

Article

Regeneration in the Understory of Declining Overstory Trees Contributes to Soil Respiration Homeostasis along Succession in a Sub-Mediterranean Beech Forest

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Abstract: *Research Highlights:* Tree decline can alter soil carbon cycling, given the close relationship between primary production and the activity of roots and soil microbes. *Background and Objectives:* We studied how tree decline associated to old age and accelerated by land-use change and increased drought in the last decades, affects soil properties and soil respiration (R_s). *Materials and Methods:* We measured R_s over two years around centennial European beech (*Fagus sylvatica* L.) trees representing a gradient of decline in a sub-Mediterranean forest stand, where the number of centennial beech trees has decreased by 54% in the last century. Four replicate plots were established around trees (i) with no apparent crown dieback, (ii) less than 40% crown dieback, (iii) more than 50% crown dieback, and (iv) dead. *Results:* Temporal variations in R_s were controlled by soil temperature (T_s) and soil water content (SWC). The increase in R_s with T_s depended on SWC. The temperature-normalized R_s exhibited a parabolic relationship with SWC, suggesting a reduced root and microbial respiration associated to drought and waterlogging. The response of R_s to SWC did not vary among tree-decline classes. However, the sensitivity of R_s to T_s was higher around vigorous trees than around those with early symptoms of decline. Spatial variations in R_s were governed by soil carbon to nitrogen ratio, which had a negative effect on R_s , and SWC during summer, when drier plots had lower R_s than wetter plots. These variations were independent of the tree vigor. The basal area of recruits, which was three times (although non-significantly) higher under declining and dead trees than under vigorous trees, had a positive effect on R_s . However, the mean R_s did not change among tree-decline classes. These results indicate that R_s and related soil physico-chemical variables are resilient to the decline and death of dominant centennial trees. *Conclusions:* The development of advanced regeneration as overstory beech trees decline and die contribute to the R_s homeostasis along forest succession.

Keywords: successional stage; forest decline; tree old-age; old-growth forest; soil carbon; soil nitrogen; rhizosphere; root biomass

1. Introduction

Soil respiration (R_s) has been measured in most terrestrial biomes, from deserts, grasslands and shrublands, to forests in temperate, boreal, tropical and Mediterranean regions [1]. From forest studies where carbon (C) fluxes have been partitioned among their components, it has been shown that R_s typically accounts for more than half of the total annual ecosystem respiration [2–4]. Tree ageing and forest succession involve changes in the overstory and, in turn, in water, nutrient and C fluxes between the atmosphere and the rhizosphere that can translate into different rates of R_s [5,6]. Global change-related episodes of forest decline and die off are accelerating forest succession worldwide, particularly in forests weakened by tree ageing, overcrowding and sub-optimal climate conditions where ancient land uses have changed [7]. To date, however, relatively little is known on how forest succession [8,9] and decline affect R_s [10]. In general, the impact of forest decline on R_s is likely to vary among ecosystems depending on how sensitive the roots of dominant species are to defoliation, and how fast microbes are in the decomposing litter and other plant species in colonizing the canopy gaps opened by defoliation and tree death. However, complex plant-soil-climate interactions mediate tree mortality impacts on R_s [10]. For example, Ávila et al. [11] found that the effect of tree decline on soil variables differed in woodlands and closed forests, varied between seasons, and was higher as declining trees were larger.

The net impact of forest decline on R_s is not straightforward, due to direct and indirect effects of the decline on soil C and nutrient inputs and microclimate. Soil areas close to vigorous trees with a high foliage volume might exhibit higher R_s than those close to declining trees with smaller crowns due to higher supply rates of respiratory substrates to roots and their mycorrhizal symbionts [12,13]. Moreover, the microbial respiration could increase around vigorous trees as a result of higher root exudation rates and leaf and fine root litter supply to the soil [14]. In support of this reasoning it has been observed that variables related with photosynthetic C uptake, such as the leaf area index, sap flow, tree size, or tree proximity to and live tree basal area around R_s sampling locations are positively related to R_s [11,15–20]. Nevertheless, R_s can be relatively homeostatic across successional stages or after perturbations [8,21–23]. A progressive decline in the vigor (and crown and root volume) of mature trees may prompt space colonization by woody recruits and herbaceous species, so that the R_s remains similar across successional stages. Further, because R_s results to a large extent from the activity of decomposing microorganisms—contributing 40 to almost 100% of the total R_s at some periods of the year [6,21,24]—it can also be envisaged that R_s is higher close to declining than vigorous trees due to higher litter input and soil metabolic activity [19]. In fact, the plant litter quality and amount affect the soil microclimate and microbial community composition and activity [25,26].

Reducing the leaf area associated to the tree decline may affect R_s via increasing the soil temperature due to enhanced penetration of solar radiation through the canopy. Temperature is one of the main drivers of temporal and spatial variations in R_s , e.g., [27]. Given the positive effect of temperature on enzymatic reactions of all living organisms, R_s can increase in warmer soils of declining stands under non-limiting soil C and nitrogen (N) conditions. Similarly, the soil water content (SWC) has a profound effect on the metabolic activity of roots, associated microorganisms (e.g., mycorrhiza) and soil microbial decomposers [28,29], which is reflected in the positive correlations between R_s and SWC, over time [30–32] and space [31,33,34] at multiple scales. The limiting effect of low SWC on R_s is observed in many studies in seasonally dry ecosystems where, below a threshold of SWC, R_s exhibits a weak sensitivity to temperature. For example, in Mediterranean-type ecosystems where the dry period occurs at the warmer time of the year, low SWC results in the saturation of the response of R_s to temperature [35]. On the contrary, a high SWC can restrict CO₂ diffusion from soils, and also make soil oxygen concentrations to drop to limiting thresholds for R_s [36,37]. The effects of vegetation die-off on soil water availability are not easy to predict. Tree dieback might result in lower water consumption, but also in higher soil water evaporation due to canopy defoliation [38–40].



Figure 1. Examples of centennial beech trees around which soil respiration measurements were conducted. Tree decline classes were established in relation to the crown dieback: Vigorous (**a**; no apparent crown dieback), affected (**b**; less than 40% crown dieback), severely affected (**c**; more than 50% crown dieback), and dead (**d**) trees.

