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Article

Experimental Precipitation Reduction Slows Down Litter Decomposition but Exhibits Weak to No Effect on Soil Organic Carbon and Nitrogen Stocks in Three Mediterranean Forests of Southern France

Mathieu Santonja ^{1,*}, Susana Pereira ^{1,†}, Thierry Gauquelin ¹, Elodie Quer ¹, Guillaume Simioni ², Jean-Marc Limousin ³, Jean-Marc Ourcival ³, Ilja M. Reiter ⁴, Catherine Fernandez ^{1,‡} and Virginie Baldy ^{1,‡}

¹ Aix Marseille Université, Avignon Université, CNRS, IRD, IMBE, Marseille, France

² Ecologie des Forêts Méditerranéennes, INRAE, Domaine Saint Paul, Site Agroparc, CEDEX 09, 84914 Avignon, France

³ Centre d'Ecologie Fonctionnelle et Evolutive—CEFE UMR CNRS, 1919 Route de Mende, CEDEX 05, 34293 Montpellier, France

⁴ Ecosystèmes Continentaux et Risques Environnementaux CNRS, FR3098 ECCOREV, Domaine du Petit Arbois, Avenue Louis Philibert, Bâtiment du CEREGE—BP 80, CEDEX 04, 13545 Aix-en-Provence, France

* Correspondence: mathieu.santonja@imbe.fr

† These authors contributed equally to this work.

‡ These authors contributed equally to this work.



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Abstract: Forest ecosystems are some of the largest carbon (C) reservoirs on earth. *Pinus halepensis* Mill., *Quercus ilex* L. and *Quercus pubescens* Willd. represent the dominant tree cover in the Mediterranean forests of southern France. However, their contributions to the French and global forest C and nitrogen (N) stocks are frequently overlooked and inaccurately quantified and little is known about to what extent the ongoing climate change can alter these stocks. We quantified the soil organic C (SOC) and N (SN) stocks in Mediterranean forests dominated by these tree species and evaluated to what extent an experimental precipitation reduction (about −30% yearly) affects these stocks and the litter decomposition efficiency. Litter mass losses were 55.7, 49.8 and 45.7% after 24 months of decomposition in *Q. ilex*, *Q. pubescens* and *P. halepensis* forests, respectively, and were 19% lower under drier climatic conditions. The SOC stocks were 14.0, 16.7 and 18.5 Mg ha^{−1} and the SN stocks were 0.70, 0.93 and 0.88 Mg ha^{−1} in *Q. ilex*, *Q. pubescens* and *P. halepensis* forests, respectively. The shallowness and stoniness of these Mediterranean forests could explain these limited stocks. By distinguishing the organic from the organo–mineral layer, we showed 74% less SOC in the organic layer of the *P. halepensis* forest under drier conditions, while no difference was detected in the organo–mineral layer or in the two oak forests. This last finding deserves further investigation and points out the necessity to distinguish the organic from the organo–mineral layer to detect the first impacts of climate change on SOC stocks.

Keywords: climate change; *Pinus halepensis*; *Quercus ilex*; *Quercus pubescens*; forest soil; carbon sequestration

1. Introduction

Globally, soils store about three to four times more organic carbon (C) (1500–2400 Gt) than vegetation (450–650 Gt) and almost twice as much as is present in the atmosphere (860 Gt) [1]. As the C residence time is longer in soils than in the vegetation biomass [2,3], the stable incorporation of C in soils is of great interest to reduce atmospheric CO₂ concentration in order to contribute to climate change mitigation [4–6]. Covering about 31% of the world's land surface, forest ecosystems play a crucial role in the global C stock [7–9]. Indeed, C sequestration in forests, which performs a key role as CO₂ sinks, can help mitigate the effects of climate change [4,5,10]. During 2004–2013, the world's forests sequestered

between 2.5 and 3.9 Gt of C per year, which represents nearly 30% of global fossil fuel emissions [9]. Pan et al. [7] reported that the C sinks in temperate forests increased by 17% from 2000 to 2007 compared with the period 1990 to 1999. The global C stock of forests is estimated at 662 Gt [11], with almost half stored in the soil [7], making soil C sequestration a powerful strategy for mitigating climate change [4,5,10]. However, strong concerns in this climate change mitigation potential persist due to uncertainties in forest soil C stock responses to future climatic conditions, as relatively small changes in soil C stocks can have large effects on the forest greenhouse gas balance [4,5,12].

Plant litter, microorganisms and roots are the major input sources of soil organic carbon (SOC) in forests [13–15], while heterotrophic respiration by litter decomposers, leaching and erosion account for the main SOC loss processes [16]. Climate has been identified as one of the main factors influencing SOC [17]. Indeed, many studies of the spatial distribution of C stocks under natural vegetation have revealed positive correlations with precipitation and negative correlations with temperature, either on the global scale [17,18] or in more restricted territories, such as in France [19,20]. With the ongoing climate change, drier conditions have become more frequent and severe in many terrestrial ecosystems [21,22]. Over the past decades, there has been a growing interest in predicting the impact of climate change on forested ecosystems as they play a major role in C sequestration and climate regulation [23–26].

