

Assessment of the impact of forestry treatments on Soil Carbon balance

ACTION C4

Beneficiary responsible: CTFC

September 2022



LIFE16 CCM/ES/000065

CLIMARK

Forest management promotion for climate change mitigation through the design of a local market of climatic credits

DELIVERABLE

38

TABLE OF CONTENTS

SUMMARY.....	3
1. Aim	5
2. Contribution on soil C stocks of forest debris remaining in the forest after thinning (C4.1).....	5
2.1. Soil C stocks.....	6
Field sampling and laboratory analysis	6
Organic and mineral soil C stocks.....	7
2.2. Leaf and woody debris resulting from forest treatments.....	7
2.3. Short- term green and brown leaf litter decomposition.....	9
2.3. Short-term woody debris decomposition.....	11
2.4. Biochemical quality of woody debris.....	12
2.5. Simulation of organic C derived from leaf and woody debris remaining in the forest floor	14
2.6. References	17
3. Carbon sequestration in agroforestry systems. Do plant-mycorrhizal associations promote carbon storage? (C4.2)	19
3.1. Carbon sequestration in agroforestry systems.....	19
3. 2. Contribution of mycorrhizas to carbon sequestration.....	21
3.3. References	25
Appendix	29
Appendix 1. Leaf and woody debris resulting from forest treatments	29
Appendix 2. Woody debris decomposition experiment	30
Appendix 3. Decomposition k-Olson parameters for different debris types and tree species.....	31

Assessment of the impact of forestry treatments on soil carbon balance

LIFE CLIMARK Project Report

Jordi Garcia-Pausas¹, Carla Fuentes¹, Noemí Palero², Teresa Baiges², Aitor Ameztegui^{1,3}, Pere Casals¹

¹ Joint Research Unit CTFC – AGROTECNIO CERCA.

² Catalan Forest Ownership Centre (CPF). Generalitat de Catalunya.

³ Department of Agriculture and Forest Engineering, University of Lleida.

SUMMARY

The Action C4 aimed to assess the **contribution of forest management practices to soil organic carbon (SOC) balance**. This deliverable reports the main results and discusses the implications for soil carbon (C) balance of specific management practices. Specifically, in C4.1 we analyse the contribution on soil C stocks of forest debris remaining in the forest after thinning, and in C4.2. C sequestration in agroforestry systems.

There is much uncertainty of the forest carbon (C) sink-source dynamics, and particularly how this balance is affected by forest management activities. In this context, the assessment of the effect leaving the forest debris in the forest is key to understand management effects. In the action C4.1 we estimated the soil C stocks and the amount of debris (i.e., leaves, fine and coarse branches, and logs) left in the stand after forest management and simulated their decomposition over time in forest stands of 6 landscape units (LUs) of NE Iberian Peninsula. Soil C stocks were quantified in paired managed and non-managed forest stands in each LUs. It included the forest floor (i.e., soil organic horizons) and mineral topsoil. After this initial C quantification, the dynamics of C stocks will be monitored in the post-LIFE. The amount of forest debris remaining in the stand after thinning was estimated in a total of 12 stands distributed in the 6 LUs. Leaf and woody debris were estimated as the difference of several tree and shrub fractions between pre- and post-management forest inventories. The dasometric information gathered in the inventories was transformed to C stocks in leaves, fine and coarse branches, and trunks by applying available allometric equations. Decomposition rates of woody debris of a conifer (*Pinus nigra* Arnold) and a broadleaved (*Quercus faginea* Lam.) species of different sizes were quantified in a field experimental set-up. A placement of a C-poor soil below the incubated woody debris will allow

in the long term to estimate the amount of debris C incorporated into the soil. As forest thinning and clearing leaves green leaves on the forest floor, differences in decomposition rates between brown and green leaf litter of three species (*P. nigra*, *Q. faginea* and *Fagus sylvatica* L.) were assessed by incubating leaf litter in pots under controlled conditions indoors. For woody debris, we analysed the biochemical characteristics of each fraction per species to improve their decomposition rates in the post-LIFE. Decomposition rates of both woody debris and leaf litter were then applied to the litter and woody debris left in the forest after management in the 12 stands to predict the remaining forest debris C along the time.

Organic C stocks in the organic layers of LUs ranged from ca. 6 Mg C ha⁻¹ to 36 Mg C ha⁻¹, and in the first 25 of mineral soil, between ca. 21 Mg C ha⁻¹ and 63 Mg C ha⁻¹. C left in the stands as litter and forest debris was estimated in about 7 and 16 Mg C ha⁻¹ (in conifer and evergreen forests stands, respectively). Based in our simulations, the C remaining in the forest floor 15 years after management, represents about 17% and 50% of the initial C debris, depending on the fraction and the conifer or evergreen functional group of dominant trees in the stand. Thus, in the 12 stands distributed in the 6 LUs, we estimated that the forest floor increased 15 y after thinning in about 3 Mg C ha⁻¹ and 7 Mg C ha⁻¹ in conifer and evergreen stands respectively.

Agroforestry systems are considered a viable C sequestration option in agricultural soils. Within the Action C4.2 we reviewed from the literature the contribution of agroforestry systems to C sequestration of agricultural soils and the role that mycorrhizas play in this process. In agroforestry systems trees increase the C inputs to the soil and change its placement to deeper soil. Furthermore, the low biochemical quality of the debris increases the residence time of organic matter inputs, allowing an increase of C stocks. C accumulation rates is variable and depends on the species involved, tree density, climate, soil characteristics, and management. According to the literature, in a Mediterranean context, the additional (compared with agricultural systems without trees) soil C sequestration could be on average around 0.25 Mg C ha⁻¹ y⁻¹ in the top 30 cm (0.35 Mg C ha⁻¹ y⁻¹ at 0.8-1.0 m depth), while aboveground C accumulation rate could be between 0.8 and 1.8 Mg C ha⁻¹ y⁻¹. Mycorrhizas increase the C inputs into the soil by promoting the tree growth and through the high turnover of the extra-radical hyphae. They also reduce the C outputs by slowing the decomposition and increasing the soil aggregation. Trees associated to ectomycorrhizal fungi may be more appropriate than those with arbuscular mycorrhizal fungi when the aim is to maximise the C storage, as ectomycorrhizas reduce the decomposability of the roots.

1. Aim

To promote multifunctional forest management through the design of a local market of climate credits, LIFE CLIMARK implemented different demonstrative forestry treatments on representative forest typologies across representative forest Landscape Units (LUs) in the north-eastern Iberian Peninsula (Catalonia, Spain). The Action C4 aimed to assess the **contribution of forest management practices on soil organic carbon (SOC) balance**.

This deliverable reports the main results and discusses the implications for soil carbon (C) balance of specific management practices. Specifically, in C4.1 we analyse the contribution on soil C stocks of forest debris remaining in the forest after thinning, and in C4.2. explore C sequestration in agroforestry systems and the role that mycorrhizas play.

2. Contribution on soil C stocks of forest debris remaining in the forest after thinning (C4.1)

Forest management affects the soil C sink-source dynamics. Tree thinning and understory clearing produce leaf litter and fine and coarse woody debris. This debris plays an important role in forest ecosystem services, especially in terms of C sequestration and soil functioning. Leaving coarse woody debris (CWD) also has other benefits such as providing habitat and food for many organisms. Therefore, one of the key aspects to assess the effect of forest management practices on soil C storage is the contribution of forest debris to the SOC. Leaving litter and woody debris in the forest floor is one of the practices that contributes to the system's C sequestration (e.g., Nave et al, 2010; Wiebe et al., 2014). Leaf litter decomposition and the factors that regulate its decay rates in temperate and boreal forests have largely been studied. However, there is a clear lack of information regarding the decay rate of forest woody debris and the C flux from decomposing debris into the soil (Magnússon et al, 2016). CWD is structurally and chemically different from leaf litter and its fate in soils differs from the leaf litter (Cotrufo et al., 2013). The low biochemical quality of woody debris results in slow decomposition rates and long residence times compared to leaf litter. As wood decomposes, an important fraction of its C may be respired and lost as CO₂, while some amount may be incorporated into the soil. The decomposition rates and the C fluxes into the soil depend on the debris size, its biochemical quality (i.e., species), and the environmental conditions of each specific site. Understanding the differences between debris types (leaf, fine and coarse woody debris) is, therefore, important for modelling the effects of forest management on C sequestration. In the case of leaf litter, Rubino et al (2010) estimated that most (67%) of poplar (*Populus nigra*) litter C lost during decomposition had been incorporated

into the soil after 11 months, while only about 30% of lost C had been lost as CO₂, but these figures may be different for woody debris and may vary among species and environmental conditions.

The main objective of this section was to estimate the contribution to soil C stocks of forest debris left in the stand after thinning in 6 LUs. We first quantified SOC in the forest floor and mineral soil and then estimated the amount of debris left in the forest floor due to forest thinning and clearing. To account for the contribution of short-term decomposition forest debris we estimated the rates of decomposition of leaf litter and different fractions of woody debris under controlled conditions (in the laboratory and in a fenced forest stand) and, applying these rates, we estimated the amount of forest debris remaining in the forest floor of each LUs after 15 years. This estimation will be improved in the post-LIF using decomposition models which are currently being developed within the framework of the H2020 HoliSoils project.