Studying the interactions between environmental conditions and forest decline on R_s helps to understand and predict the C balance of forest ecosystems. We measured R_s in a marginal beech (*Fagus sylvatica* L.) forest stand in south Europe during two consecutive years. This population is suffering an accelerated loss of centennial trees. Among the probable causes are (i) the 21% reduction

in annual rainfall occurred in the last decade relative to the previous one; (ii) the ancient practice of pollarding, which has favored the development of large horizontal branches, prone to fall from mechanical instability, and being infected from open wounds; and (iii) competition with recruits, mainly for water. One objective of this work was to compare R_s among sites of different degrees of decline, from mature climax stages where vigorous centennial trees dominate, to stages of increasing tree decline and understory colonization with recruits (Figure 1). We hypothesized that tree decline would result in a reduction in R_s that would be partly related to a change in the soil physico-chemical properties and a reduction in the fine root density. As a second objective, we explored the influence of soil temperature and water availability on R_s seasonality in two years of contrasted climate conditions, and whether soil temperature, SWC or their influence on R_s changed across the tree decline stages. We expected that R_s was driven by temperature across periods of high SWC, and by SWC below a certain threshold of SWC.

2. Materials and Methods

2.1. Area and Design of the Study

The study was conducted in a 1-ha beech stand located within a 120-ha mixed forest in central Spain (3°30' W, 41°07' N; at 1240–1550 m a.s.l.). Trees of beech and oak (*Quercus petraea* and *Quercus pyrenaica*) formed an open overstory during most of the 19th and 20th centuries when the forest was managed as an open woodland used for sheep and cattle grazing, resting and breeding [41]. In the 1960s, domestic livestock was banned, and the understory was rapidly colonized by the progeny of overstory trees and other woody species from surrounding forests (hereafter recruits). The climate of the area is sub-Mediterranean, with a mean annual temperature of 9.7 °C and a mean annual rainfall of 873 mm (data from 1994 to 2017, recorded in a meteorological station within the forest). Both variables exhibit a strong seasonality: Minimum and maximum temperatures are reached in January and July, respectively, and a dry period (i.e., 131 mm rainfall on average) typically extends from June through September. Soils are formed from mica schists and mica gneiss substrates.

In 2015, sixteen circular plots of 10 m radius were established around centennial beech trees with different degrees of vigor, or dead approximately 15–25 years ago (Figure 1; Table 1). The slope ranged from 15% to 22%, and the orientation from 40° N to 87° N across the study area. Four plots were selected for each of the four tree-decline classes: Healthy trees with no symptoms of decay (vigorous; V), trees with less than 40% of the crown dead (affected; A1), trees with severe symptoms of decay and most of the crown dead (severely affected; A2), and finally snags, i.e., dead trees with part of the main trunk still standing up (dead; D). The stem density and basal area were computed within the plots, including all stems taller than 1.5 m. Given the high contribution of the central tree to the basal area, the data were also given for the recruits only. Beech and occasionally holly (*Ilex aquifolium*) were the main recruits in the understory, while shrubs (mostly *Genista florida*) and herbaceous species (mostly *Melica uniflora*) were rare. Table 1 summarizes the structural and edaphic properties of the plots.

2.2. Soil Respiration

On each plot, four 19-cm diameter polyvinyl chloride (PVC) collars were inserted 2 cm deep into the soil and fixed with brackets and nails. Soil respiration (R_s) was measured in situ with a close-path infrared gas analyzer (LI-6400, Li-Cor, Inc., Lincoln, NE, USA) coupled to a closed dynamic soil chamber (LI-6400-09). The collars were larger than those supplied by the manufacturer to sample a larger soil surface area (284 cm²). A custom-made PVC lid with a central hole slightly larger than the soil chamber was fit to the collar, and the soil chamber was inserted in the hole with a foam gasket to ensure an appropriate seal. The four collars were placed forming a square of 5–8 m side around the centennial beech tree/snag, avoiding fallen branches and rare rock outcrops. If at the time of measuring there was evidence that the collar had been moved by animals, the location was not measured, and the collar was re-installed for subsequent dates. Despite the tree rhizosphere is laterally expansive and

belowground delimitation of individual trees is challenging, previous studies in denser forests than the one surveyed here have shown that single trees are determinant of the surrounding R_s [19,21] and soil microbial communities [42].

Table 1. Characterization of the four types of plots established around centennial beech trees of different declines—vigorous, affected, severely affected—and dead. Crown dieback of the centennial beech tree (%); cover of herbaceous (Herb.) species (%) and cover of shrub species (%); stem density (stems ha^{-1}) and basal area ($\text{m}^2 \text{ha}^{-1}$) of either all trees in the plot (ALL) or only saplings >1.5 m height and <0.5 m diameter at breast height (i.e., of recruits established after the woodland was banned to cattle grazing in the 1960s; REC); fine litter depth (i.e., leaves and small twigs; cm); soil water content over 0–20 cm topsoil (SWC; %) averaged for all or only summer measurement dates, and soil bulk density (g cm^{-3}), organic matter (OM; %), total carbon (C, %), total nitrogen (N, %), C to N ratio, and fine root density (<2 mm; g m^{-2}) of 0–10 cm topsoil; average soil respiration measured across two years (R_s ; $\mu\text{mol m}^{-2} \text{s}^{-1}$), and modeled at mean annual soil temperature (11°C), and constant temperature and soil water content (20%). Data are means ($\pm\text{SE}$) from four replicate plots per tree decline class. Different letters separate significantly different means of crown dieback, the only variable for which significant differences among tree classes were found at $p < 0.05$.

