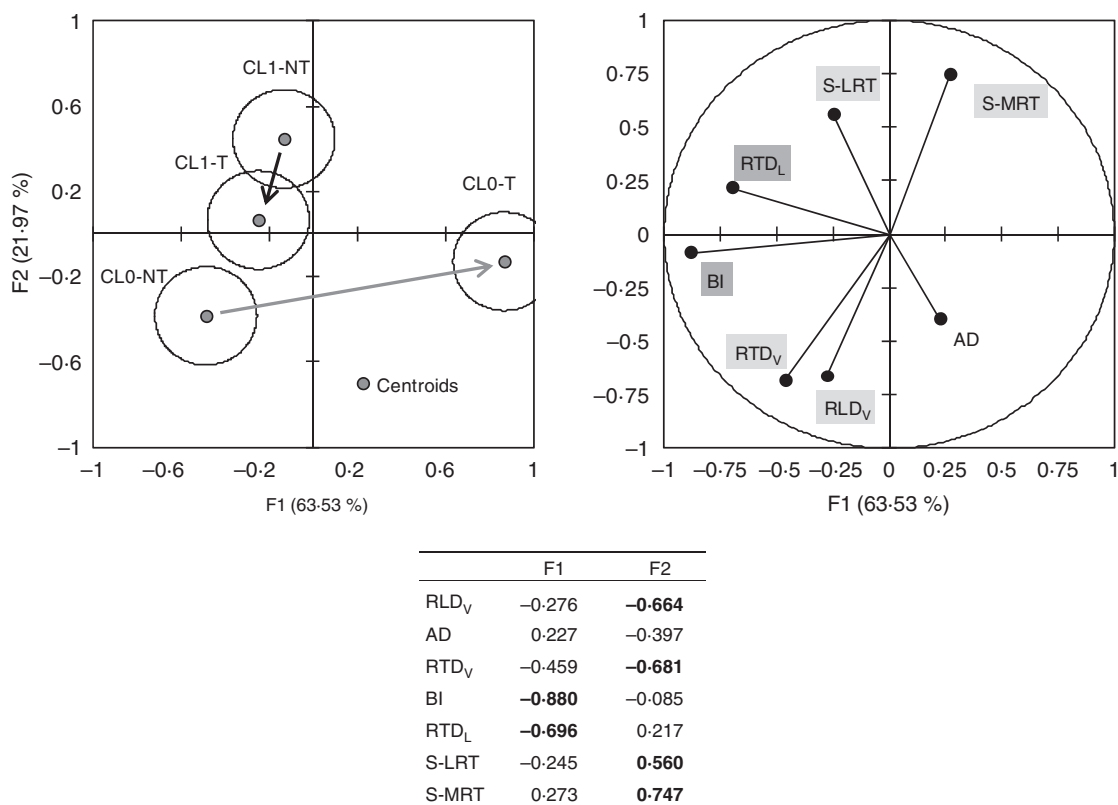


FIG. 3. Discriminant analysis (DA; left) and principal component analysis (PCA; right) for two classes of decline (CL0, non-declining; CL1, declining) combined with silvicultural treatment (T, thinning; NT, no thinning) in the second observation year (July 2005–January 2006). Parameter loadings for two main dummy variables F1 and F2 are reported below (highlighted values $> |0.5|$). Circles in the DA include 25 % of cases in each group. Arrows: changes of centroid position due to thinning in declining (grey) and non-declining (black) trees.

DISCUSSION

Tree decline is an important syndrome currently causing damage to a wide variety of forest species, including conifers and deciduous broadleaves, regardless of their age or site. Many species of oak are damaged, and symptoms are typically observed above-ground, while only sparse information is available on fine root production. Auclair (1993) suggested that, in cold climates, root death and cavitation might be involved in the syndrome, leading to reductions in both fine root biomass and living roots. Root mycorrhizal colonization diversity and abundance have been related to tree health in the pedunculate oak forest considered in this study (Mosca *et al.*, 2007), as these authors found a lower proportion of vital and ECM tips, together with lower ECM biodiversity in declining trees compared with healthy trees before thinning. Although concerning evergreen oak species, ECM fungal richness was also found to be associated with low tree mortality in cork oak (*Quercus suber* L.) (Azul *et al.*, 2010), and a higher number of living root tips characterized healthy holm oak trees (*Quercus ilex*) (Corcobado *et al.*, 2015).