2.1. Soil C stocks

Field sampling and laboratory analysis

We estimated soil C stocks in managed and unmanaged (control) plots of each LU (Table 1). Sampling was performed before forest management to define the initial conditions of each plot. Sampling followed the protocols defined in the LIFE CLIMARK deliverable 4 (Garcia-Pausas et al. 2018). Briefly, we sampled 16 points systematically distributed in a 15 x 15 m plot. At each point, we collected the organic horizons (L, F+H layers) by using a round frame of 24.5 cm diameter (area: 471.4 cm²) and sampled the mineral soil using a prismatic soil auger (5 x 5 x 30 cm) within the frame. Mineral soil was divided into 0 – 5 cm, 5 – 15 cm, > 15 cm depths. Total depth depended upon the presence of large stones. In each plot we characterized a soil profile and collected samples at different depths to estimate the OC content. F+H samples were then separated in the lab into two fractions (F: > 4 mm; H: < 4 mm) and weighed. Subsamples of H and mineral layers were air-dried, sieved (2 mm mesh), grounded and analysed for OC by dichromate oxidation (Mebius, 1960). A subset of three L and F organic samples per site were analysed using an elemental analyser (Termo Fischer Scientific). The median of C stocks per plot of organic layers was calculated considering the sampled area, the C concentration, and the weight of the different organic layers (L, F, and H). For mineral soil, median C stocks were calculated using the bulk density and the organic C concentration.

Organic and mineral soil C stocks

Organic C in the organic layers of LUs ranged from 5.8 Mg ha⁻¹ to 35.8 Mg ha⁻¹ (mean \pm standard error: 21.6 \pm 2.9, n = 13), and in the first 25 cm of mineral soil, between 20.5 Mg ha⁻¹ and 62.6 Mg ha⁻¹ (mean \pm standard error 42.8 \pm 4.4, n = 13) (Table 1). The H layer accounted for the 59% of the C content in the three organic layers and the SOC in the first 5 cm of soil mineral represented about 34% of the stocks in the first 25 cm.

Table 1. Organic carbon stocks (median \pm standard error) in the organic horizons and the top mineral soil of the managed and unmanaged (control) stands of each landscape unit (LU) before forest management treatments were implemented.

Landscape Unit	Stand	Tree species	Plot	Organic layer (Mg C ha ⁻¹)	Mineral soil 0-25 cm (Mg C ha ⁻¹)	Total* (Mg C ha ⁻¹)
Aspres	Agullana	Q. ilex, Q. suber	Managed	5.8 \pm 1.3	24.6 \pm 1.5	34.9 \pm 2.1
			Control	6.3 \pm 0.9	24.5 \pm 1.6	31.5 \pm 2.2
Montmell	Aiguaviva	P. halepensis	Managed	14.4 \pm 2.4	26.3 \pm 0.4	49.3 \pm 6.2
			Control	15.4 \pm 2.4	20.5 \pm 0.2	37.3 \pm 2.6
Serres d'Ancosa	Llacuna	P. nigra	Managed	19.5 \pm 3.3	51.6 \pm 3.3	71.6 \pm 3.1
			Control	25.5 \pm 2.5	36.5 \pm 2.6	58.0 \pm 3.8
Vall Rialb	Confòs	P. nigra	Managed	33.5 \pm 3.6	43.2 \pm 2.1	77.9 \pm 4.1
			Control	35.8 \pm 3.3	42.8 \pm 1.8	78.1 \pm 4.1
	Cerdanyès	P. sylvestris	Managed	29.5 \pm 2.2	57.4 \pm 5.5	84.7 \pm 6.2
			Control	n.a.	n.a	n.a
Capçaleres del Llobr.	Vallcebre	P. sylvestris	Managed	22.4 \pm 2.2	23.2 \pm 1.4	44.8 \pm 2.7
			Control	25.2 \pm 2.5	55.7 \pm 2.9	79.2 \pm 3.6
Replans de Berguedà	Cercs	P. sylvestris	Managed	11.9 \pm 3.2	62.6 \pm 4.0	74.5 \pm 3.8
			Control	35.4 \pm 3.5	61.4 \pm 2.7	93.8 \pm 4.5

*Total = Organic layers plus mineral soil 0-25 cm

2.2. Leaf and woody debris resulting from forest treatments

We estimated the amount of forest debris remaining in the stand after thinning in a total of 12 stands distributed in 6 LUs (Table 2). Two stands of Aspres LU were mixed evergreen *Quercus ilex* and *Q. suber* forests, while the rest of the stands were dominated by different species of *Pinus*. Management was performed in the C2 action. The biomass per fractions existing in the stands before and after management (Appendix Table A.1) was estimated using allometric equations described by Montero et al. (2005) on data recorded in forest inventories.

Except in the “Vallcebre feixes” stand, no logs were left in the stand. The amount of leaves and branches left in the forest floor after overstory thinning ranged from 5.6 Mg ha⁻¹ to 48.4 Mg ha⁻¹ in conifer stands and from 23.2 Mg ha⁻¹ to 27.7 Mg ha⁻¹ in evergreen stands (median 16.8 Mg ha⁻¹ and 25.5 Mg ha⁻¹ respectively). Even assuming that the understory was totally cleared, the amount of debris resulting from understory was clearly lower than that of the overstory, and was mostly composed of leaves and small branches. Woody debris are mostly composed by small diameter branches (more than 80%, debris with diameter < 7 cm) in *Pinus* stands, while thicker branches are dominant in evergreen tree debris (more than 70% with diameter > 7 cm) (Figure 1). This suggests that woody debris in evergreen oak stands will persist on the floor for longer time.

Table 2. Total overstory and understory and trunk biomass before management, biomass of logs extracted and debris per typologies left into the stand per each landscape unit. Data in Mg ha⁻¹. Total, trunk and debris fractions biomass were estimated applying allometric equations (Montero et al. 2005) to dasometric information recorded in forest inventories before and after management. Woody understory biomass (Understory) was estimated applying specific allometries to shrub volume estimated by cover and mean height per dominant understory species (De Caceres et al. 2018).

Landscape unit	Stand	Tree species	Stand id.	Total			Trunk biomass			Overstory debris				Underst.
				Mg ha ⁻¹			Mg ha ⁻¹			Mg ha ⁻¹				Mg ha ⁻¹
				Before	Before	Extracted	Before	Before	Extracted	Trunk	Leaves	Branches (per dia. class in cm)		Before
												<2	2-7	>7
Aspres	Agullana	Q.s/Q.i	C2.1.1a	164.6	61.50	17.1	0.0	0.7	1.2	5.0	20.8			9.9
	CanBudo	Q.s/Q.i	C2.1.2a	124.2	52.47	17.7	0.0	0.8	1.5	5.7	15.3			11.7
Montmell	Aiguaviva	P.h.	C2.2.1a	55.3	28.41	6.2	0.0	0.0	3.8	1.3	0.5			2.0
Serra	Can Vich	P.h.	C2.3.2a	135.5	68.56	16.6	0.0	0.0	9.8	3.6	1.9			0.1
Ancosa	La Llacuna	P.h.	C2.3.1a	124.3	63.06	26.7	0.0	0.0	14.0	5.4	3.2			1.9
	La Llacuna	P.n.	C2.3.1a	138.2	91.97	35.3	0.0	0.1	12.2	4.8	1.1			0.6
Vall de	Confos	P.n.	C2.6.1a	209.2	139.16	18.7	0.0	0.1	7.0	2.1	0.2			0.6
Rialb	Sardanyes	P.n./Ps.	C2.6.2a	191.4	150.77	54.4	0.0	5.3	7.0	7.5	0.9			0.8
Repl Berg.	Cercs	P.s.	C2.5.3a	171.4	122.37	56.1	0.0	9.2	12.6	9.9	0.3			3.2
Capçal.	Vallcebre f.	P.s.		214.6	162.97	0.0	87.9	14.2	18.8	15.6	0.1			1.5
Llobregat	Vallcebre1	P.s.	C2.5.1a	93.9	67.53	22.5	0.0	4.7	6.2	4.5	0.1			4.1
	Vallcebre2	P.s.	C2.5.2a	123.5	88.55	21.8	0.0	4.6	6.1	4.3	0.1			0.7

Q.s. *Quercus suber*, Qi. *Q. ilex*, Ph. *Pinus halepensis*, Pn. *P. nigra*, Ps. *P. sylvestris*

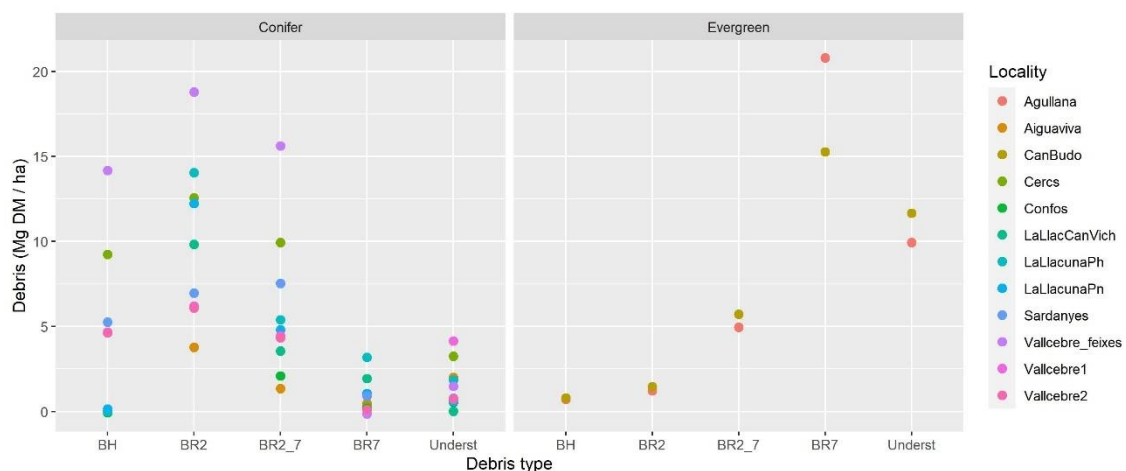


Figure 1. Amount of forest debris left in the stand after management per debris type in conifer and evergreen forests. BH. Leaf biomass, BR. branches biomass of 2, 2-7 and 7 cm of diameter; Underst. woody biomass in the understory. Evergreen stands are in the Aspres LU (n=2), and conifer in the rest of LUs (n= 10).