Although Mediterranean forests represent only 1.8% (80 Mha) of the world's forest cover, they are an important niche for biodiversity [27,28] and they provide a wide array of essential ecosystem services to society [28–30]. Compared with temperate and tropical forests, C assessments in Mediterranean ecosystems remain scarce [31–33], arguing that Mediterranean ecosystems (i) have a low productivity (and therefore are of low economic value) and (ii) are characterized by heterogeneous historical land-use practices and soil characteristics (e.g., shallow, high rock content) that hamper the reliable and accurate estimation of C pools [28,32,34]. Mediterranean ecosystems are among the most sensitive terrestrial biomes to climate change [28,35]. Regional climate models for the Mediterranean basin predict for 2100 a warming of 3.4 °C of the annual average temperature and a decrease of annual precipitations by 30–40%, which will result in an intensification of severe droughts (according to the RCP 8.5 scenario) [36–38]. Drier conditions will severely impact biological processes that are already limited by water availability in Mediterranean forests [30,39], with cascading effects on litter decomposition efficiency and C dynamics [40,41] and consequently on the ability of Mediterranean forests to sequester C.

In France, Mediterranean forests represent about 8% of the total forest cover (1.4 over 16.9 Mha) [42], with almost 80% dominated by *Pinus halepensis* Mill. (0.21 Mha), *Quercus ilex* L. (0.38 Mha) and *Quercus pubescens* Willd. (0.33 Mha) [43]. These three tree species also structure the three main forests of the northern part of the Mediterranean basin [27]. As French forest cover has been increasing by 0.8% per year since 1985 mainly due to the cessation of traditional farming [28,42], the Mediterranean forests dominated by these three trees species may constitute an important C sink for the future. However, to date, little is known about their C stocks that remain to be accurately quantified.

In order to improve our knowledge on their contribution to the global C and N stocks, the present study aimed (i) at quantifying the soil organic C and N concentrations and stocks in the three dominant Mediterranean forests (i.e., *P. halepensis*, *Q. ilex* and *Q. pubescens*) at three long-term study sites located in southern France and (ii) at evaluating whether an experimental precipitation reduction (about –30% yearly) affects the efficiency of the litter decomposition process and the SOC and SN concentrations and stocks in these forests. We hypothesized a negative effect of an experimental precipitation reduction on litter decomposition efficiency and on SOC and SN concentrations and stocks, as water availability is the most important environmental constraint for soil microbial and faunal activities in Mediterranean ecosystems [39,41,44–46].

2. Material and Methods

2.1. Study Sites

The study was conducted in the three dominant forests north of the Mediterranean basin. The *P. halepensis* forest was represented by the experimental site of Font-Blanche (hereafter, FB; 43°14'27" N, 5°40'45" E), the *Q. ilex* forest was represented by the experimental site of Puéchabon (hereafter, PC; 43°44'29" N, 3°35'46" E) and the *Q. pubescens* forest was represented by the experimental site of Oak Observatory at the Observatoire de Haute Provence (hereafter, O₃HP; 43°56'16" N, 5°42'64" E) (Table 1). Each study site included a control plot (natural drought ND) and a rain exclusion plot (amplified drought AD) to mimic the future precipitation reduction predicted by the climatic model for the Mediterranean region (about −30% yearly) [36–38]. In the *Q. pubescens* forest, the rain exclusion started in 2012 with the implementation of a 15 m × 20 m rainout shelter above the canopy that dynamically excluded precipitations by deploying automated shutters during rainfall events of the vegetation period (i.e., from spring to autumn). In the *P. halepensis* and *Q. ilex* forests, the rain exclusion is performed by using fixed PVC gutters (in an area of 25 m × 25 m) installed below the forest canopy, excluding about 30% at each rainfall event throughout the year. Control plots had the same system, but the PVC gutters were fixed upside down and did not exclude rainfall. The corresponding rain exclusion plots were settled in 2003 and 2008 in the *Q. ilex* and *P. halepensis* forests, respectively. The three study sites are part of the research infrastructure AnaEE-France (<https://www.anaee-france.fr/en/>; accessed on 11 August 2022) and offer experimental facilities, including a set of sensors for long-term monitoring of environmental data. The study site characteristics are detailed in Table 1.

Table 1. Main characteristics of the three studied forests. Mean annual temperature (MAT) and precipitation (MAP) correspond, respectively, to the annual mean values of temperature and precipitation between 2008 and 2019 in natural precipitation (ND) and amplified drought (AD) plots.

Forests	<i>Pinus halepensis</i>	<i>Quercus ilex</i>	<i>Quercus pubescens</i>
Sites	Font-Blanche (FB)	Puéchabon (PC)	Oak Observatory at Observatoire de Haute Provence (O ₃ HP)
Location	43°14'27" N, 5°40'45" E	43°44'29" N, 3°35'46" E	43°56'16" N, 5°42'64" E
Altitude a.s.l. (m)	425	270	650
MAT (°C)	13.7	14.0	12.6
MAP ND (mm)	605.0	955.4	866.3
MAP AD (mm)	441.6	698.9	639.5
Soil type	Leptosol	Rhodo-chromic luvisol	Pierric calcosol
Soil texture	Clay	Clay loam	Clay
Soil pH	6.8	6.6	6.8
Surface rock cover (%)	50	75	23
Dominant tree species	Mixed <i>Pinus halepensis</i> Mill./ <i>Quercus ilex</i> L.	<i>Quercus ilex</i> L.	<i>Quercus pubescens</i> Willd.
Other dominant plant species	<i>Quercus coccifera</i> L., <i>Phyllirea latifolia</i> L.	<i>Buxus sempervirens</i> L., <i>Phyllirea latifolia</i> L., <i>Pistacia terebinthus</i> L., <i>Juniperus oxycedrus</i> L.	<i>Acer Monspeulanum</i> L., <i>Cotinus coggygria</i> Scop.
Tree density (stems·ha ^{−1})	3368	4500	3503
Forest structure	Uneven age (61 years)	Even age (74 years)	Even age (70 years)
Rain exclusion device	Permanent system: PVC gutters	Permanent system: PVC gutters	Dynamic system: moving roof device
Rain exclusion system dimensions (m ²)	625	140	300
Rain exclusion device installation	2009	2003	2012