This study considered a 60-year-old oak forest at Basalghelle, quite close to the Italian Alps, where aerial symptoms of decline (particularly transparency) were due to a strong reduction in the root network. With regard to the root system, at the beginning of the experiment it was not possible to obtain a

clear picture of decline, although affected trees displayed negative trends for root length and tip densities, and positive trends for root branching and RTD_L. Seasonal variations in root proliferation, and the wide variability of individual trees in particular, may have partially masked the true effects of decline at the root level.

It has already been demonstrated that inadequate silvicultural practices, which fail to control tree competition, play an important role in the health status of several conifer species (López *et al.*, 1998; Sword *et al.*, 1998), but little is known about pedunculate oak, particularly regarding what happens at the root level after removal of neighbouring competition. Although it concerns a different species, in scattered cork oak forests with extensive silvo-pastoral exploitation or with soil tillage and periodic shrub cutting, healthy trees have been found to have high root ECM biodiversity (Azul *et al.*, 2010). At Basalghelle, the effects of thinning appeared late, after an unaffected lag period lasting 1 year, during which there were very small variations in the main root descriptors and no dominant trend. This result was unexpected, as reduced below-ground competition for soil resources should give rise to increased root growth and greater root vitality and mycorrhizal colonization. Very probably, water and nutrients cannot be considered limiting factors in the study site with its eutrophic soil, which is characterized by abundant spring and summer rainfall and a shallow water table, whereas reduced transpiration by thinning may have

temporarily caused adverse anoxic root conditions before the forest attained a new equilibrium. The dense ditch network in the surrounding farmland is evidence that the forest is more liable to waterlogging rather than to summer drought, as suggested by the mottled B soil horizon.

The 2 year study period was probably insufficient to monitor tree response to thinning thoroughly, but the situation clearly changed from April of the second year, when PCA and DA, together with regressions over time of normalized values of root parameters, revealed the probable evolution of decline and the effects of thinning. In declining trees, thinning increased RLD_V and RTD_V , but reduced tip mycorrhizal colonization and the number of living tips compared with non-thinned controls, whereas the opposite occurred in healthy trees, together with marked increases in branching. It seems that, in critical conditions, silvicultural treatment has a positive effect on root elongation, thereby reducing the association with ectomycorrhiza. On the other hand, healthy trees are less dependent on mycorrhizal colonization and maintain good root elongation and soil exploration, a condition that appeared to be altered by the removal of neighbouring trees.

On the basis of the loadings and fractions of variance explained by two dummy variables in the PCA, it is demonstrated that the most representative root descriptors are root branching (BI) and root tip density (RTD_L), followed by mycorrhizal colonization, RLD_V and the number of living root tips. Root diameter was the least important parameter. These results are consistent with the findings of Montecchio *et al.* (2004), which indicate that roots of declining trees (holm oak in their case) may take on a bristle-like configuration, roughly like above-ground epicormic shoots.

Fine roots are commonly subject to seasonal variations in their dynamics, an effect particularly evident in terms of RLD_V , reaching maximum values in spring and minimum in winter (Cheng and Bledsoe, 2002). Several tree species regulate their root life span according to resource uptake from the soil (Adams *et al.*, 2013), with considerable species-specific life span plasticity. In this study, RLD_V was positively correlated with the BI, although Stover *et al.* (2010) found small roots to be associated with a shorter life span. A positive correlation between spring root production and water availability is generally expected in oak forests (Coll *et al.*, 2012), as volumetric root length density is one of the best descriptors for estimating water uptake (Molz, 1971; Taylor and Klepper, 1973, 1975). Although not investigated in this study, the patterns of root descriptors among root orders over time are also important for the acquisition of soil resources in *Q. robur* L., as recently highlighted by Zadworny *et al.* (2015). These authors found stable root diameters and specific root lengths across differing soil fertilities and seasons, but large variations in nitrogen concentrations in root tissues. High nitrogen was assigned to the most distal, absorptive roots in spring and summer, and to the higher order transport roots in autumn. This would support new growth during spring, when environmental conditions inhibit high uptake from cold soil, and represents a way of limiting nutrient losses from thin, lower order roots potentially damaged by winter frost. In light of this, it is hypothesized that functional disorders in declining oaks cause significant root losses over observed time periods.

Pedunculate oak (*Q. robur* L.) is a hygrophilous species, particularly sensitive to changes in soil water content, and much more sensitive to water-induced transportation dysfunction than other oak species, such as *Q. petraea* and *Q. pubescens*. Both pedunculate oak and holm oak mainly make use of rain-water, suggesting that fine root proliferation in the investigated shallow soil is essential for preserving tree functionality and health. In this regard, recovery in RLD_V due to thinning in declining trees may be considered positive in sustaining leaf transpiration, although water and N availability may not be considered limiting factors at the Basalghelle site. The dynamics of nutrient availability over time were not monitored, but a good fertility was assessed at the beginning of the experiment.