2.3. Short-term green and brown leaf litter decomposition

Leaf litter decomposition and the factors affecting decomposition rates has been widely studied in several forest ecosystems across the world. Most of the studies use senescing brown litter, as naturally fallen leaves are. However, forest thinning leaves green litter on the forest floor, which have different biochemical quality and nutrient content compared to brown litter (Berg and Ekbohm, 1991; Berg et al, 2003; Sariyildiz and Anderson, 2005). These different structural and nutrient contents between brown and green litter may influence the rates at which litter decomposes and the litter-derived C flux into the soil and the atmosphere.

Here we compared the decomposition rates of brown and green litter of three species (*P. nigra*, *Q. faginea* and *F. sylvatica*) incubated indoors in pots. Leaf litter was placed over vermiculite substrate in plastic pots, which in turn were placed in large trays with an organic layer surrounding the pots (Appendix 2). The organic layer material had been collected from a forest floor dominated by the same species. Incubation started on May 2019. Remaining litter and C in vermiculite substrate were sampled in August 2021. The trays were regularly watered with tap water using little sprinklers. Although the incubation conditions are not comparable with the natural conditions in the forest floor, the use of pots with C-depleted vermiculite could allow to know whether the different litter types influence the C flux to the soil.

Decomposition rates were calculated assuming an exponential model of litter mass loss (Olson, 1963): $X_T = X_0 e^{-kT}$, where X_T and X_0 are the litter mass remaining at the times T and 0 (initial) respectively and k is the decomposition rates (y^{-1}). Table 3 shows the obtained decomposition rates of brown and green litter together with some examples of k values for pine and oak litter found in the literature. Mean decomposition rates of *P. sylvestris*, *P. halepensis* and

Q. ilex leaf litter were used for the simulation of the remaining debris along time (Table 7, Figure 3).

Table 3. Leaf litter decomposition ratios (k from Olson, 1963) for different species and leaf type taken from the literature and estimated indoors in the LIFE CLIMARK action 4.

Reference*	Species	Type	k Olson y^{-1}	Locality	Mean rainfall mm	Mean temperature °C
1	<i>Pinus sylvestris</i>	Brown	0.3006	Jädraås, SE		
		Green	0.4246	Jädraås, SE		
1	<i>Pinus sylvestris</i>	Brown	0.4344	Monte Tabumo, IT		
		Green	0.4808	Monte Tabumo, IT		
2	<i>Pinus halepensis</i>	Brown	0.107	Maials, ES	521.6	15.7
2	<i>Pinus halepensis</i>	Brown	0.177	Desert, ES	476.6	16.2
2	<i>Pinus halepensis</i>	Brown	0.250	Montesquiu, ES	886.3	13.0
2	<i>Quercus ilex</i>	Brown	0.163	Maials, ES	521.6	15.7
2	<i>Quercus ilex</i>	Brown	0.191	Desert, ES	476.6	16.2
3	<i>Pinus radiata</i>	Brown	0.328	Sta Coloma F., ES	750	15.6
		Green	0.362	Sta Coloma F., ES	750	15.6
4	<i>Pinus nigra</i>	Brown	0.217±0.01	Indoors	-	-
		Green	0.370±0.05	Indoors	-	-
4	<i>Quercus faginea</i>	Brown	0.365±0.05	Indoors	-	-
		Green	0.432±0.03	Indoors	-	-
4	<i>Fagus sylvatica</i>	Brown	0.181±0.01	Indoors	-	-
		Green	0.370±0.04	Indoors	-	-

* Literature references: 1. Berg et al. 2003; 2. Garcia-Pausas et. al. 2004; 3. Cortina & Vallejo, 1994, k values estimated from Fig. 2; 4. LIFE-Climark

Climate and litter quality are the main drivers of litter decomposition rates. Green leaves decomposed faster than brown litter, which is consistent with some previous studies (Berg et al, 2003), and indicates that higher biochemical quality of green litter promotes its decomposition. However, Cortina and Vallejo did not find significant differences in the case of *P. radiata* needles. Possibly, short term decomposition rates are higher in green than in brown needles due to their higher biochemical quality. But this effect may disappear at later stages of decomposition, when most of the labile components has already been decomposed, as Berg et al (1982) showed in decomposing *P. sylvestris* needles.

2.3. Short-term woody debris decomposition

Decomposition of woody debris was assessed by field incubation in three fenced plots in a *P. nigra* forest stand located in Montpol (Solsonès, 42.082°N, 1.427°E, 720 m a.s.l.), at the southern face of eastern Pre-Pyrenees (Appendix 2). The mean annual temperature and precipitation of the site is 12.1°C and 643 mm (Meteorological station of Lladurs, 1999-2021, at about 600-700 m from the plots, 785 m a.s.l.). Three plots of about 50 m² were fenced to avoid eventual disturbances by people or animals. Woody debris of different species and diameters were placed in each plot, over a 5-cm-depth layer of C-poor soil. In addition to recent cut logs, 4-year-old logs and partially burnt coarse woody debris were included. Woody debris incubation started on the 22nd August 2019. The remaining debris and soil under them were sampled in 12th April 2022. We incubated CWD of two diameters (logs and branches) of two species: *P. nigra* ssp *salzmannii* and *Q. faginea*. Logs were between 45 and 80 cm long and 10-15 cm thick and were placed on the forest floor. Branches had similar lengths but were about 2-3 cm thick and were placed on the forest floor in groups of 5 branches. We also incubated fine woody debris (FWD, branches 0.3-0.6 cm thick) of *P. nigra* and *Q. faginea* in litterbags made of fiberglass mesh (2 mm mesh).

Table 4. Species and debris types included in the decomposition activity. Age states for the number of decomposing years before including them in this activity

Species	Diameter (cm)	Burnt	Age (y)	Replicates	Initial date
<i>Pinus nigra</i>	10 – 15	No	0	9	22/08/2019
			4	9	04/10/2019
		Yes	0	5	04/10/2019
			4	9	04/10/2019
	2 – 3	No	0	9	22/08/2019
	0.3-0.6	No	0	9	04/09/2019
<i>Quercus faginea</i>	10 – 15	No	0	9	22/08/2019
		Yes	0	8	04/10/2019
	2 – 3	No	0	9	22/08/2019
	~0.6	No	0	9	04/09/2019
None	-	-	-	5	-

Our results show that FWD (0.3-0.6 cm diameter) decomposed faster than large logs and branches, but the *k* values of these latter two types did not differ significantly. In general, oak woody debris decomposed slightly faster (*p*= 0.050), but looking specifically for each size, we do

not see significant differences between species in the decomposition rates of logs and branches, but only the oak FWD decomposed faster than pine FWD. Table 5 shows the obtained k values, together with some decomposition rates found in the literature. For CWD, Mattson and collaborators (1987) gave an average rate of 0.083 y^{-1} for different species in southern North Carolina (USA); while the rates given by Herrmann and colleagues (2015) for southern Germany are quite lower (Table 5). These latter authors estimate different rates depending on the species and do not find that climate is a factor that contribute to explain the variability within the species.

Obtained decomposition rates (k values) for the woody debris of *P. nigra* and *Q. faginea* (Table 5) were then used for the simulation of remaining woody debris along time in the different landscape units (Table 7, Figure 3)

Table 5. CWD decomposition rates (k from Olson, 1963) for different species and wood diameters taken from the literature

Reference*	Species	Diameter	k Olson	Locality	Mean rainfall	Mean temperature
		cm	y^{-1}		mm	$^{\circ}\text{C}$
1	Several species	--	0.083	N. Carolina, US	1820	12.6
2	<i>Fagus silvatica</i>	<20	0.078	S. Germany	657-1700	8.5-11.0
		20-40	0.055			
2	<i>Pinus sylvatica</i>	<20	0.050	S. Germany	657-1700	8.5-11.0
		20-40	0.030			
2	<i>Picea abies</i>	<20	0.034	S. Germany	657-1700	8.5-11.0
		20-40	0.036			
3	<i>Pinus nigra</i>	~0.6 cm	0.082	This activity.	643	12.1
		2-3 cm	0.041	Solsonès		
		10-15 cm	0.068			
3	<i>Quercus faginea</i>	~0.6 cm	0.119	This activity.	643	12.1
		2-3 cm	0.091	Solsonès		
		10-15 cm	0.054			

* Literature references: 1. Mattson et al. 1987; 2. Herrmann et. al. 2015; 3 Action4, Life Climark

2.4. Biochemical quality of woody debris

Decay rates of CWD are mainly controlled by the environmental parameters, substrate quality, and decomposer community (Russell et al, 2015). Woody debris is a low-quality litter compared with leaves, resulting in low decomposition rates. Decomposition models allow estimating the long-term remaining mass and therefore the persistence of CWD carbon in the forest system. In most models, the effect of species on wood decomposition is represented by

their initial quality, based on the chemical composition and recalcitrance. For instance, the Century model (Parton, 1996) predicts the decomposability based on the lignin content of the substrate. Here we analysed the initial chemical characteristics of the incubated CWD to model in the post-LIFE the long-term decomposition of CWD of different sizes and species (*P. nigra* and *Q. faginea*).