	Vigorous	Affected	Severely Affected	Dead
Crown dieback ¹	6.3 \pm 3.8 a	25 \pm 5.4 a	68.8 \pm 8.3 b	100 \pm 0 c
Herb. cover ¹	3.7 \pm 1.3	3.2 \pm 2.0	11.1 \pm 4.4	12.7 \pm 4.7
Shrub cover ¹	4.2 \pm 3.2	10.4 \pm 4.9	13.3 \pm 8.4	12.3 \pm 3.9
Stem density (ALL)	1003 \pm 486	2045 \pm 543	2690 \pm 592	2355 \pm 185
Basal area (ALL)	48.8 \pm 9.7	36.9 \pm 5.7	37.7 \pm 6.6	27.4 \pm 9.5
Stem density (REC)	963 \pm 491	2021 \pm 538	2658 \pm 592	2340 \pm 183
Basal area (REC)	5.3 \pm 1.8	12 \pm 4.1	15.6 \pm 6.6	18 \pm 1.3
Soil litter depth	5.3 \pm 0.3	5.3 \pm 0.3	5.3 \pm 0.8	4.1 \pm 0.6
SWC	18.8 \pm 0.2	19.4 \pm 0.1	18.5 \pm 0.1	21.4 \pm 0.3
SWC summer	10.7 \pm 0.2	11.0 \pm 0.2	9.6 \pm 0.2	10.7 \pm 0.4
Soil bulk density	0.77 \pm 0.01	0.78 \pm 0.01	0.76 \pm 0.01	0.73 \pm 0.01
Soil OM	9.7 \pm 1.0	8.3 \pm 0.6	8.9 \pm 0.4	8.8 \pm 0.6
Soil total carbon	7.7 \pm 0.8	6.5 \pm 0.5	7.2 \pm 0.4	7.4 \pm 0.7
Soil total nitrogen	0.48 \pm 0.05	0.41 \pm 0.04	0.45 \pm 0.04	0.49 \pm 0.06
Soil C/N	15.8 \pm 0.1	16.1 \pm 0.2	16.1 \pm 0.1	15.2 \pm 0.2
Root density	232 \pm 4	208 \pm 11	229 \pm 6	224 \pm 8
R_s	4.67 \pm 0.08	3.95 \pm 0.06	4.49 \pm 0.14	4.29 \pm 0.05
$R_{s_{11^\circ\text{C}}}$	4.51 \pm 0.07	3.92 \pm 0.07	4.31 \pm 0.15	4.15 \pm 0.07
$R_{s_{11^\circ\text{C}, 20\%}}$	5.19 \pm 0.06	4.41 \pm 0.07	5.20 \pm 0.12	4.81 \pm 0.04

¹: Variables visually estimated by the same observer.

Fourteen measurement campaigns were conducted between May 2015 and November 2016, at 1–3 months intervals. On each campaign, all measurements were conducted in one day, typically from 10:00 am to 17:00 pm, alternating V, A1, A2, and D plots to avoid differences in soil temperature among tree-decline classes due to the sampling time. Three measurement cycles were recorded per collar and campaign. The topsoil temperature (T_s) was measured at the same time than R_s with a 6000-09TC temperature probe 5–10 cm away from the collar.

2.3. Soil Physico-Chemical Properties and Root Density

A soil pit excavated in one location of the study area revealed a 46 cm deep A horizon (29.3% coarse sand, 51.4% fine sand, 7.3% silt, 12% clay), a 61 cm deep B horizon (53.5% coarse sand, 27.8% fine sand, 8.3% silt, 10.4% clay) and an 18 cm deep C horizon (33.5% coarse sand, 38.5% fine sand, 12.8% silt, 15.2% clay).

Moreover, on each plot and campaign, the volumetric soil water content (SWC) was measured in the top 20 cm with a quasi-time-domain-reflectometry system (TDR; TRIME-FM, IMKO Micromodultechnik GmbH, Germany) coupled to a TRIME-T3 tube probe. The frequency of measurements was the same

than for R_s . To account for soil physical properties specific of the forest, TDR measurements were calibrated with independent gravimetric SWC measurements, transformed to volumetric SWC values using the soil bulk density ($r^2 = 0.97$, $p < 0.001$, $n = 13$).

In November 2015, one sample from the 0–10 cm topsoil per plot was taken for analyses of organic matter (OM) and total C and N content using the Dumas method [43] with an elemental analyzer (Elementar N/CN; VarioMax, Hanau, Germany). Two 10 cm deep soil samples per plot were also taken with a corer and used to measure the bulk density, as the ratio of soil sample mass dried at 105 °C to the core volume. Litter depth was measured with a ruler in four locations per plot, near the soil collars.

At the end of the study, two cores of 19 cm in diameter and 10 cm depth were taken per plot to collect small roots. In the laboratory, roots were separated from successive small volumes of soil with the help of a 2-mm sieve, first, and a 200 µm sieve afterwards, using water to carefully disaggregate numerous soil aggregates holding fine roots. Soil-free roots were oven-dried at 70 °C and weighted separately according to their diameter: <2 mm (fine) or 2–5 mm (small). We are confident that roots belonged to beech, given the low abundance of herbaceous and shrub species in the study plots (Figure 1; Table 1) and the same morphology of roots across soil samples.

2.4. Data Analyses

For each single date, cycles higher than 1.5 times the 25th to 75th interquartile range were considered outliers and thus removed from the data set. These outliers accounted for 6.1% of the total data set. Thereafter, we calculated the mean R_s per collar, and the mean R_s per plot after averaging the four (occasionally 2–3) collars per plot on each of the 14 field campaigns.

Mixed-effects models were used to analyze the temporal relationship between the plot-based mean R_s and tree-decline classes (i.e., V, A1, A2, and D), seasons, T_s and SWC. We evaluated all possible models combining the tree-decline classes, season, T_s , SWC and their first order interactions (saturated model) in order to achieve the minimum adequate model according to the corrected Akaike information criterion (AICc). Once we had the minimum adequate models, we calculated the adjusted r^2 . Before running the model selection, we tested the variance inflation factor (VIF) in the saturated model to assess collinearity between T_s and SWC; VIF was lower than two, indicating low collinearity [44]. Since the magnitude of the mixed effects models is dependent on the units of each variable, we scaled and centered all variables to improve the performance and interpretability of the output models [45]. A plot identity and correlation structure was included in the random part of the model to control for the temporal autocorrelation between measurements.