Decline has a complex aetiology, due to dynamic interactions between the host plant and several abiotic and biotic factors. Manion (1991) proposed a model with three categories of factor according to their action: long-term pre-disposing factors, which weaken tree vitality (e.g. genetic susceptibility, climate changes); intense short-term inciting factors, causing symptoms of decline (e.g. low temperature, drought, tree density, competition); and last-acting contributing factors, which accelerate decline, such as insects and fungal pathogens. Root infection by *Phytophthora* spp., particularly *P. cinnamomi*, has very recently proven to be a direct contributor to tree decline in Europe (Camilo-Alves *et al.*, 2013), but root losses depend on the sensitivity of individual oak species (McConnell and Balci, 2015). The involvement of *Phytophthora* in dieback and mortality has been demonstrated in *Q. ilex* (Linaldeddu *et al.*, 2014). *Phytophthora* was not recorded in the forest at Basalghelle, and the literature does not provide evidence that it interacts with mycorrhizal abundance (Corcobado *et al.*, 2015).

Among abiotic factors, water stress (drought) is widely believed to be one of the major causes of decline, and climate changes are probably exacerbating the syndrome. In Italy, the first reports of oak decline date back to 1980 (Ragazzi *et al.*, 1986) in central Italy, and it is estimated that up to 25–30 % of several oak species are currently subject to decline throughout the country. Within the last two decades, severe summer droughts across the whole of Europe were recorded in 2003, 2006, 2012 and 2015, probably increasing the intensity of the phenomenon. Fine root turnover is generally sensitive to drought conditions, with longer survival expected when roots are thicker (Stover *et al.*, 2010). Indeed, root diameter was not significantly affected at any time during this study, indicating that it varies over a small range typical of the species and that environmental pressure was probably limited (Zadworny and Eissenstat, 2011). Instead, root vitality (S-LRT) was sensitive to decline and generally fell after thinning, probably due to reduced root turnover. S-LRT progressively decreased in the thinned, acute declining trees, and the structure of the ECM community recovered its previous level of biodiversity in about 10 months (Mosca *et al.*, 2007). In this regard, the behaviour of root tip mycorrhizal colonization (S-MRT) was interesting, being generally higher in declining trees, an index of the tree's need to co-operate with aiding fungi. In the studied forest, 80 % of the root tips were found in the upper 10 cm of soil, where decaying organic litter is concentrated and where the annual fine root turnover of oak can involve the production of approx. 3 t ha⁻¹ of organic matter per year (Usman *et al.*, 1999),