From each wood fraction we performed a chemical characterisation of the incubated debris (logs and branches) that will allow to track the biochemical changes along the decomposition process. A sample of each fractions was analysed for organic C and N content by the dichromate oxidation method, following the Mebius (1960) method. Then, a biochemical fractionation was carried out by performing sequential extractions with water and sulphuric acid, obtaining 4 fractions: hydrosoluble, hydrolysate I (*hemicelluloses*), hydrolysate II (*celluloses*), and lignin (Klason). The carbohydrate and phenol content of the hydrosoluble fraction and the hydrolysates I and II was then determined.

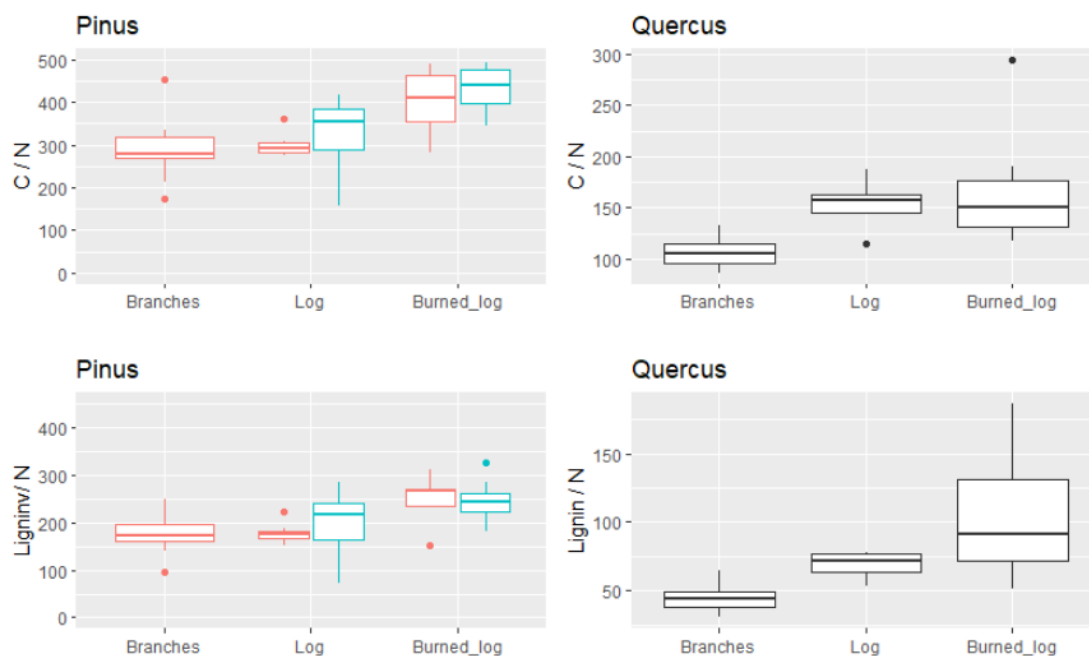


Figure 2. Carbon to N and Lignin to N ratios of branches and logs in *Pinus* and *Quercus*. In *Pinus*, recently cutted (red), and 4-y old logs (blue)

P. nigra woody debris had higher C/N than *Q. faginea* debris (Figure 2), due to their lower N content. Also, lignin content of logs and branches was higher in *P. nigra* than in *Q. faginea*, as well as the lignin-to-N ratio (Figure 2). While carbohydrate content in logs and branches did not differ significantly between both species, phenol content was much higher in the logs and branches of *Q. faginea* than in *P. nigra* (Table 6). Charred *P. nigra* logs had lower N

content than non-charred logs, resulting in higher C/N ratios. In contrast, charred *Q. faginea* logs did not differ from the uncharred ones. Charring did not have any significant effect on lignin content (Table 6).

Table 6. Initial content of carbohydrates, phenols and lignin in the incubated oak and pine logs and branches

Species	Diameter	Charred	Total carbohydrates	Total phenols	Klason lignin
	cm		ppm	ppm	%
<i>Q. faginea</i>	2-3	No	2661.6±102.4	184.8±9.3	18.4±1.0
	10-15	No	2555.3±194.2	218.9±10.9	19.6±0.5
	10-15	Yes	2647.0±53.1	153.1±22.0	22.2±1.6
<i>P. nigra</i>	2-3	No	2457.8±92.1	84.7±13.9	28.2±0.6
	10-15	No	2270.9±51.8	70.2±9.5	27.7±0.6
	10-15	Yes	2354.2±104.7	46.1±2.0	28.4±1.8

2.5. Simulation of organic C derived from leaf and woody debris remaining in the forest floor

We estimated the remaining C content in leaves, FWD and CWD short-term after forest thinning by applying over time the different decomposition rates (k , Olson, 1963) obtained in this action C4 (section 2.2 and 2.3), and from the literature (Table 7, Figure 3). The resulting decomposition curves can be visualized in the Appendix 3, figure 1.

Table 7. Decomposition rates (Olson k , y^{-1} and standard error in brackets) used to estimate leaf and woody debris remaining in the forest floor with time since treatments. See sections 2.2 and 2.3 for details.

	Species ¹	Leaves	Species ¹	Woody debris ²		
				FWD	CWD, 2-7 cm	CWD >7 cm
Conifer	Ps	0.3675				0.050
	Ph	0.142	Pn	0.082 (0.009)	0.041 (0.022)	0.068 (0.010)
Evergreen	Qi	0.177	Qp	0.119 (0.003)	0.091 (0.013)	0.054 (0.020)

¹ Ps. *Pinus sylvestris*, Pn. *P. nigra*, P.h. *P. halepensis*, Qi. *Quercus ilex*, Qp. *Q. pubescens*

² FWD, fine woody debris diameter < 2 cm; CWD, 2-7 cm and >7cm, coarse debris with diameter between 2 and 7 cm, and > 7cm, respectively

Figure 3 showed the C remaining over time depending on the debris type and size and for conifer or evergreen *Quercus* stands in the LUs. A large part of the leaf C is lost short-term after thinning, while woody debris remains in the forest floor in the long-term (Figure 3).

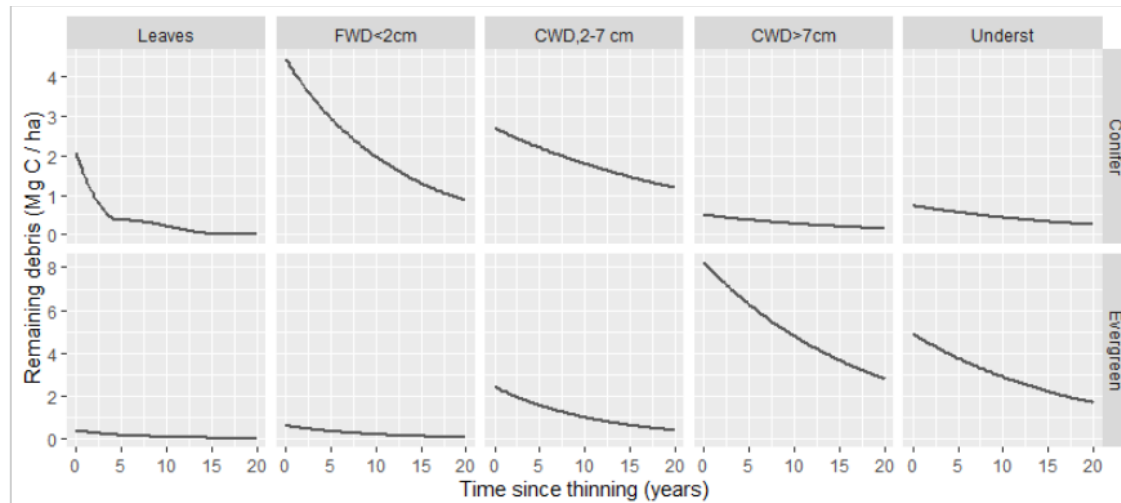


Figure 3. Simulated C remaining after thinning in the forest floor with time for leaves and woody debris typology for conifer or evergreen LU stands. Remaining debris were simulated applying K-Olson parameters indicated in table 7, for woody debris with diameter lower than 2 cm (FWD <2cm), between 2-7 cm (CWD, 2-7 cm) and higher than 7 cm (CWD >7 cm). Note the different scales of remaining C debris between Conifer and Evergreen plots.

Our simulations suggested that ca. 40% of the woody debris left in the forest floor will remain after 15 years (3.0 Mg C ha⁻¹ and 6.6 Mg C ha⁻¹, including overstory and understory, in conifer and evergreen stands respectively: Table 8).

Part of the C lost was emitted into the atmosphere in the form of CO₂, while another part has been incorporated into the soil in particulate form or dissolved in rainwater. Mattson and colleagues estimate that approximately 2/3 of the lost C has been emitted into the atmosphere. Our C remaining estimations did not take into account the specific quality of debris, such as the ratios C/N or Lignin/N (see section 2.4), and the climate of LU stands. In the post-LIFE we will account for these variables in the simulation by using specific models such as Century (Parton, 1996) and Yasso (Liski et al, 2005).

Table 8. Estimated debris (Mg C ha⁻¹) in the forest floor of conifer and evergreen stands just after thinning and after 15 y. See table 2 for details of after thinning estimates. Remaining were estimated applying the decomposition k-Olson parameter for each debris indicated in Table 7.