2.2. Litter Decomposition Experiment

Senescent leaves and needles were collected in the three studied forests during the abscission period that occurred from April to May 2014 for *Q. ilex* leaves, from June to September 2014 for *P. halepensis* needles and from October to November 2014 for *Q. pubescens* leaves, using litter traps suspended under the concerned trees. Immediately after collection, the leaves were air dried at room temperature and stored until the beginning of the experiment.

Leaf litter decomposition was studied over 24 months using the litterbag method [47]. Ten grams of dry leaf litter were placed in a 4 mm mesh litterbag (20 cm × 20 cm), with the mesh size designed to allow colonization by microbes, soil mesofauna and some soil macrofauna [41]. A total of 84 litterbags (3 forest types × 2 precipitation treatments × 2 sampling times × 7 replicates) were thus used during the experiment. Litterbags were placed perpendicularly to the gutter systems in the *P. halepensis* and *Q. ilex* forests and under the rain exclusion device in the *Q. pubescens* forest in December 2014. They were placed on the ground after the removal of the litter layer and fixed to the soil with galvanized nails to prevent movement by animals or wind. A litter layer was then replaced over the litter bags.

The litter mass loss was measured after 12 and 24 months of decomposition, i.e., in December 2015 and 2016, respectively. At each sampling date, 14 litter bags were retrieved from each forest. When taken off, the litterbags were immediately sealed in plastic bags to prevent litter material loss and transported to the laboratory. In the laboratory, the litter contained in the litter bags was cleaned of soil and other plant detritus and stored at −18 °C. All the samples were freeze dried (Lyovac GT2) and weighted.

2.3. Soil Sampling and Carbon and Nitrogen Measurements

At each forest site, fifteen soil cores were randomly collected up to 10 cm depth in both ND and AD plots in 2016. Soil layers were identified and mechanically separated according to the World Reference Base for Soil Resources system [48] in order to distinguish the organic (O) from the organo–mineral (A) layer. In total, 180 soil samples (3 forest types × 2 precipitation treatments × 2 soil layers × 15 replicates) were collected and transported to the laboratory in plastic bags prior to further analysis.

In the laboratory, each soil sample was sieved at 2 mm mesh prior to being oven dried at 65 °C for 48 h. Stones and other litter detritus (>2 mm) were retrieved and only fine soil fraction (<2 mm) was used for soil C and N measurements.

Total carbon (TC) and nitrogen (N) concentrations of the 180 soil samples were determined by thermal combustion on a Flash EA 1112 series C/N elemental analyzer (Thermo Scientific®, Waltham, MA, USA). As the bedrock of our study sites is limestone (Table 1), C can also be present as calcium carbonate (CaCO₃) in the soil, necessitating that this inorganic form of C in our soil analysis is taken into account. SIC concentrations were estimated using the loss-on-ignition (LOI) procedure followed by Wang et al. [49]:

$$\text{SIC}_{\text{LOI}} \left(\text{g kg}^{-1} \right) = \left(\frac{\text{Weight}_{375\text{ }^{\circ}\text{C}} - \text{Weight}_{800\text{ }^{\circ}\text{C}}}{\text{Weight}_{105\text{ }^{\circ}\text{C}}} \right) \times 0.273 \times 1000$$

Soil organic C (SOC) was calculated by the differences between TC and soil inorganic C (SIC). SOC and SN stock (Mg ha^{−1}) were estimated for both organic and organo–mineral layers and scaled to ha by taking into consideration the rock surface estimations (Table 1).

2.4. Statistical Analysis

All statistical analyses were performed with the R software (version 3.1.3, R Development Core Team, 2017). Data were log-transformed when necessary to meet the assumption of normality and homoscedasticity.

A three-way analysis of variance (ANOVA), followed by post hoc Tukey tests, were used to compare litter mass loss during the decomposition process according to forest type, precipitation treatment, time of decomposition and their interactions.

A linear mixed effects model (“nlme” package), followed by Tukey HSD tests for post hoc comparisons, were used to test the effects of forest type (*P. halepensis*, *Q. ilex* or *Q. pubescens*), precipitation treatment (natural or amplified drought), soil layers (organic or organo–mineral) and their interactions on SOC and SN concentrations and stocks. To consider the fact that the two soil layers within a soil core were not independent, the random part of the model indicated that the soil layers were nested soil cores.

A two-way analysis of variance (ANOVA), followed by post hoc Tukey tests, were used to compare total SOC and SN stocks up to 10 cm depth according to forest type, precipitation treatment and their interactions.

3. Results

3.1. Litter Mass Loss during the Decomposition Process

During the whole experiment, we found that the average litter mass loss was 35.0% and 45.6% after 12 and 24 months of decomposition, respectively (Table 2, Figure 1).