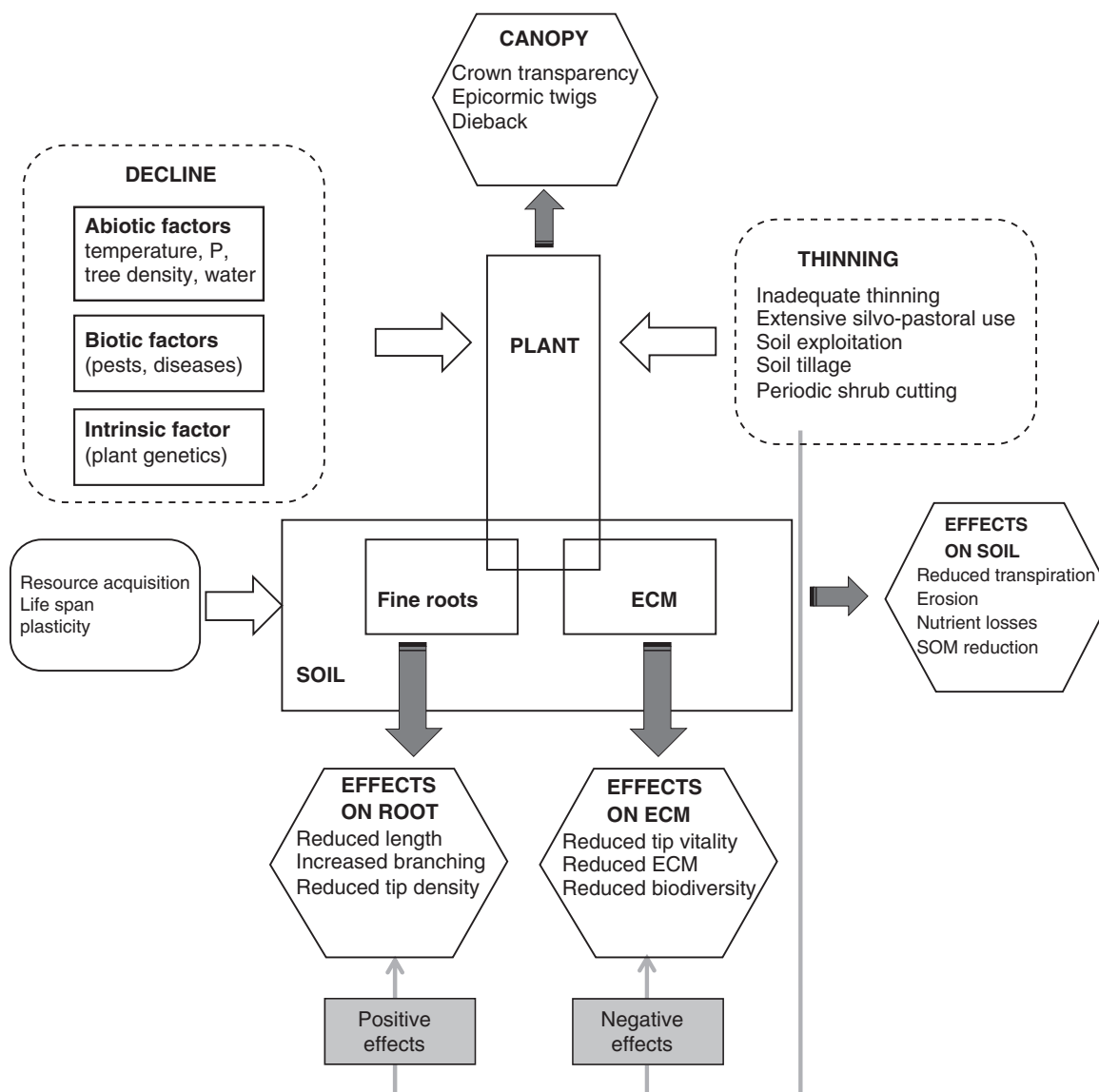


FIG. 4. Flow chart relating effects of decline and silvicultural treatment to above- and below-ground responses in *Quercus robur* L., and their relationship with forest ecosystem.

suggesting that a lack of nutrients as a possible cause of decline may be excluded.

Findings from this study integrate knowledge at the root level on the complex syndrome of oak decline and its causes or contributing factors, showing some consequences of thinning (Fig. 4). Dieback of the dominant oak stand is expected to have dramatic long-term effects at the ecosystem level, due to soil erosion, loss of organic matter and nutrients, which are associated with reduced plant transpiration, and altered root plasticity. The absence of any silvicultural treatment was thought to be one cause of the widespread and progressive decline at Basalghelle, but thinning did not in fact have the hoped-for effects. Very probably, an integrated management approach, including periodic shrub cutting, should be applied to revitalize the forest.

This study probably has some weaknesses and some issues that deserve further investigation. In particular, possible regrowth and root deepening as a consequence of soil disturbance by soil coring in the top 10 cm of soil were not considered (Prévost and Pargney, 1985; Bruns, 1995; Bakker et al., 2000), nor was misclassification of ECM branches as root tips within the WinRHIZO software. Also the dynamics of soil temperature, moisture or nitrogen availability were not monitored, although this study does highlight the importance of seasonal fine root variations in relation to decline status and thinning. The common feature of all root parameters was the large effect of the tree factor, meaning that root dynamics are tree specific, probably as a result of different stages of decline.

CONCLUSIONS

It is concluded that, together with the canopy transparency and other aerial symptoms, loss of fine roots is a result of decline in pedunculate oak, and that removal of nearby tree competition has some positive effects, although it does not appear to be essential to the syndrome in the short term, confirming the complexity of this phenomenon. The close correlation observed between root vitality and mycorrhizal colonization and their deterioration after thinning does not support the hypothesis of limited water or nutrient availability as a possible cause of the syndrome at the studied site. Currently, the most important below-ground trait related to decline is the abundance and biodiversity of the ECM fungal community, since it can help trees to cope with environmental factors, variations in the availability of resources and resistance to pathogens. Further studies of root responses to thinning and other silvicultural treatments would provide better understanding of the factors predisposing trees to the syndrome and also indicate ways of countering its effects.

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