Forest type	Debris type	After thinning ¹		15y remaining ¹		Remaining
		Mg C ha ⁻¹		Mg C ha ⁻¹		%
Conifer (n = 10 stands)						
	Leaves	1.3	±2.3	0.1	±0.0	1.1
	FWD (<2cm)	3.8	±2.1	1.1	±0.6	29.2
	CWD (2-7 cm)	2.1	±1.9	1.1	±1.0	54.1
	CWD (>7cm)	0.3	±0.4	0.1	±0.1	47.2
	Total Overstory	7.0	±3.9	2.7	±1.6	39.1
	Total Understory	0.5	±0.5	0.2	±0.3	44.5
Evergreen (n = 2 stands)						
	Leaves	0.4	±0.0	0.1	±0.0	7.0
	FWD (<2cm)	0.6	±0.1	0.1	±0.0	16.8
	CWD (2-7 cm)	2.4	±0.2	0.6	±0.1	25.5
	CWD (>7cm)	8.2	±1.8	3.7	±0.8	44.5
	Total Overstory	11.3	±1.5	4.4	±0.7	38.9
	Total Understory	4.9	±0.6	2.2	±0.3	44.5

¹ median ± standard error (n=2 stands for Evergreen, n=10 for Conifer)

2.6. References

- Berg B, Ekbohm G. 1991. Litter mass-loss rates and decomposition patterns in some needle and leaf litter types. Long term decomposition in a Scots pine forest. VII. Canadian Journal of Botany 69: 1449-1456
- Berg, B., Virzo De Santo, A., Rutigliano, F.A., Fierro, A., Ekbohm, G., 2003. Limit values for plant litter decomposing in two contrasting soils Influence of litter elemental composition. Acta Oecologica 24, 295–302.
- Berg B, Wessén B, Ekbohm G. 1982. Nitrogen level and decomposition in Scots pine needle litter. Oikos 38: 291-296.
- Cortina J, Vallejo VR. 1994. Effects of clearfelling on forest floor accumulation and litter decomposition in a radiata pine plantation. Forest Ecology and Management 70: 299-310
- Cotrufo, M.F., Wallenstein, M.D., Boot, C.M., Denef, K., Paul, E., 2013. The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? Glob. Change Biol. 19 (4), 988–995.
- De Cáceres M, Casals P, Gabriel E, Castro X, 2019. Scaling-up individual-level allometric equations to predict stand-level fuel loading in Mediterranean shrublands. Ann. For. Sci. 76, <https://doi.org/10.1007/s13595-019-0873-4>.
- Garcia-Pausas, J., Casals, P., Romanyà, J., 2004. Litter decomposition and faunal activity in Mediterranean forest soils: Effects of N content and the moss layer. Soil Biol. Biochem. 36, 989–997. <https://doi.org/10.1016/j.soilbio.2004.02.016>
- Garcia-Pausas J, Baiges T, Casals P. 2018. Field indicators and monitoring protocols to assess forest ecosystem services (FES): climate regulation, water cycle regulation / water provision, and biodiversity. LIFE CLIMARK, deliverable 4, pp. 28.
- Herrmann, S., Kahl, T., Bauhus, J., 2015. Decomposition dynamics of coarse woody debris of three important central European tree species. For. Ecosyst. 2. <https://doi.org/10.1186/s40663-015-0052-5>
- Liski J, Palosuo T, Peltoniemi M, Sievänen R. 2005. Carbon and decomposition model Yasso for forest soils. Ecological Modelling 189: 168-182
- Magnússon RI, Tietema A, Cornelissen JHC, Hefting MM, Kalbitz K. 2016. Tamn Review: Sequestration of carbon from coarse woody debris. Forest Ecology & Management 377: 1-15
- Mattson, K.G., Swank, W.T., Waide, J.B., 1987. Decomposition of woody debris in a regenerating, clear-cut forest in the Southern Appalachians. Can. J. For. Res. 17, 712–721. <https://doi.org/10.1139/x87-114>
- Mebius LJ. 1960. A rapid method for the determination of organic carbon in soil. Analytica Chimica Acta 22: 120-124
- Montero G., Ruiz-Peinado R., Muñoz M., 2005. Producción de biomasa y fijación de CO₂ por los bosques españoles. Monografías INIA, Serie Forestal 13. Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Ministerio de Educación y Ciencia, Madrid, España, 270 pp. [In Spanish]
- Nave LE, Vance ED, Swanston CW, Curtis PS. 2010. Harvest impacts on soil carbon storage in temperate forests. Forest Ecology & Management 259: 857-866
- Olson, J.S., 1963. Energy storage and the balance of producers and decomposers in ecological systems. Ecology 44, 322–331
- Parton WJ. 1996. The CENTURY model. In: Powlson, D.S., Smith, P., Smith, J.U. (eds) Evaluation of Soil Organic Matter Models. NATO ASI Series, vol 38. Springer, Berlin, Heidelberg.
- Rubino M, Dungait JAJ, Evershed RP, Bertolini T, De Angelis P, D’Onofrio A, Lagomarsino A, Lubritto C, Merola A, Terrasi F, Cotrufo MF. 2010. Carbon input belowground is the major C flux contributing to leaf litter mass loss: Evidences from a ¹³C labelled-leaf litter experiment. Soil Biology and Biochemistry 42: 1099-1016

- Russell MB, Fraver S, Aakala T, Gove JH, Woodall CW, D'Amato AW, Ducey MJ. 2015. Quantifying carbon stores and decomposition in dead wood: A review. *Forest Ecology and Management* 350: 107-128
- Sariyildiz T, Anderson JM. 2005. Variation in the chemical composition of green leaves and leaf litters from three deciduous tree species growing on different soil types. *Forest Ecology and Management* 210: 203-319
- Shorohova S, Kapitsa E, Vanha-Majamaa I. 2008. Decomposition of stumps in a chronosequence after a clear-felling vs clear-felling with prescribed burning in a southern boreal forest in Finland. *Forest Ecology and Management* 255: 3606-3612
- Wiebe SA, Morris DM, Luckai NJ, Reid DE. 2014. The influence of coarse woody debris on soil carbon and nutrient pools 15 years after clearcut harvesting in black spruce-dominated stands in northwestern Ontario, Canada. *Ecoscience* 21: 11-20

3. Carbon sequestration in agroforestry systems. Do plant-mycorrhizal associations promote carbon storage? (C4.2)

3.1. Carbon sequestration in agroforestry systems

The large extension of agricultural lands makes their carbon (C) dynamics highly relevant for accounting for the regional C stocks and balances. Croplands generally act as a major source of greenhouse gases (Freibauer et al, 2004), having lost an important fraction of their original soil organic matter. As a result, their soil C content is generally low compared to natural or semi-natural ecosystems (e.g. Álvaro-Fuentes et al, 2011). However, because of their C depletion, agricultural soils could have now the potential to act as C sinks if adequate management practices were implemented (Singh et al, 2018). Among the proposed practices that could contribute to C sequestration, the implementation of agroforestry systems appears as a useful practice (Aertsens et al, 2013) that entails several environmental benefits.

Agroforestry refers to the practice of growing woody perennials (i.e. trees or shrubs) and annual crops on the same land unit (Nair, 2011). This is an old practice, but only in the last decades has been acknowledged as a viable C sequestration option in agricultural soils. Besides C sequestration, agroforestry also provides several benefits in comparison to conventional monospecific crops, such as improved soil fertility, improved water retention and quality, control of soil erosion, and enhanced biodiversity (Jose, 2009).

The increase of C storage in agroforestry systems is achieved by the enhanced organic matter inputs at both above- and below-ground, by strengthening soil organic matter physical stabilisation, and by reducing the rate of biomass decomposition. Trees have extensive root systems that are critical sources of soil organic C. This, together with the aboveground litter fall allows the accumulation of more C under the tree rows compared to the inter-row soils (Bambrick et al, 2010; Lorenz and Lal, 2014). The effect of trees is clear, as the observed effects are gradually reduced with distance from the tree row (Pardon et al, 2017). But trees not only cause an increase of the C inputs into the soil, but also a shift in their placement. Compared to annual crops, trees tend to accumulate organic matter in deeper horizons, which may have higher organic matter stabilisation potential than topsoil. For instance, Howlett et al (2011) showed that silvopastoral systems with *Pinus radiata* stored more C in macroaggregates in the subsoil than in adjacent treeless pastures. This effect on deep soil can be improved by choosing deep-rooting tree species. Also, Cardinael et al (2015) showed in Mediterranean alley cropping agroforestry stands, that competition with annual crops can induce the trees to deeper rooting in comparison to tree monocultures, contributing to the deep soil C storage.

Organic matter derived from the planted woody plants also differs from that of the annual plants in their biochemical quality. The chemical composition of tree litter is generally considered more resistant to decay due to its higher content of lignin, tannins, and other secondary products. This may cause a shift in the quality of particulate organic matter when woody vegetation is introduced in croplands, as occurs in other grassland-to-woodland transitions (Filley et al, 2008). This may contribute to increasing the residence time of organic matter inputs into the soil, allowing higher C accumulation, at least in the particulate organic matter pool. And the organic matter quality may be behind the large relative contribution of the particulate coarse organic fraction to the C accumulation reported by Cardinanel et al. (2015b) in agroforestry systems of France.