Table 2. Effects of forest type (*P. halepensis*, *Q. ilex* and *Q. pubescens*), precipitation treatment (natural or amplified drought), time (12 or 24 months) and their interactions on litter mass loss. *F*-values and associated *P*-values (* for $P < 0.05$ and *** for $P < 0.001$) are indicated. Significant values are indicated in bold.

	Litter Mass Loss		
	d.f.	<i>F</i> -Value	<i>P</i> -Value
Forest type (F)	2	3.2	*
Precipitation treatment (P)	1	13.3	***
Time (T)	1	43.0	***
F × P	2	0.2	
F × T	2	4.7	*
P × T	1	5.2	*
F × P × T	2	0.3	

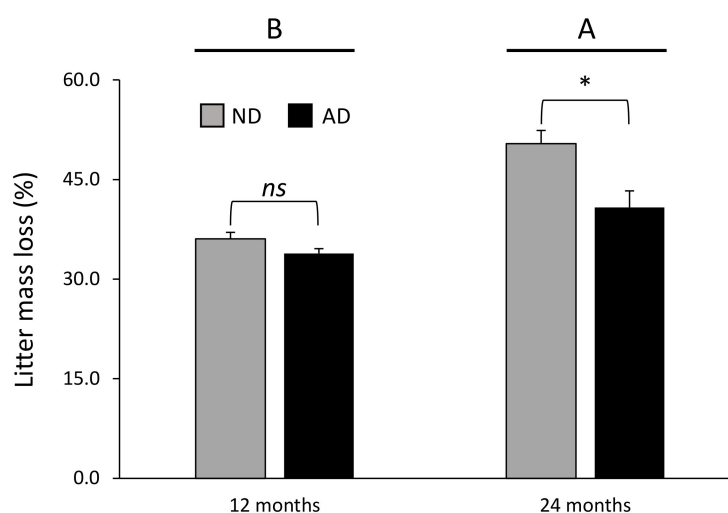


Figure 1. Litter mass loss according to the significant precipitation treatment × time of decomposition interaction (Table 2). Data are mean values ± SE and expressed as % of mass loss; $n = 21$. Different capital letters denote significant differences between time of decomposition with A > B (post hoc Tukey tests results). Stars indicate significant differences between precipitation treatments (* for $P < 0.05$). ND—natural drought, AD—amplified drought, *ns*—non significant.

The differences in litter mass loss according to precipitation treatment were dependent on the time of decomposition (significant precipitation treatment × time of decomposition interaction, Table 2). Litter mass loss was not affected by the precipitation treatment after 12 months of decomposition (Figure 1); on the contrary, litter mass loss was 19% lower

under amplified drought compared with natural drought treatment after 24 months of decomposition (Figure 1).

The differences in litter mass loss according to forest type were also dependent on the time of decomposition (significant forest type \times time of decomposition interaction, Table 2). *Pinus halepensis* litter mass loss was higher (37.0%), compared with the two oak species after 12 months of decomposition (Figure 2). *Quercus ilex* showed higher litter mass loss (51.5%) compared with *P. halepensis* (41.7%) and *Q. pubescens* (43.6%) after 24 months of decomposition (Figure 2).

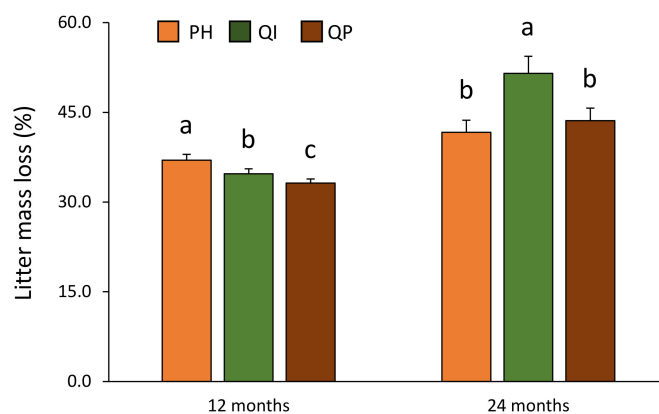


Figure 2. Litter mass loss according to the significant forest type \times time of decomposition interaction (Table 2). Data are mean values \pm SE and expressed as % of mass loss; $n = 14$. Different lowercase letters denote significant differences between forest types with $a > b > c$ (post hoc Tukey tests results). PH—*Pinus halepensis*, QI—*Quercus ilex*, QP—*Quercus pubescens*.

3.2. SOC and SN Concentrations

SOC and SN concentrations differed according to forest type and soil layer but were not affected by precipitation treatment (Table 3; Supplementary Table S1).