The spatial variation in R_s across the 16 plots was studied for the mean R_s measured over the two-year study period, the mean R_s measured on each season, and standardized R_s at constant T_s , or constant T_s and SWC (i.e., at the mean annual or seasonal annual T_s and SWC values). The standardization allowed to account for small differences in T_s and SWC among plots (Figure 2) when studying the influence of OM, C, N, C/N, soil density, and fine and small root density on spatial variations in R_s . To do so, we first model R_s as a linear function of T_s over dates with SWC not limiting to R_s (i.e., >15%; Figure 3a). The 15% SWC threshold was determined as the SWC value below which R_s was best explained by SWC ($r^2 = 0.44$, $p < 0.001$, $n = 73$). Second, the residuals between the observed and modeled values of R_s from T_s across the entire range of SWC were modeled as a quadratic function of SWC (Figure 4b). Finally, the standardized R_s was derived from the following equation using the observed T_s and SWC:

$$R_s = (a + b T_{\text{soil}}) + (c + d \text{SWC} + e (\text{SWC})^2) \quad (1)$$

where a through e were coefficients specific to each tree-decline class (see Table 2).

Differences in all soil physico-chemical variables among the tree-decline classes were tested with general linear models, using a repeated measures analysis for T_s and SWC. Post-hoc Tukey's HSD tests were conducted to separate the mean values among the tree classes and measurement dates when these factors were significant at $p < 0.05$. Analyses were performed with Statistica (StatSoft, Tulsa, OK, USA), and R using the packages SMATR [46], nlme [47] and MuMIn [48].

3. Results

3.1. Environmental Conditions

The two years of study were climatically anomalous: Compared to the 25-year averages of the study site, the spring through summer period of 2015 was 1.4 °C warmer, while the summer rainfall in 2016 was 67% lower. The temperature and rainfall followed the typical seasonal pattern of Mediterranean climates, with warmer temperatures being registered during the drier period in the summer (Figure 2a). This resulted in an inverse relationship between T_s and SWC ($r^2 = 0.59$, $p < 0.001$, $n = 214$; Figure 2a,b).

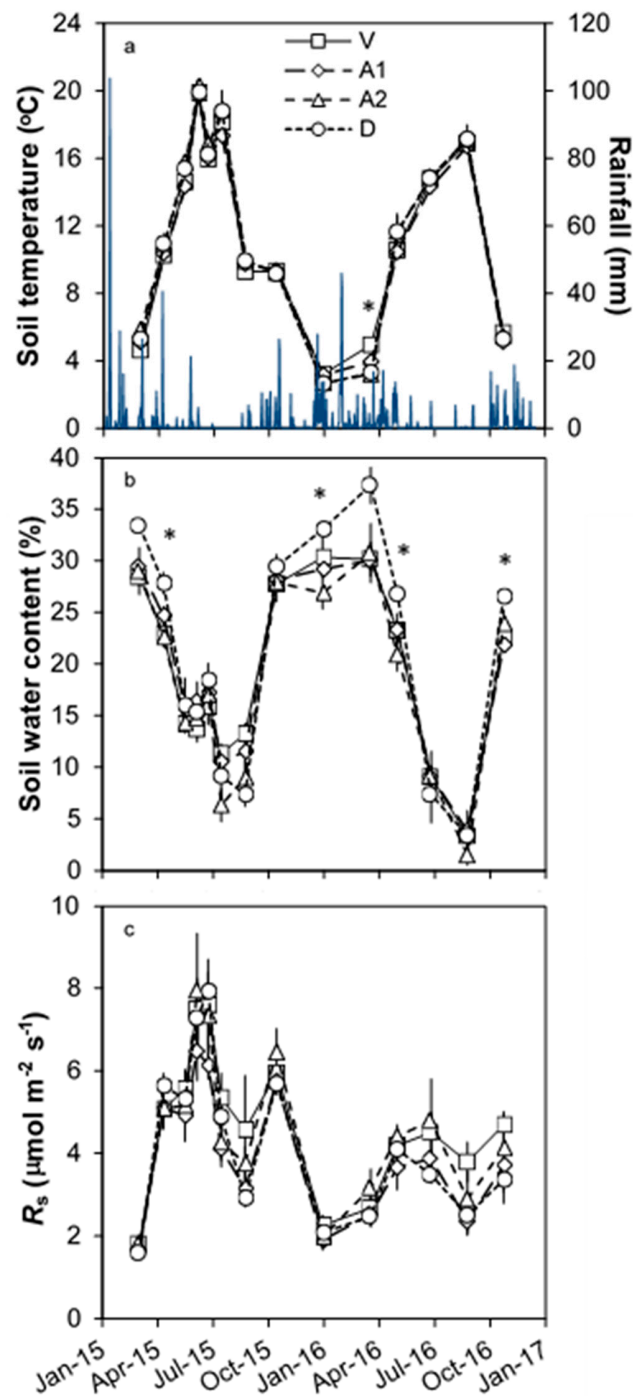


Figure 2. Temporal changes in (a) soil temperature and daily rainfall (blue line), (b) soil water content at 0–20 cm depth, and (c) soil respiration (R_s) around centennial beech trees of different decline classes

– vigorous (V), affected (A1), severely affected (A2) – and dead (D). Values are means (\pm SE) of $n = 4$ replicate plots, except for rainfall, recorded in a meteorological station in the study area. Asterisks indicate dates in which significantly different means among tree-decline classes were found at $p = 0.05$.

Neither T_s nor SWC, nor their temporal variations were significantly different among the tree-decline classes. Still, in some dates of spring and autumn, SWC was consistently higher near the dead trees (Figure 2b).

Table 2. Mixed effects model of temporal variations in soil respiration as a function of soil temperature (T_s), soil water content (SWC), tree-decline class (vigorous [V], affected [A1], severely affected [A2] and dead [D]) and season. The model explained 83% of the variation in R_s and was significant at $p < 0.001$.