But, for long-term soil organic matter preservation, eventual changes in the physicochemical stabilisation would be essential. Organic matter occlusion into aggregates is known that confers long-term physical protection against microbial degradation (Balesdent et al, 1998; Six et al, 2002). And the organic matter inputs generally promote soil aggregation. In tropical agroforestry systems, Gama-Rodrigues et al (2010) considered that the organic matter occlusion was a major mechanism for C preservation. In NW Iberian Peninsula, Howlett et al (2011) showed that silvopastoral systems with *Pinus radiata* promoted the C storage in macroaggregates in the subsoil, which were considered a consequence of increased C inputs from afforestation together with the cessation of tillage. It is known that plowing disrupts soil aggregates, exposing large amounts of protected organic matter to microbial degradation and, therefore causing the loss of soil C (Singh et al, 2020). So, reduced tillage in agroforestry systems also promotes the physical protection of the organic matter and therefore its long-term preservation.

Although the magnitude may largely depend on the agroforestry system, tree density, species involved, climate conditions, soil type and characteristics, and management, estimations of C sequestration potential of agroforestry systems are always positive in comparison to the traditional monocultures. In a global meta-analysis Feliciano et al (2018) reported that silvopastoral systems were the agroforestry systems that on average have higher soil C sequestration rates ($4.38 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$), while the highest aboveground C sequestration occurred in improved fallows ($11.29 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$). But in general, agroforestry systems in tropical and subtropical areas show higher C accumulation rates than under temperate and arid or semiarid climates (Lorenz and Lal, 2014; Feliciano et al, 2018; Singh et al, 2018), and the C accumulation rates increase with the age of the stand (Cardinanel et al, 2017).

Reported rates of soil C accumulation in agroforestry systems of temperate areas range between 0.1 and 6.4 Mg C ha⁻¹ yr⁻¹ (Singh et al, 2018). For instance, Cardinael et al (2017) estimated mean C accumulation rates in the soil (0 – 30 cm) of 0.24 Mg C ha⁻¹ yr⁻¹ (range: 0.09 – 0.46 Mg C ha⁻¹ yr⁻¹) in six agroforestry systems in France, and Cardinael et al (2015) estimated rate of 0.25 Mg C ha⁻¹ yr⁻¹ in the top 30 cm soil (0.35 Mg C ha⁻¹ yr⁻¹ in 0-100 cm) in Mediterranean alley cropping agroforestry systems. Similar values were reported by Oelbermann et al (2006) in a poplar alley cropping system in southern Canada, with a soil C accumulation rate of 0.30 Mg C ha⁻¹ yr⁻¹. In North American agroforestry systems, Udawatta and Jose (2012) estimated that the potential C sequestration rates (biomass plus soil) were 6.1 Mg C ha⁻¹ yr⁻¹ for silvopastures, 3.4 Mg C ha⁻¹ yr⁻¹ for alley cropping systems and 2.6 MgC ha⁻¹ yr⁻¹ for riparian buffers. And Wotherspoon et al (2014) estimated in southern Ontario (Canada) net C sequestration potentials (soil plus biomass) between 0.84 and 2.12 Mg C ha⁻¹ yr⁻¹ in tree-based intercropping systems with different tree species and soybean. These values contrasted with the estimated net rates of -1.15 Mg C ha⁻¹ yr⁻¹ for soybean monocrops. Also, in a meta-analysis of soil C sequestration (0 – 60 cm depth) of Chinese agroforestry systems, Hübner et al (2021) estimated rates of 2.16, 1.61, and 0.33 Mg C ha⁻¹ yr⁻¹ for shelterbelts, agrosilvicultural and silvicultural systems respectively.

Biomass C accumulation rates are also very variable, depending on the tree density, age, management, species involved, and climate and soil conditions. Based on literature data, Kay et al (2019) indicated that biomass C accumulation rates in European agroforestry systems ranged between 0.09 and 7.29 Mg C ha⁻¹ yr⁻¹, with the lowest values related to systems with low tree density, and the highest corresponding to high tree density systems with fast-growing species, growing in good soil conditions. For instance, in agroforestry systems of France, Cardinael et al (2017) showed an average C accumulation rate in biomass of 0.65 Mg C ha⁻¹ yr⁻¹ (range: range 0.004 – 1.85 Mg C ha⁻¹ yr⁻¹), and Wotherspoon et al (2014) estimated accumulation rates in Canadian intercropping systems that ranged between 0.52 and 1.06 Mg C ha⁻¹ yr⁻¹, depending on the tree species.

3. 2. Contribution of mycorrhizas to carbon sequestration

To test the effect of the symbiotic association between plants and fungi (mycorrhizas) on C sequestration under realistic field conditions is a difficult task. In practice, to test the net effect of mycorrhizas, non-mycorrhizal controls are required. These controls are difficult to obtain without severe perturbations of soil microflora and fauna; therefore, it is very difficult to evaluate the effect of mycorrhizas on plant production and fitness under field conditions (Jones and Smith, 2004) and, consequently, on the eventual C sequestration improvement.

However, it is known that mycorrhizal associations can contribute to soil C storage in different ways (Figure 1). Mycorrhizas are known to cause an increase in plant C translocation to roots (Reid et al, 1983). From the roots, plants transfer photosynthates to the intra-radical hyphae and subsequently to the extra-radical hyphae, which are finally released into the soil matrix. The amount of C transferred from the host plant to the fungi can be considerable. In controlled conditions, it was estimated that more than 20% of the net primary production can be allocated to ectomycorrhizas (Hobbie, 2006). At the landscape scale, although there is much uncertainty in the estimations, ectomycorrhizal mycelia production in forests can be several hundred kilograms per hectare and year (Ekblad et al, 2013). The net production in agroforestry systems will depend, among other factors, on the tree density. But as the turnover rate of extra-radical hyphae can be of few days (Staddon et al, 2003), mycorrhizas could be an important pathway of C inputs into the soil. For instance, in poplar plantations of central Italy, it has been estimated that the mycorrhizal mycelium was the predominant pathway (62%) by which organic C entered to the soil organic matter pool, exceeding the leaf litter and root C inputs (Godbold et al, 2006). But, as mentioned above, the lack of non-mycorrhizal controls does not allow us to test the net effect of mycorrhizas on the C inputs into the soil.

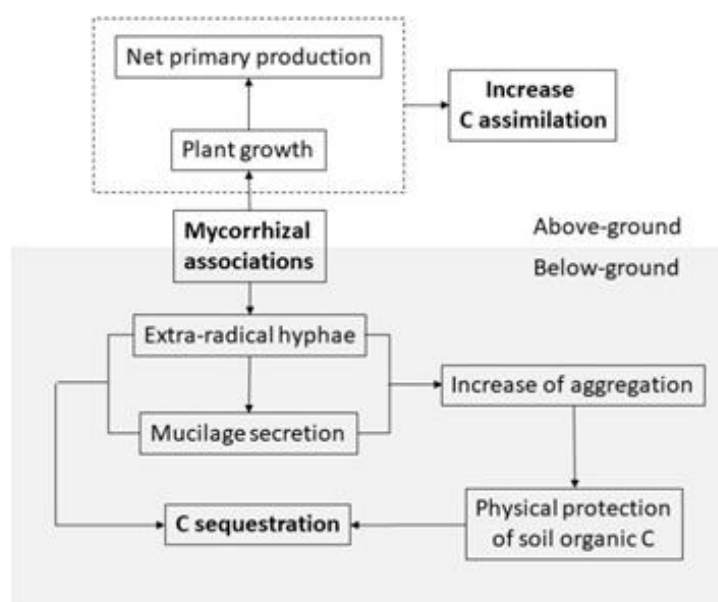


Figure 1. Main mechanisms by which mycorrhizal association can promote carbon accumulation above- and below-ground. Modified from Zhu and Miller (2003).

Mycorrhizal plants translocate C to the roots and the fungal hyphae. To compensate for this C loss, and thanks to the improved nutrition provided by the mycorrhizal fungi, plants increase their photosynthetic rate (Doskey et al, 1990; Rousseau and Reid, 1990) and fitness. This higher

productivity not only increases the C stocks in the biomass but also the C inputs into the soil through the leaf and root litter production. However, this positive effect on plant productivity, which usually is taken for granted, is far from being universal, with cases of positive, null, or even negative effects of mycorrhizas on plant growth (Corrêa et al, 2012). For instance, it has been shown in boreal forests that, under some conditions, nitrogen retention by mycorrhizal fungi can aggravate the N limitation for tree growth (Näsholm et al, 2013).

But, what about the effect of mycorrhizas on the C outputs? Some observations indicate that litter decomposition is reduced when mycorrhizal roots are present (Gadgil and Gadgil, 1975), the so-called Gadgil effect. Although there is little empirical evidence due to methodological limitations (Frey, 2019), it has been suggested that efficient nutrient or water uptake by ectomycorrhizal fungi may reduce the availability of nutrients for other microorganisms or increase water limitation for decomposition (Ekbad et al, 2013). On the other hand, ectomycorrhizal hyphae could increase the microbial decomposition of recalcitrant organic compounds by priming the co-metabolism of these substrates by saprophytic microorganisms through the release of labile substrates to the hyphosphere and mycorrhizosphere (Gorka et al, 2019) and the interaction with other microorganisms that are efficient in priming of soil organic matter mineralisation (Paterson et al, 2016).

Also, it has been suggested the mycorrhizas can alter the decomposability of roots through changes in their biochemical quality (Langley and Hungate, 2003). Mycorrhizal tissues have a higher concentration of N than roots, which apparently could hasten their decomposition. However, this N is bound to compounds like chitin, which is recalcitrant to decomposition, making N-rich mycorrhizal roots less decomposable than non-colonised roots with lower N concentration (Langley and Hungate, 2003). Indeed, in a study with *Pinus edulis*, Langley et al (2006) showed that non-colonised roots decomposed three times faster than those with ectomycorrhizal fungi, suggesting that they could contribute to the C accumulation in soils. However, plants associated with arbuscular mycorrhizal fungi tend to produce leaf (Cornelissen et al, 2001) and root (Langley et al, 2006) litter of higher quality and decomposability in comparison to ectomycorrhizal species. So, if the aim is to maximise the soil C accumulation in an agroforestry system, choosing ectomycorrhizal plant species may be more appropriate than those associated with arbuscular mycorrhizal fungi.