Table 3. Effects of forest type (*P. halepensis*, *Q. ilex* and *Q. pubescens*), precipitation treatment (natural or amplified drought), soil layer (organic or organo–mineral) and their interactions on SOC and SN concentrations and stocks. Chi-square values and associated P -values (* for $P < 0.05$ and *** for $P < 0.001$) are indicated. Significant values are indicated in bold.

	d.f.	SOC Concentration		SN Concentration		SOC Stock		SN Stock	
		Chisq	P -Value	Chisq	P -Value	Chisq	P -Value	Chisq	P -Value
Forest type (F)	2	23.0	***	40.2	***	16.6	***	1.6	
Precipitation treatment (P)	1	1.9		0.2		0.0		0.1	
Soil layer (S)	2	1709.5	***	200.0	***	2264.8	***	1892.4	***
F \times P	2	2.4		0.0		5.6		2.2	
F \times S	2	33.6	***	3.5		82.0	***	20.6	***
P \times S	1	2.1		0.4		0.0		0.2	
F \times P \times S	2	1.7		1.2		7.4	*	0.0	

SOC concentration was three times higher in the organic layer (from 35.9 to 45.5%) compared with the organo–mineral layer (from 12.0 to 15.2%) (Table 3; Figure 3). Differences in SOC concentration between forest types were dependent on the soil layer considered (significant forest type \times soil layer interaction, Table 3). SOC concentration in the organic layer decreased according to the gradient *P. halepensis* $>$ *Q. ilex* $>$ *Q. pubescens* forests, and was, respectively, 14% and 21% higher in the *P. halepensis* forest compared with the *Q. ilex* and *Q. pubescens* forests (Figure 3). Concerning the organo–mineral layer, the SOC concentration was 19% lower in the *Q. ilex* forest compared with the *P. halepensis* and *Q. pubescens* forests (Figure 3).

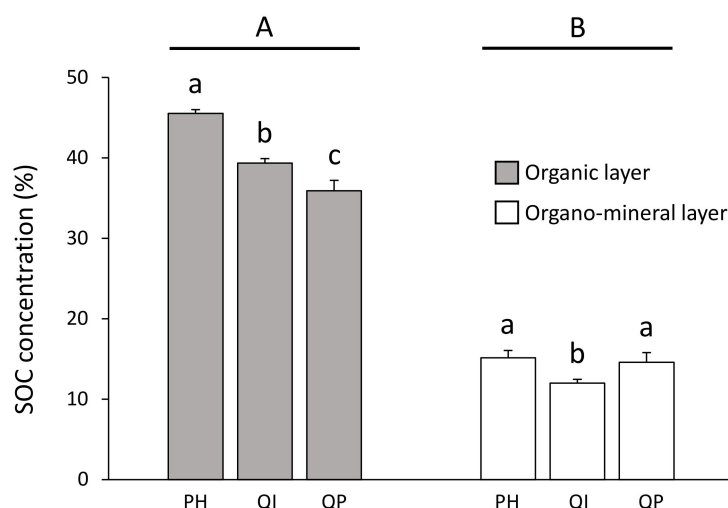


Figure 3. Soil organic carbon concentration according to the significant forest type \times soil layer interaction (Table 3). Data are mean values \pm SE and expressed as % per g of DW soil; $n = 30$. Different capital letters denote significant differences between soil layers with $A > B$ (post hoc Tukey tests results). Different lowercase letters denote significant differences between forest types for each soil layer with $a > b > c$ (post hoc Tukey tests results). SOC—soil organic carbon, PH—*Pinus halepensis*, QI—*Quercus ilex*, QP—*Quercus pubescens*.

SN concentration was 38% higher in the organic compared with the organo–mineral layer (Table 3; Figure 4a). SN concentration decreased according to the gradient *Q. pubescens* $>$ *P. halepensis* $>$ *Q. ilex* forests, and was, respectively, 18% and 24% higher in the *Q. pubescens* forest compared with the *P. halepensis* and *Q. ilex* forests (Table 3; Figure 4b).

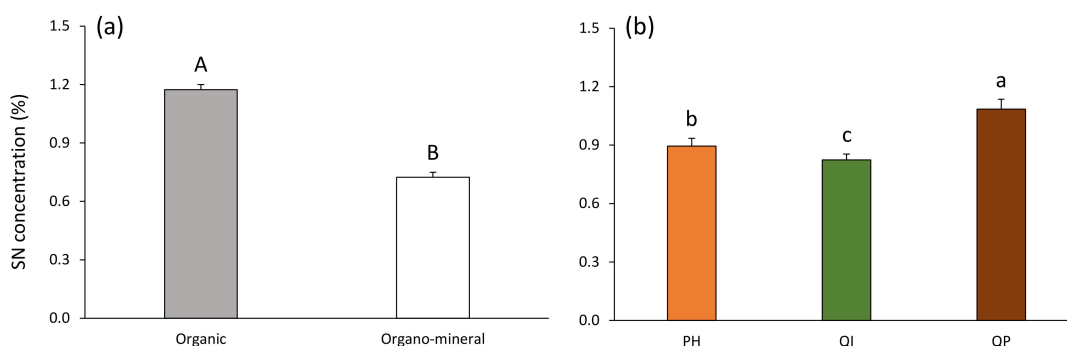


Figure 4. Soil nitrogen concentration according to (a) soil layer and (b) forest type (Table 2). Data are mean values \pm SE and expressed as % per g of DW soil; $n = 30$. Different capital letters denote significant differences between soil layers with $A > B$ (post hoc Tukey tests results). Different lowercase letters denote significant differences between forest types with $a > b > c$ (post hoc Tukey tests results). SN—soil nitrogen, PH—*Pinus halepensis*, QI—*Quercus ilex*, QP—*Quercus pubescens*.

3.3. SOC and SN Stocks

SOC and SN stocks differed according to forest type and soil layer (significant forest type \times soil layer interaction) and only SOC stock was affected by a shift in precipitation (significant forest type \times precipitation treatment \times soil layer interaction, Table 3; Supplementary Table S1).