	Estimate	SE	DF	t-Value	p-Value
<i>Intercept</i>					
Intercept	0.226	0.184	186	1.225	0.222
T_s	0.829	0.163	186	5.088	<0.001
SWC	0.103	0.154	186	0.665	0.507
$T_s \times$ SWC	0.233	0.088	186	2.646	0.009
<i>Tree-decline</i>					
V	-	-	-	-	- a
A1	-0.376	0.245	12	-1.536	0.151 a
A2	-0.026	0.245	12	-0.105	0.918 a
D	-0.131	0.249	12	-0.525	0.609 a
<i>Season</i>					
Spring	-	-	-	-	- a
Summer	0.796	0.189	186	4.211	<0.001 b
Fall	1.828	0.478	186	3.821	<0.001 c
Winter	0.231	1.931	186	0.12	0.905 abc
<i>$T_s \times$ season</i>					
$T_s \times$ spring	-	-	-	-	- a
$T_s \times$ summer	0.093	0.225	186	0.411	0.681 a
$T_s \times$ fall	1.017	0.393	186	2.589	0.01 b
$T_s \times$ winter	0.188	1.237	186	0.152	0.879 ab
<i>SWC \times season</i>					
SWC \times spring	-	-	-	-	- a
SWC \times summer	1.009	0.198	186	5.09	<0.001 b
SWC \times fall	-0.507	0.378	186	-1.341	0.182 a
SWC \times winter	0.117	0.43	186	0.273	0.786 ab
<i>$T_s \times$ tree-decline</i>					
$T_s \times$ V	-	-	-	-	- a
$T_s \times$ A1	-0.252	0.092	186	-2.738	0.007 b
$T_s \times$ A2	-0.105	0.091	186	-1.157	0.249 ab
$T_s \times$ D	-0.147	0.097	186	-1.517	0.131 ab

3.2. Temporal Changes in R_s

The mean values of R_s varied by 4-fold over the course of the experiment (Figure 2c), and significantly among seasons (Table 2). The lowest values were registered in winter/early spring and the highest in early summer, when T_s was high and storms prevented SWC to drop (Figure 2). This reflects the interaction effect of T_s and SWC on R_s : At a low SWC, the effect of T_s on R_s was lower than at a high SWC (Table 2). The lower sensitivity of R_s to T_s during the summer drought resulted in an asymptotic relationship between R_s and T_s (Figure 3a). Similarly, a lower T_s during periods of high SWC (from fall through spring), resulted in a parabolic relationship between SWC and R_s across the entire range of SWC observed (Figure 3b). Exploring the relationship between both variables at increasing SWC intervals revealed that a positive significant relationship existed between SWC and R_s

below 15% SWC (Figure 4a), i.e., mostly during the summer (Table 2). This explains that the sensitivity of R_s to SWC was highest in the summer (when a low SWC was limiting R_s), and the sensitivity of R_s to T_s was highest in fall (when a high SWC did not limit R_s ; Table 2). The only significant effect of the factor “tree decline” was an interaction with T_s , which indicated that R_s increased with increasing T_s faster near vigorous trees than near trees with early symptoms of decline (Table 2, Figure 3). The best regression model explaining the relationship between T_s and R_s excluding values with SWC <15% was a linear model (Figure 3b), which contrasts with the logarithmic relationship between both variables when all data were included (Figure 3a). The residuals of the R_s vs T_s linear regression model were related with SWC, with larger deviations of observed values from the predicted R_s values occurring at a low SWC (Figure 4b). The R_s standardized at the mean annual T_s (11) continued to be non-linearly related with SWC (Figure 4c).

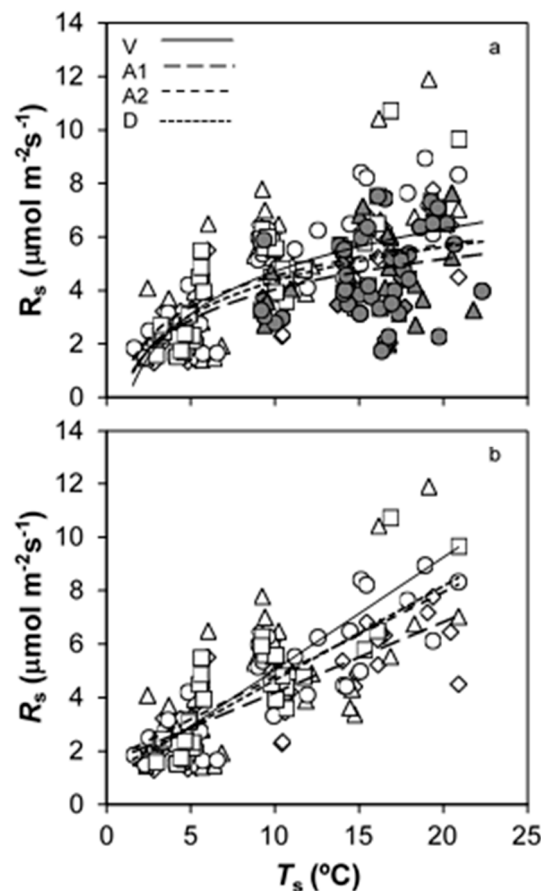


Figure 3. Soil respiration (R_s) as a function of soil temperature (T_s) throughout the entire study period (a) or after excluding observations above 15% soil water content (b); non-limiting SWC for R_s ; see text), observed around centennial beech trees of different decline classes—vigorous (V; squares), affected (A1; diamonds), severely affected (A2; triangles), and dead (D; circles). Data points are mean values per plot and sampling campaign. In (a) open and closed symbols account for R_s values above and below 15% SWC, respectively; non-linear models are fitted to all data points, separately among tree-decline classes.

Modeled R_s as a function of both T_s and SWC exhibited a good agreement with observed R_s values (Figure 5). However, the slope and intercept of the linear relationship between both variables differed significantly from one and zero, respectively.