Mycorrhizas can promote soil aggregation. This confers physical protection to organic matter against microbial degradation and also reduces the risk of C loss by erosion. The mechanisms by which aggregation is promoted by mycorrhizas include biophysical mechanisms (entanglement and physical pressures on soil particles by hyphae), biochemical mechanisms

(mucilage secretion that acts as a soil binding agent), and biological mechanisms (indirect effect by modifying soil microbial communities in the rhizosphere) (Lehman et al, 2017). In the case of arbuscular mycorrhizal fungi, although the aggregation capacity may vary between fungal species (Piotrowski et al, 2004), Wilson et al (2009) showed a close correlation between hyphal abundance and soil aggregation. And Leifheit et al (2014) showed in a meta-analysis a global positive effect of arbuscular mycorrhizas on soil aggregation. Less studied is the influence of ectomycorrhizas on soil aggregation and structure. However, some studies indicate an increase in aggregate stability when trees are inoculated with ectomycorrhizas (Caravaca et al, 2002; Graf and Frei, 2013). And Zheng et al (2014) showed that several ectomycorrhizal fungal species associated with *Pinus sylvestris* seedlings increased both water-stable aggregates by 6-12% and/or the mean diameter of the aggregates, depending on the fungal species.

Root architecture is also greatly modified by ectomycorrhizal fungi, which can alter root decomposability (Langley and Hungate, 2003). Roots colonised by ectomycorrhizal fungi lack fine root hairs and have a short, rounded shape. This, together with the protection with the hyphal sheath substantially reduces the surface area exposed to herbivores and pathogens, and also could even protect dead roots against decomposition. By contrast, architectural changes caused by arbuscular mycorrhizal fungi are subtle and do not seem that this could have a significant influence on root decomposability (Langley and Hungate, 2003).

In summary, although the comparison between inoculated and non-inoculated plants under field conditions is difficult, the known effects of mycorrhizas on plants and soil indicate that they alter the C cycling, generally promoting the accumulation of C in soil and biomass. Mycorrhizas increase the C inputs by promoting tree growth and through the turnover of extra-radical hyphae. Also reduce the C output by slowing down the root decomposition and the promotion of the soil aggregation, but this could partly be offset by the promotion of priming of soil organic matter mineralisation. Apparently, trees associated to ectomycorrhizal fungi are more appropriate when the aim is to maximise the C storage, as reduce the decomposability of roots.

3.3. References

- Aertsens J, De Nocker L, Gobin A. 2013. Valuing the carbon sequestration potential for European agriculture. *Land Use Policy* 31: 584-594
- Álvaro-Fuentes J, Easter M, Cantero-Martínez C, Paustian K. 2011. Modelling soil organic carbon stocks and their changes in the northeast Spain. *European Journal of Soil Science* 62: 685-695
- Balesdent J, Barsnard E, Arrouays D, Chenu C. 1998. The dynamics of carbon in particle-size fractions of soil in a forest cultivation sequence. *Plant and Soil* 201: 49-57
- Bambrick AD, Whalen JK, Bradley RL, Cogliastro A, Gordon AM, Olivier A, Thevathasan NV. 2010. Spatial heterogeneity of soil organic carbon in tree-based intercropping systems in Quebec and Ontario, Canada. *Agroforestry Systems* 79: 343-353
- Caravaca F, Garcia C, Hernández MT, Roldán A. 2002. Aggregate stability changes after organic amendment and mycorrhizal inoculation in the afforestation of a semiarid site with *Pinus halepensis*. *Applied Soil Ecology* 19: 199-208
- Cardinael R, Chevallier T, Barthès BG, Saby NPA, Parent T, Dupraz C, Bernoux M, Chenu C. 2015b. Impact of alley cropping agroforestry on stocks, forms and spatial distribution of soil organic carbon – A case study in a Mediterranean context. *Geoderma* 259-260: 288-299
- Cardinael R, Chevallier T, Cambou A, Béral C, Barthès BG, Dupraz C, Durand C, Kouakoua E, Chenu C. 2017. Increased soil organic carbon stocks under agroforestry: a survey of six different sites in France. *Agriculture, Ecosystems and Environment* 236: 243-255
- Cardinael R, Mao Z, Prieto I, Stokes A, Dupraz C, Kim JH, Jourdan C. 2015. Competition with winter crops induces deeper rooting of walnut trees in a Mediterranean alley cropping agroforestry system. *Plant and Soil* 391: 219-235
- Cornelissen JHC, Aerts R, Cerabolini B, Werger MJA, van der Heijden MGA. 2001. Carbon cycling traits of plant species are linked with mycorrhizal strategy. *Oecologia* 129: 611-619
- Côrrea A, Gurevitch J, Martins-Loução MA, Cruz C. 2012. C allocation to the fungus is not a cost to the plant in ectomycorrhizae. *Oikos* 121: 449-463
- Doskey MG, Linderman RG, Boersma L. 1990. Carbon-sink stimulation of photosynthesis in Douglas fir seedlings by some ectomycorrhizas. *New Phytologist* 115: 269-274
- Eklblad A, Wallander H, Godbold DL, Cruz C, Johnson D, Baldrian P, Björk RG, Epron D, Kieliszewska-Rokicka B, Kjoller R, Kraigher H, Matzner E, Neumann J., Plassard C. 2013. The production and turnover of extramatrical mycelium of ectomycorrhizal fungi in forest soils: role in carbon cycling. *Plant and Soil* 366: 1-27
- Feliciano D, Ledo A, Hillier J, Nayak DR. 2018. Which agroforestry options give the greatest soil and above ground carbon benefits in different world regions? *Agriculture, Ecosystems and Environment* 254: 117-129
- Filley TR, Boutton TW, Liao JD, Jastrow JD, Gamblin DE. 2008. Chemical changes to nonaggregated particulate organic matter following grassland-to-woodland transition in a subtropical savanna. *Journal of Geophysical Research* 113: G03009, doi:10.1029/2007JG000564
- Freibauer A, Rounsevell MDA, Smith P, Verhagen J. 2004. Carbon sequestration in the agricultural soils of Europe. *Geoderma* 122: 1-23
- Frey S. 2019. Mycorrhizal fungi as mediators of soil organic matter dynamics. *Annual Review of Ecology, Evolution, and Systematics* 50: 237-259

- Gadgil RL, Gadgil PD. 1975. Suppression of litter decomposition by mycorrhizal roots of *Pinus radiata*. *New Zealand Journal of Forest Science* 5: 33-41
- Gama-Rodrigues EF, Nair PKR, Nair VD, Gama-Rodrigues AC, Baligar VC, Machado RCR. 2010. Carbon storage in soil size fractions under two cacao agroforestry systems in Bahia, Brazil. *Environmental Management* 45: 274-283
- Godbold DL, Hoosbeek MR, Lukac M, Cotrufo MF, Janssens IA, Ceulemans R, Polle A, Velthorst EJ, Scarascia-Mugnozza G, De Angelis P, Miglietta F, Peressotti A. 2006. Mycorrhizal hyphal turnover as a dominant process for carbon input into the soil organic matter. *Plant and Soil* 281: 15-24
- Gorka S, Dietrich M, Mayerhofer W, Gabriel R, Wiesenbauer J, Martin V, Zheng Q, Imai B, Prommer J, Weidinger M, Schweiger P, Eichorst SA, Wagner M, Richter A, Schintmeister A, Wobken D, Kaiser C. 2019. Rapid transfer of plant photosynthates to soil bacteria via ectomycorrhizal hyphae and its interaction with nitrogen availability. *Frontiers in Microbiology* 10: 168. doi: 10.3389/fmicb.2019.00168
- Graf F, Frei M. 2013. Soil aggregate stability related to soil density, root length, and mycorrhiza using site-specific *Alnus incana* and *Melanogaster variegatus* s.l. *Ecological Engineering* 57: 314-323
- Hobbie EA. 2006. Carbon allocation to ectomycorrhizal fungi correlates with belowground allocation in culture studies. *Ecology* 87: 563-569
- Howlett DS, Mosquera-Losada MR, Nair PKR, Nair VD, Rigueiro-Rodríguez A. 2011. Soil carbon storage in silvopastoral systems and treeless pasture in northwestern Spain. *Journal of Environmental Quality* 40: 825-832
- Hübner R, Kühnel A, Lu J, Dettmann H, Wang W. 2021. Soil carbon sequestration by agroforestry systems in China: A meta-analysis. *Agriculture, Ecosystems & Environment* 315: 107437
- Jones MD, Smith SE. 2004. Exploring functional definitions of mycorrhizas: Are mycorrhizas always mutualisms? *Canadian Journal of Botany* 82: 1089-1109
- Jose S. 2009. Agroforestry for ecosystem services and environmental benefits: an overview. *Agroforestry Systems* 76: 1-10
- Kay S, Rega C, Moreno G, den Herder M, Palma JHN, Borek R, Crous-Duran J, Freese D, Giannitsopoulos M, Graves A, Jäger M, Lamersdorf N, Memedemin D, Mosquera-Losada R, Pantera A, Paracchini ML, Paris P, Roces-Díaz JV, Rolo V, Rosati A, Sandor M, Smith J, Szerencsits E, Varga A, Viaud V, Wawer R, Burgess PJ, Herzog F. 2019. Agroforestry creates carbon sinks whilst enhancing the environment in agricultural landscapes in Europe. *Land Use Policy* 83: 581-593
- Langley JA, Chapman SK, Hungate BA. 2006. Ectomycorrhizal colonization slows root decomposition: the *post-mortem* fungal legacy. *Ecology Letters* 9: 955-959.
- Langley JA, Hungate BA. 2003. Mycorrhizal controls on belowground litter quality. *Ecology* 84: 2302-2312.
- Lehman A, Leifheit EF, Rillig MC. 2017. Mycorrhizas and Soil Aggregation. In: Johnson NC, Gehring C, Jansa J. *Mycorrhizal Mediation of Soil. Fertility, Structure, and Carbon Storage*. Elsevier.
- Leifheit EF, Veresoglou SD, Lehmann A, Morris EK, Rillig MC. 2014. Multiple factors influence the role of arbuscular mycorrhizal fungi in soil aggregation – a meta-analysis. *Plant and Soil* 374: 523-537
- Lorenz K, Lal R. 2014. Soil carbon sequestration in agroforestry systems. A review. *Agronomy for Sustainable Development* 34: 443-454