While SOC stock was higher in the organo–mineral layer (from 13.1 to 18.1 Mg ha⁻¹) compared with the organic layer (from 0.4 to 2.0 Mg ha⁻¹) (Table 3), the differences between forest types were dependent on the soil layer considered (significant forest type \times soil layer interaction, Table 3) and the differences between precipitation treatments were dependent on both the soil layer and the forest type considered (significant forest type \times soil layer \times precipitation treatment interaction, Table 3). SOC stock in the organic

layer was 69% higher in the *Q. pubescens* forest compared with the *P. halepensis* and *Q. ilex* forests (Figure 5a), while SOC stock in the organo–mineral layer was 24% higher in the *P. halepensis* forest compared with the two oak forests (Figure 5b). Only the SOC stock in the organic layer of the *P. halepensis* forest was affected by a shift in precipitation, with 74% less SOC in amplified drought compared with natural drought conditions (Figure 5a).

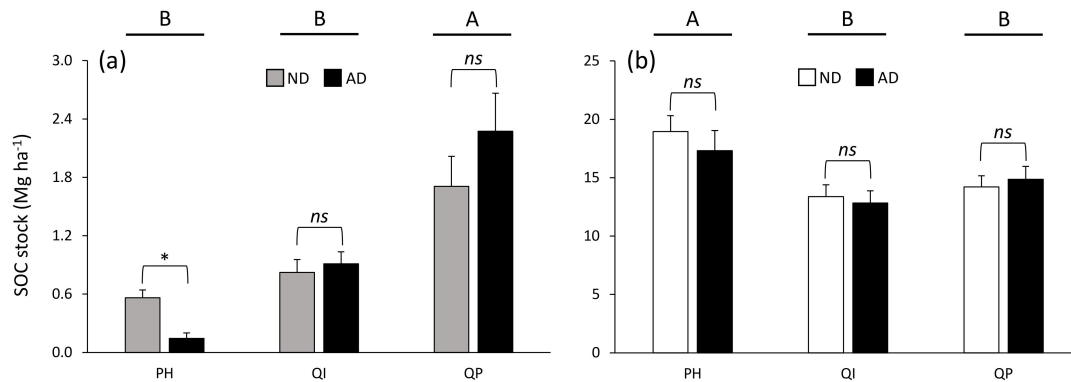


Figure 5. Soil organic carbon stock in soil (a) organic and (b) organo–mineral layer according to the significant forest type \times precipitation treatment \times soil layer interaction (Table 3). Data are mean values \pm SE and expressed as Mg per ha; $n = 15$. Different capital letters denote significant differences between forest types for each soil layer with $A > B$ (post hoc Tukey tests results). Stars indicate significant differences between precipitation treatments ($*$ for $P < 0.05$). SOC—soil organic carbon, ND—natural drought, AD—amplified drought, PH—*Pinus halepensis*, QI—*Quercus ilex*, QP—*Quercus pubescens*.

SN stock concentration was lower in the organic layer (from 0.01 to 0.09 Mg ha⁻¹) compared with the organo–mineral layer (from 0.66 to 0.87 Mg ha⁻¹) (Table 3; Figure 6). Differences in SN stock between forest types were dependent on the soil layer considered (significant forest type \times soil layer interaction, Table 3). SN stock in the organic layer was five times higher in the *Q. pubescens* forest compared with the *P. halepensis* and *Q. ilex* forests (Figure 6). Concerning the organo–mineral layer, SN stock was 21% lower in the *Q. ilex* forest compared with the *P. halepensis* and *Q. pubescens* forests (Figure 6).

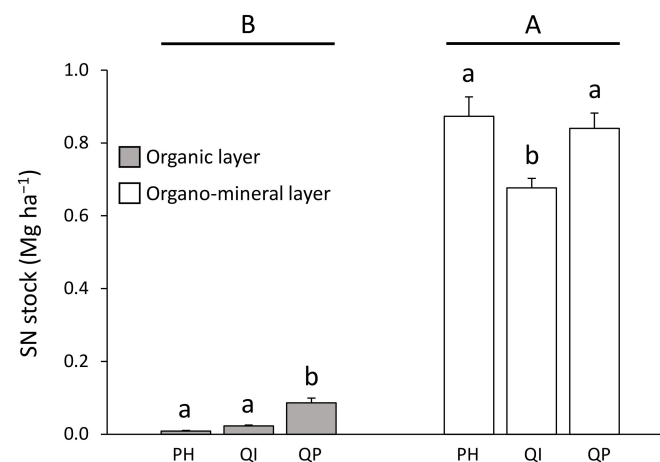


Figure 6. Soil nitrogen stock according to the significant forest type \times soil layer interaction (Table 2). Data are mean values \pm SE and expressed as Mg per ha; $n = 30$. Different capital letters denote significant differences between soil layers with $A > B$ (post hoc Tukey tests results). Different lowercase letters denote significant differences between forest types for each soil layer with $a > b > c$ (post hoc Tukey tests results). SN—soil nitrogen, PH—*Pinus halepensis*, QI—*Quercus ilex*, QP—*Quercus pubescens*.