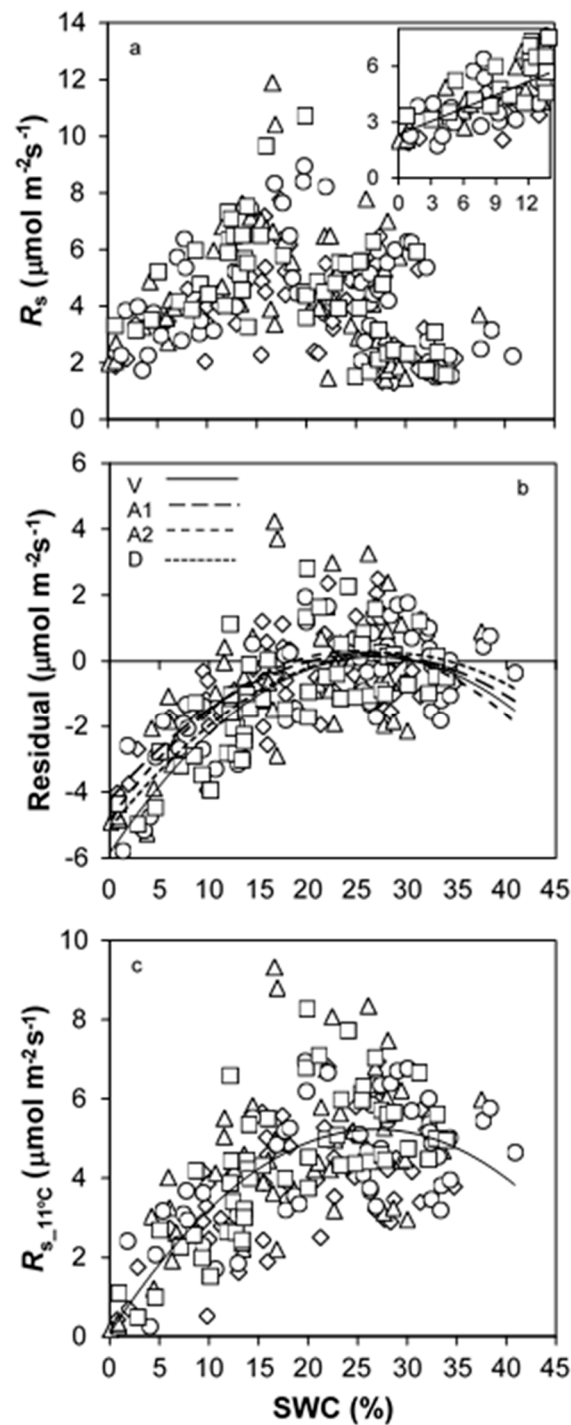


Figure 4. Observed soil respiration (R_s ; a) and derived parameters (b,c) modeled as a function of soil water content (SWC) in plots established around centennial beech trees of different decline classes – vigorous (V; squares), affected (A1; diamonds), severely affected (A2; triangles), and dead (D; circles). Data points are mean values per plot and sampling campaign. In (a) the inset depicts the relationship below 15% SWC. b,c) relationship of SWC with residuals between observed R_s values and R_s values modeled from T_s above 15% SWC (b), and standardized R_s at 11 °C (mean annual T_s ; c). Non-linear regression models were fitted pooling all tree-decline classes or separately among tree-decline classes to account for the significant relationship of T_s with factor “Decline” (see Table 2).

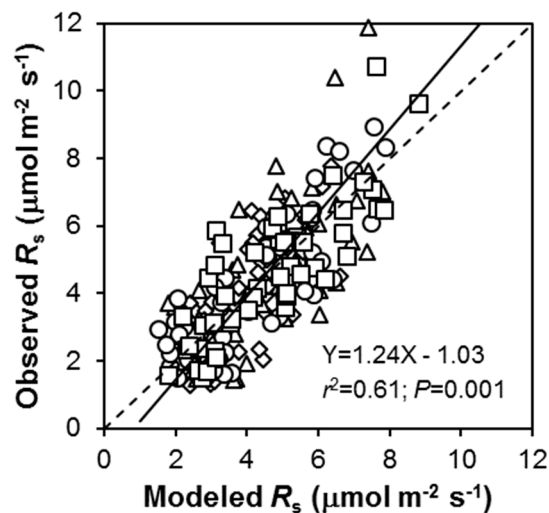


Figure 5. Relationship between observed soil respiration (R_s) and R_s modeled from temporal variations in soil water content and soil temperature. Different symbols are used to distinguish between vigorous (squares), slightly affected (diamonds), severely affected (triangles) and dead (circles) trees, but a single regression model is given for all tree-decline classes pooled for clarity. The major axis regression analysis was used to analyze the relationship between the observed and the modeled R_s . Dotted line depicts the 1:1 line.

3.3. Spatial Changes in Soil Physico-Chemical Variables and R_s

Spatial patterns of most variables analyzed were not related to the degree of beech decline. Compared to large changes in the percentage of crown dieback of each plot's central tree, changes in T_s and SWC (Figure 2a,b), soil physico-chemical variables, fine root density, and R_s were insignificant among tree-decline classes, irrespective of the season, or whether R_s was standardized at a given T_s and SWC or not (Tables 1 and 2).

Nonetheless, spatial variations in R_s were considerable and partly explained by soil physico-chemical variables, mostly C/N and SWC during summer. Litter depth, which was not significantly related to aboveground canopy features (e.g., basal area or stem density), was related with soil ground area covered by herbaceous species ($r = -0.54$; $p = 0.032$), soil density ($r = 0.52$; $p = 0.039$) and summer SWC (Figure 6a) across the 16 studied plots. SWC controlled spatial variability in R_s during summer, when wetter plots had higher standardized R_s at 17 °C (the average T_s during summer) than drier ones (Figure 6b). On the other hand, wetter plots during summer had higher soil N concentrations and lower C/N than drier plots (Figure 6c), whereas, in turn, C/N was marginally significantly related to the mean annual R_s standardized at the mean annual T_s and SWC (i.e., taking differences in T_s and SWC among plots into account; Figure 6d). Soil total C and N were positively correlated ($r = 0.92$; $p < 0.001$).

Of the vegetation-related features examined, only the basal area of recruits was negatively related to the soil C/N ($r = -0.51$; $p = 0.046$), which is consistent with the trend of R_s (standardized at the mean annual T_s and SWC) to increase in plots with a higher basal area of recruits ($r = 0.48$; $p = 0.06$).

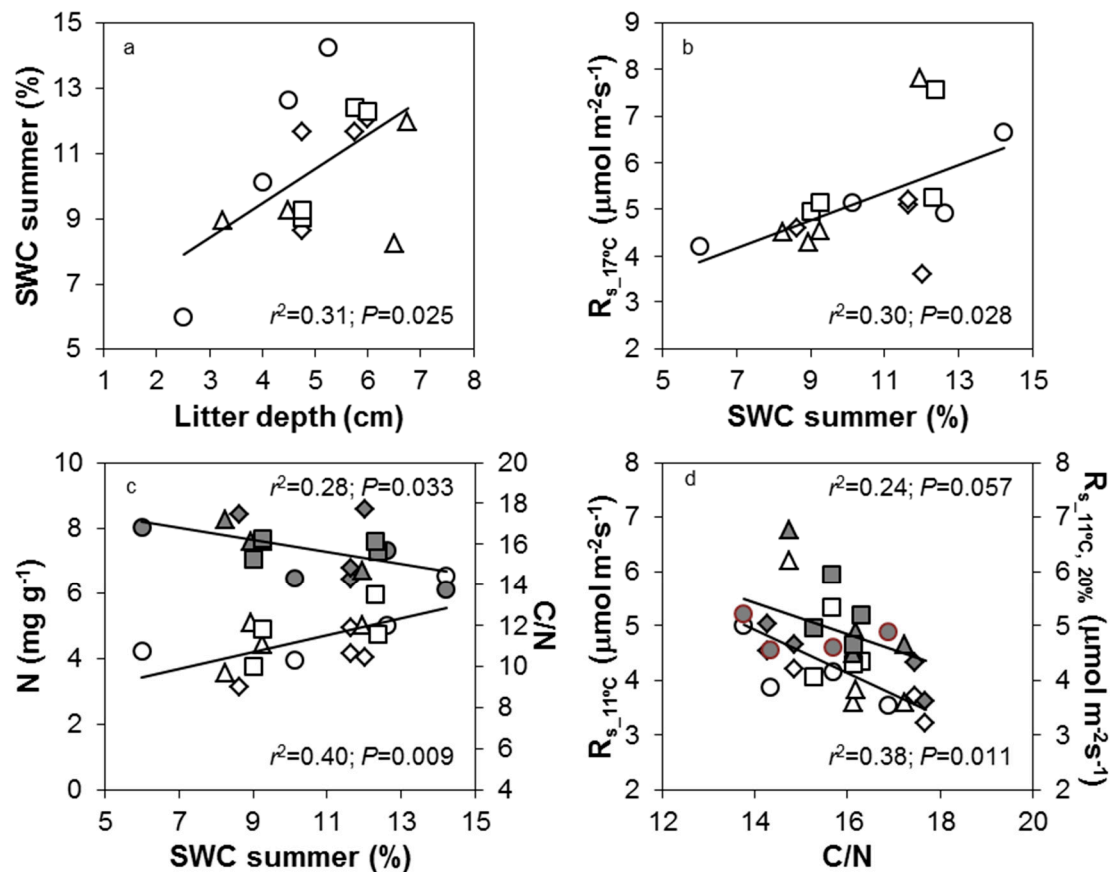


Figure 6. Relationships between soil variables across 16 plots established around centennial beech trees of different decline classes – vigorous (squares), affected (diamonds), severely affected (triangles) – and dead (circles): (a) Litter depth versus soil water content (SWC) during summer; (b) summer SWC versus standardized soil respiration (R_s) during summer at 17 °C (the average soil temperature (T_s) during summer); (c) summer SWC versus soil nitrogen concentration (open symbols) and C to N ratio (C/N; closed symbols); and (d) C/N versus mean R_s during the entire study period standardized at 11 °C (the mean annual T_s over the study period; open symbols) and at 11 °C and 20% SWC (the mean annual SWC over the study period; closed symbols). The coefficient of determination (r^2) and p -value of linear regression models fitted to all tree-decline classes pooled are shown.

4. Discussion

4.1. Does R_s Change with Tree Decline in a Mature Beech Forest?

The spatial variation of R_s was controlled by the soil C/N, basal area of recruits and, during summer, by SWC. However, the spatial variation in R_s was independent of the overstory tree decline. Previous studies have reported a homeostatic response of R_s to successional changes in forest structure involving either unchanged forest species composition [8] or species replacement [21]. Here, a minor influence of tree decline on R_s is consistent with non-significant changes in soil properties across tree-decline classes (Table 1). Large differences in the living crown volume among beech trees contrasted with non-significant changes in the soil density, litter depth, C, N, C/N or fine root density across tree-decline classes. Only SWC tended to be higher near snags from autumn to spring (Figure 2b), but this did not translate into higher R_s than in the other tree-decline classes because, at this time period, SWC did not control R_s (Figure 4a). The impact of the tree dieback on above- and below-ground processes depends on the speed at which different processes are co-occurring. The progressive degradation of ancient beech trees in the studied forest might have been lately enhanced by (i) competition from their own recruits, starting to fill the open woodland in 1960s when grazing by domestic livestock ceased,

and (ii) drought, exacerbated by a 21% reduction in the summer rainfall in this decade relative to the previous one. Still, several decades can elapse between the incipient to large decline and eventually death of centennial beech trees in the studied forest. During this time, regeneration under declining dominant beech trees is evidenced by the increasing understory population and size of beech seedlings (and more rarely holly recruits) that benefit from the increase in available resources (i.e., irradiance, soil water and nutrients) resulting from the progressive decline of overstory trees [10,21]. Reductions in the leaf biomass, root biomass, nutrients uptake and litter fall from these trees are compensated for by the development of understory trees, as discussed by others [10,21,49]. In support of this, there was a 3-fold increment in the density and basal area of recruits under declining trees than under vigorous ones, although not significant at $p < 0.05$ (Figure 1; Table 1), and R_s tended to increase as the basal area of recruits was higher. Consistently with these results, Søe and Buchmann [16] found that the stand structure was not related to the vegetation area index in another unmanaged, uneven-aged beech stand; Barba et al. [19] measured a similar root biomass in areas nearing dead and live trees; and Pereira-Blanco et al. [50] measured similar respiration rates in fine roots near dead and living trees. The progressive decline of dominant trees favors a gradual filling of the gaps left by the branch dieback that buffer its impact on the soil microclimate, root biomass and rate of decomposition processes [51]. Moreover, the late-successional character of beech allows trees of this species to establish in the understory for decades “waiting” for the canopy to open, and precludes that other species replace it, which contributes to moderate changes in the soil climate, chemistry and R_s compared to successional changes involving species replacement [19]. Future research should address how the impact of the sudden tree death on soil processes compares with a progressive process of decline.