3.4. Total SOC and SN Stocks in the First 10 cm Soil Depth

The total SOC stocks ranged from 14.0 Mg ha⁻¹ in the *Q. ilex* forest to 18.5 Mg ha⁻¹ in the *P. halepensis* forest (Figure 7a). This total SOC stock was 24% higher in the *P. halepensis*

forest compared with the *Q. ilex* forest (Table 4; Figure 7a). When pooling both soil layers to estimate these SOC stocks in the first 10 cm soil depth, the precipitation treatment effect previously observed in the *P. halepensis* forest (Figure 6) disappeared as the forest type \times precipitation treatment interaction was non-significant (Table 4). The total SN stocks ranged from 0.70 Mg ha⁻¹ in the *Q. ilex* forest to 0.93 Mg ha⁻¹ in the *Q. pubescens* forest (Figure 7b). This total SN stock was 22% lower in the *Q. ilex* forest compared with the *P. halepensis* and *Q. pubescens* forests (Table 4; Figure 7b).

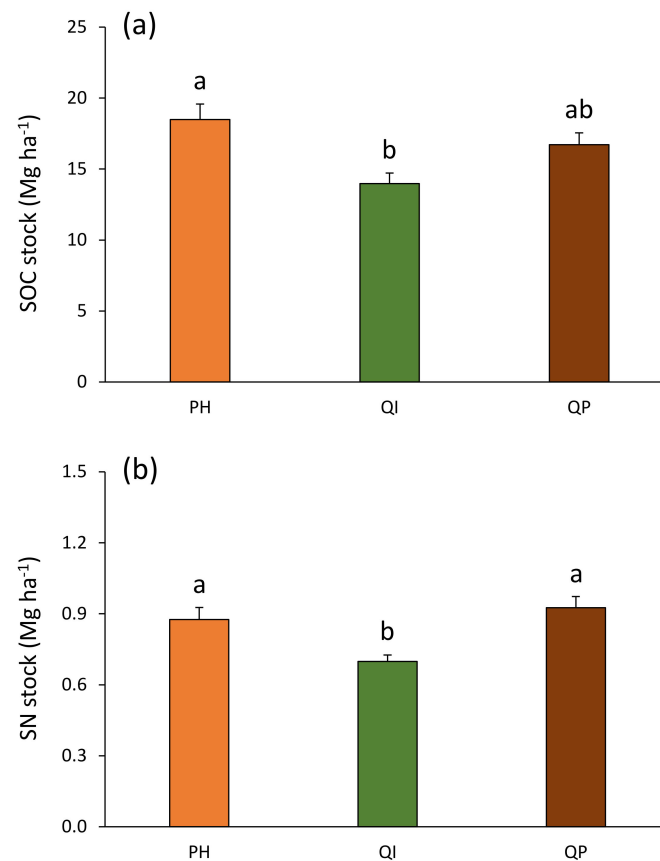


Figure 7. (a) Soil organic carbon and (b) soil nitrogen stocks according to forest type (Table 2). Data are mean values \pm SE and expressed as Mg per ha; $n = 30$. Different lowercase letters denote significant differences between forest types with $a > b$ (post hoc Tukey tests results). SOC—soil organic carbon, SN—soil nitrogen, PH—*Pinus halepensis*, QI—*Quercus ilex*, QP—*Quercus pubescens*.

Table 4. Effects of forest type (*P. halepensis*, *Q. ilex* and *Q. pubescens*), precipitation treatment (natural or amplified drought) and their interactions on total SOC and SN stocks within the first 10 cm soil depth. *F*-values values and associated *P*-values (** for $P < 0.01$ and *** for $P < 0.001$) are indicated. Significant values are indicated in bold.

	d.f.	<i>F</i> -Value	<i>P</i> -Value
<i>Total SOC stock</i>			
Forest type (F)	2	6.3	**
Precipitation treatment (P)	1	0.1	
F \times P	2	1.0	
<i>Total SN stock</i>			
Forest type (F)	2	7.8	***
Precipitation treatment (P)	1	0.2	
F \times P	2	2.2	

4. Discussion

We assessed with common methods the litter decomposition efficiency and the soil organic carbon (SOC) and soil nitrogen (SN) stocks up to 10 cm depth in the three dominant Mediterranean forest stands in southern France. After 24 months of the experiment, *P. halepensis* showed a lower litter decomposition rate compared with the two oak species. The lower litter quality of pine needles compared with oak leaves can explain this difference in decomposition rates [50–53]. Indeed, *P. halepensis* needle litter exhibits higher C:N and C:P ratios and lower water-holding capacity (WHC) than *Q. ilex* and *Q. pubescens* leaf litters [53]. These three litter traits control the decomposition process [50,51,54,55], as higher high C:N and C:P ratios make the organic matter more recalcitrant to decomposition, while WHC is directly linked to the ability of the litter to keep the humidity necessary for decomposer and detritivore activities. *Quercus ilex* showed the highest litter decomposition rate after 24 months of the experiment, probably due to the higher N and P concentrations [53] that made the litter more palatable [56,57].

As expected, we observed a negative effect of reduced precipitation on litter decomposition efficiency, but only after 24 months of the experiment. A recent study performed by Pereira et al. [53] reported no significant effect of amplified drought conditions on the litter decomposition process in 2015 in the same AnaEE-France experimental sites as the present study. Pereira et al. [53] explained this lack of effect by the extremely dry year during which their study was performed. Indeed, other studies performed in the same AnaEE-France experimental sites in 2012 and 2013 reported lower *Q. pubescens* and *P. halepensis* litter decomposition rates under amplified drought conditions [41,51]. Previous litter decomposition studies conducted in other Mediterranean ecosystems also reported a significant reduction in litter decomposition rates with reduced water availability [58–60]. These reduced litter decomposition rates can be explained by the negative effect of a decrease in water availability on soil microbial and faunal abundances and diversities and activities, as soil biodiversity drives the litter decomposition process [41,61,62]. For example, Curiel Yuste et al. [63] and Santonja et al. [41] reported lower soil microbial biomass, Sardans and Peñuelas [64] and Hueso et al. [65] reported a reduction in soil enzyme activities and Santonja et al. [41] and Aupic-Samain et al. [66] reported a lower Collembola abundance under drier climatic conditions in Mediterranean ecosystems.

SOC concentrations measured in the organic and organo–mineral layers were high and typical of Mediterranean forest calcareous soils, where the decomposition of the organic matter is slow compared with temperate forests [67–69] and leads to an accumulation of organic carbon in soils [70]. The *Pinus halepensis* forest showed higher SOC concentrations in the organic layer compared with the two oaks forests. This finding is consistent with the lower litter quality of pine needles compared with oak leaves [50–53], leading to a slower litter decomposition rate (Figure 3 of the present study) [50,51,71] and consequently to a higher accumulation of organic matter at the soil surface [70]. Regarding the SOC stocks in the three studied forests, our estimations ranging from 14.0 to 18.5 Mg C ha⁻¹ are lower than those previously reported in other Mediterranean forests (from 33.6 to 120.5 Mg C ha⁻¹) [72–77]. Our estimations of SOC stocks are also lower than the values reported for other French forests generally developing in deeper soils (from 65.0 to 94.1 Mg C ha⁻¹ considering a 30 cm soil depth) [78–80]. In addition to the differences in the protocols used to estimate SOC stocks in the present study compared with the previous ones, two main reasons could explain the strong differences reported: (i) the soils of our three studied Mediterranean forests are extremely shallow (from 10.0 to 13.2 cm depth until bedrock); (ii) they are developed on extremely stony limestone (from 23 to 75% percent surface cover of rocks, Table 1; Figure 8). Stoniness is one of the key variables affecting C stock estimation; however, this soil parameter is rarely measured [81], thus SOC stocks are often overestimated [82].



Figure 8. Trench opened in the *Q. pubescens* forest highlighting the shallow soil developed directly on limestone slab (credit: T. Gauquelin).

Surprisingly, while we observed lower litter decomposition rates in amplified drought compared with natural drought conditions in the three studied forests, total SOC concentrations and stocks up to 10 cm depth were not affected by a shift in precipitation conditions. However, by distinguishing the organic layer from the organo–mineral layer, we showed that the SOC stock in the organic layer of the *P. halepensis* forest was affected by a shift in precipitation, with 74% less SOC in amplified drought compared with natural drought conditions. This important finding points out the necessity to distinguish both soil layers to detect the first impact of climate change on SOC stocks. A lower litter decomposition rate under drier conditions cannot explain this strong decrease. We can speculate that a decrease in pine needle litter production is linked to this decrease in SOC stocks, but further investigation will be mandatory to support this statement. Finally, despite a 30% annual experimental precipitation reduction occurring between 4 and 13 years in the *Q. ilex* and *Q. pubescens* forests, respectively, and lower leaf litter decomposition rates, SOC concentrations and stocks evaluated are not affected in these two Mediterranean oak forests.

5. Conclusions

For the first time, we performed a quantification of the SOC and SN stocks of the three dominant forest stands of southern France. The SOC stocks were 14.0, 16.7 and 18.5 Mg ha⁻¹ and the SN stocks were 0.70, 0.93 and 0.88 Mg ha⁻¹ in the *Q. ilex*, *Q. pubescens* and *P. halepensis* forests, respectively. The shallowness (from 10.0 to 13.2 cm depth until bedrock) and stoniness (from 23 to 75% percent surface cover of rocks) of these Mediterranean forests could explain the limited SOC and SN stocks. By distinguishing the organic layer from the organo–mineral layer, we showed 74% less SOC in the organic layer of the *P. halepensis* forest in amplified drought compared with natural drought conditions, while the SOC stocks of the two oak forests were not affected. Further investigation will

be necessary to explain why amplified drought affected the pine forest and not the oak forests, which appeared better adapted to future environmental conditions. As French forest cover has been increasing by 0.8% per year since 1985, we can speculate that the Mediterranean forests dominated by *Q. ilex* and *Q. pubescens* constitute an important SOC sink for the future.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/f13091485/s1>, Table S1: Soil organic carbon (SOC) and nitrogen (SN) concentrations and stocks according to forest type, soil layer and precipitation condition. Data are mean values \pm SE; $n = 15$. Concentrations are expressed as % per g of DW. Stocks are expressed as Mg per ha. PH = *Pinus halepensis*, QI = *Quercus ilex*, QP = *Quercus pubescens*, O = organic, OM = organo-mineral, ND = natural drought, AD = amplified drought.

Author Contributions: T.G. and V.B. conceived the experiment. S.P., T.G., C.F. and V.B. performed the experiment. S.P., M.S. and T.G. analyzed the data and led the writing of the manuscript with contributions by E.Q., G.S., J.-M.L., J.-M.O., I.M.R., C.F. and V.B. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data presented in this study are archived in SEDOO database from BioDivMex Mistrals program (<https://mistrals.sedoo.fr/BioDivMex/>; accessed on 11 August 2022).

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