Other studies have discussed that minor changes in R_s associated to stress- or perturbation-induced forest decline can be related to a balancing contribution of autotrophic and heterotrophic respiration to total R_s [10,52]. A reduced activity of roots and mycorrhiza associated to a reduced phloem carbohydrate transport below the ground near declining trees observed in some cases [12,20], (but see [50]) can be counteracted by an increased activity of decomposing microorganisms associated to an increased litterfall [8,53]. Unfortunately, using the trenching method in a parallel experiment did not allow us to separate the autotrophic and heterotrophic components of R_s . The higher R_s temperature sensitivity near vigorous trees than near trees with incipient symptoms of decline (Table 2) could reflect a higher contribution of autotrophic respiration to R_s near vigorous trees, given the higher temperature sensitivity of the autotrophic than heterotrophic respiration [10,24,54]. However, it could also reflect a higher seasonality in plant and soil activity, co-varying with T_s , near vigorous trees [55,56]. The fact that R_s temperature sensitivity did not gradually vary with the degree of tree decline suggests that it was not simply related to the health status of the dominant trees [56].

Independently of the degree of dominant tree decline, there were considerable variations in the mean R_s across plots ($3.25\text{--}6.35 \mu\text{mol m}^{-2} \text{s}^{-1}$), that were partly controlled by the microclimate, basal area of beech recruits under the dominant tree, and soil C/N. As already observed, the spatial variability of R_s across plots was controlled by the spatial variability in SWC rather than T_s [16,57]. During summer, R_s was higher in wetter plots (Figure 6b), likely due to low-SWC limitations on the respiratory metabolism of roots and soil microorganisms [28,29,58]; whereas spatial variations in T_s were probably too small (0.86°C in autumn and 2.74°C in spring) to cause noticeable changes in R_s . The maintenance of relatively high SWC during the dry summer period favored soil N (but not soil C) concentration and resulted in a reduced C/N (Figure 6c). A higher soil total N concentration in areas of higher SWC [59] or higher precipitation [60] has been previously reported. Here, this result might be related with a higher litter and N input in sites of high SWC (Figure 6a), although soil N and litter were not significantly related. The data suggest that litter accumulation helped to retain the soil water during summer (probably by preventing soil evaporation; Figure 6a), and that a high SWC had a direct effect on R_s by stimulating the soil activity. At comparable T_s and SWC conditions, R_s was negatively related with C/N across plots (Figure 6d). This suggests that N was a better proxy of the soil metabolic activity than C, and/or that the N substrates were more limiting than the C substrates for

the respiratory metabolism of roots and microorganisms. Studies in northern European beech forests have reported the opposite result, which could be explained by lower soil C/N ratios in these forests (9.6–11.7 in the organo-mineral layer [17] and 11.1–13.2 in the mineral layer; Søe and Buchmann [16]) than in our case (13.7–17.8). Higher soil C/N in the studied sub-Mediterranean stand can be related to drier conditions favoring higher plant and litter C/N [61], and/or slowing down soil organic matter decomposition [58,62,63], relative to northern European beech stands.

4.2. How SWC and T_s Govern Temporal Changes in R_s ?

The soil temperature drove intra-annual changes in R_s . The positive effect of temperature on the activity of respiratory enzymes of roots and soil microorganisms results in the increase of R_s as T_s rises. This has worried managers and scientists about the impact that climate warming may have on the release of C from soils, which, in forests, stock 383 ± 30 Pg C [64]. Projecting how R_s is going to respond to on-going warming is hampered, among other factors, by the interaction of T_s with many biotic and abiotic factors, including SWC [9,20,21,27,58]. Here, a progressively lower SWC registered during summer had a negative effect on R_s (Figure 4a), attenuated the increase in R_s as T_s increased (Table 2), and resulted in a logarithmic fit of R_s and T_s across the two years of study, given that higher temperatures were registered during the summer drought (Figure 3a). The limiting effect of low SWC on the enzymatic activity of roots and soil microorganisms [29,58] is likely the main factor resulting in reduced R_s rates below certain SWC thresholds in the tropical [65], temperate [16,17] and Mediterranean-type [5,6] forests. Here, the threshold below which SWC controlled R_s was 15%, which is within the wide range but in the lower end of thresholds that have been reported in literature (between 10% to 35% SWC [5,16,35,65]), suggesting a variable sensitivity of soil activity to SWC across systems.

The soil water excess also appeared to exert a small negative effect on R_s (Figure 4c). The filling of a large proportion of soil pores with water could reduce the CO_2 diffusion from the soil, and also reduce oxygen diffusion to mitochondria of roots and soil microorganisms [37,65]. In temperate forests with clay-rich soils, transient periods of waterlogging can result in the suppression of R_s [16]. Moderate soil clay proportions (12% in the A horizon) and relatively high slopes in the studied forest make waterlogging less relevant to R_s suppression than recurrent summer droughts. The mean R_s normalized at a constant T_s of 11 °C and non-limiting SWC (20%)—the mean annual values—was $4.90 \mu\text{mol m}^{-2} \text{s}^{-1}$, which is higher than R_s at a similar T_s reported in temperate beech forests [16,17]. This is in spite of the higher long-term influence of drought on reducing R_s in the studied sub-Mediterranean stand. It is possible that the high soil N concentration (4.6 g K^{-1} , equivalent to 368 g m^{-2} in the mineral topsoil), partly associated to the high basal area and litterfall across plots, contributes to the high R_s in this old forest (with dominant trees >200 years and suppressed trees often >50 years; Gil et al. [41]), compared to younger beech forests.

5. Conclusions

The decline of centennial dominant beech trees in a mature forest has only minor effects on the soil microclimate, physico-chemical variables, fine root density and R_s . The gradual process of decline of these trees, even if accelerated in the last years probably by competition with their own recruits and enhanced drought, is probably contributing to the homeostasis in soil processes. Shade tolerant beech recruits establish in the understory and develop as crown dieback advances, self-perpetuating the beech stand and maintaining above- and below-ground characteristics relatively constant. Increased duration and intensity of droughts projected for the study area [66] could exacerbate the control of SWC on the soil activity seen here over time and space; however, it could also have more profound effects on soil processes by altering the successional direction and velocity. Further research oriented to separate the autotrophic and heterotrophic contribution to R_s will help to better understand how tree decline affects soil biological processes, and to eventually delineate management practices aiming at increasing soil organic carbon stocks and reducing soil heterotrophic respiration [67].

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