- Nair PKR. 2011. Agroforestry systems and environmental quality. Introduction. *Journal of Environmental Quality* 40: 784-790
- Näsholm T, Högborg P, Franklin O, Metcalfe D, Keel SG, Campbell C, Hurry V, Linder S, Högborg MN. 2013. Are ectomycorrhizal fungi alleviating or aggravating nitrogen limitation of tree growth in boreal forests? *New Phytologist* 198: 214-221
- Oelbermann M, Voroney RP, Thevathasan NV, Gordon AM, Kass DCL, Schlönvoigt A. 2006. Soil carbon dynamics and residue stabilization in a Costa Rican and southern Canadian alley cropping system. *Agroforestry Systems* 68: 27-36
- Pardon P, Reubens B, Reheul D, Mertens J, De Frenne P, Coussement T, Janssens P, Verheyen K. 2017. Trees increase soil organic carbon and nutrient availability in temperate agroforestry systems. *Agriculture, Ecosystems and Environment* 247: 98-111
- Paterson E, Sim A, Davidson J, Daniell TJ. 2016. Arbuscular mycorrhizal hyphae promote priming of native soil organic matter mineralisation. *Plant and Soil* 408: 243-254
- Piotrowski JS, Denich T, Klironomos JN, Graham JM, Rillig MC. 2004. The effects of arbuscular mycorrhizas on soil aggregation depend on the interaction between plant and fungal species. *New Phytologist* 164: 365-373
- Reid CPP, Kidd FA, Ekwebelam SA. 1983. Nitrogen nutrition, photosynthesis and carbon allocation in ectomycorrhizal pine. *Plant and Soil* 71: 415-432
- Rousseau JVD, Reid CPP. 1990. Effects of phosphorus and ectomycorrhizas on the carbon balance of loblolly pine seedlings. *Forest Science* 36: 101-112
- Singh BP, Setia R, Wiesmeier M, Kunhikrishnan A. 2018. Agricultural Management Practices and Soil Organic Carbon Storage. In: Singh BK (Ed.) *Soil Carbon Storage. Modulators, Mechanisms and Modelling*. Academic Press. Pg. 207-244. ISBN 9780128127667
- Singh S, Nouri A, Singh S, Anapalli S, Lee J, Arelli P, Jagadamma S. 2020. Soil organic carbon and aggregation in response to thirty-nine years of tillage management in southeastern US. *Soil and Tillage Research* 197: 104523
- Six J, Conant RT, Paul EA, Paustian K. 2002. Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils. *Plant and Soil* 241: 155-176
- Staddon PL, Ramsey CB, Ostle N, Ineson P, Fitter AH. 2003. Rapid turnover of hyphae of mycorrhizal fungi determined by AMS Microanalysis of ¹⁴C. *Science* 300: 1138-1140
- Udawatta RP, Jose S. 2012. Agroforestry strategies to sequester carbon in temperate North America. *Agroforestry Systems* 86: 225-242
- Wilson GWT, Rice CW, Rillig MC, Springer A, Hartnett DC. 2009. Soil aggregation and carbon sequestration are tightly correlated with the abundance of mycorrhizal fungi results from long-term field experiments. *Ecology Letters* 12: 452-461.
- Wotherspoon A, Thevathasan NV, Gordon AM, Voroney RP. 2014. Carbon sequestration potential of five tree species in a 25-year-old temperate tree-based intercropping system in southern Ontario, Canada. *Agroforestry Systems* 88: 631-643
- Zheng W, Morris EK, Rillig MC. 2014. Ectomycorrhizal fungi in association with *Pinus sylvestris* seedlings promote soil aggregation and soil water repellency. *Soil Biology and Biochemistry* 78: 326-331
- Zhu Y-G, Miller RM. 2003. Carbon cycling by arbuscular mycorrhizal fungi in soil-plant systems. *Trends in Plant Science* 8: 407-409

Conclusions

The analysed landscape units contain on average $21.6 \pm 2.9 \text{ Mg C ha}^{-1}$ in the organic horizons and 42.8 ± 4.4 in the top 25 cm of the mineral soil, with variations depending on climate, forest type and soil characteristics. As a consequence of forest thinning, between 5.6 and 48.4 Mg ha⁻¹ (median: 16.8 and 25.5 Mg ha⁻¹ for *Pinus* and evergreen *Quercus* stands respectively) of forest debris were left in the forest floor. This debris was mostly in form of leaves and fine branches in *Pinus* forests, or thick branches in evergreen *Quercus* forests, suggesting that in *Quercus* forests woody debris will remain in the forest floor for longer time. Green leaves decompose faster than brown leaf litter, at least during the first stages of decomposition. Woody debris decomposed slower than leaf litter, with higher decomposition rates in FWD than in CWD. Although *P. nigra* CWD had higher lignin and higher C/N ratios than *Q. faginea* CWD, both decompose at similar rates during the first stages of decomposition. Long term monitoring of CWD will be necessary to see the effect of their different biochemical quality on their decomposition rates and the C fluxes to the soil. By contrast, FWD of *Q. faginea* decomposed faster than that of *P. nigra*. A simulation using the decomposition rates obtained in this Action and in the literature suggested that about 40% (3.0 and 6.6 Mg C ha⁻¹ in conifer and evergreen oak stands respectively) of woody debris left in the forest floor will remain 15 years after thinning, while most of the leaf C will be lost during the first years.

Agroforestry systems are effective in increasing organic C content in agricultural soils. Although it is very variable, in Mediterranean agroforestry systems, literature reports soil C accumulation rates can be around 0.35 Mg C ha⁻¹ y⁻¹ (0 – 100 cm) more than in agricultural systems, while aboveground biomass C accumulation are between 0.8 and 1.8 Mg C ha⁻¹ y⁻¹. The presence of trees increases the C inputs and allocates more C in deep soil. The low biochemical quality of tree debris slows down its decomposition and increase the persistence of the tree C inputs in the soil. Mycorrhizas promote the tree growth and therefore the C inputs into the soil. The effect of mycorrhizas on soil aggregation improve the C stabilisation in soil, reducing the C loss. Ectomycorrhizas reduce the decomposability of the roots, so trees species associated to ectomycorrhizal fungi may be more effective in increasing soil C storage than those associated to arbuscular mycorrhizas.

Appendix

Appendix 1. Leaf and woody debris resulting from forest treatments

Appendix 1. Table A.1. Biomass (Mg/ha) per fractions estimated using allometric equations¹ on dasometric information obtained in before and after the treatments.

UP		Trunk		Leaves		Branches (2-7 cm)		Branches (<2cm)		Branches (>7 cm)		Total ABG	
		Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
Aspres	Agullana	61.5	44.3	2.5	1.8	17.2	12.3	4.3	3.0	81.6	60.8	165.0	120.7
Aspres	CanBudo	52.5	34.8	2.3	1.6	16.5	10.8	4.4	3.0	50.8	35.5	125.4	85.1
Montmell	Aiguaviva	28.4	22.2	0.2	0.2	6.8	5.5	16.9	13.2	3.1	2.7	53.0	41.8
Ancosa	LaLlacCanVich	68.6	51.9	0.0	0.0	15.5	12.0	39.5	29.7	11.9	10.0	131.4	100.1
	LaLlacunaPh	63.1	36.3	0.1	0.1	14.4	9.1	34.5	20.4	12.3	9.1	119.4	70.9
	LaLlacunaPn	92.0	56.6	0.1	0.0	12.5	7.7	30.8	18.6	2.9	1.9	139.5	85.9
Rialb	Confos	139.2	120.5	0.4	0.5	20.5	18.4	43.2	36.2	6.4	6.2	212.4	184.4
	Sardanyes	150.8	96.4	13.4	8.1	20.1	12.6	17.8	10.8	2.7	1.8	205.0	129.7
Replans Berguedà	Cercs	122.4	66.2	17.6	8.3	22.0	12.1	25.6	13.1	1.4	1.1	188.0	100.4
Capçaleres	Vallcebre_feixes	163.0	75.0	18.3	4.1	23.5	7.9	24.3	5.5	3.8	4.0	228.6	89.6
	Vallcebre1	67.5	45.0	10.7	6.0	11.7	7.3	14.1	7.9	0.5	0.4	105.0	67.4
	Vallcebre2	88.5	66.8	11.2	6.5	15.5	11.2	18.1	12.0	1.4	1.3	138.7	102.3

Appendix 2. Woody debris decomposition experiment

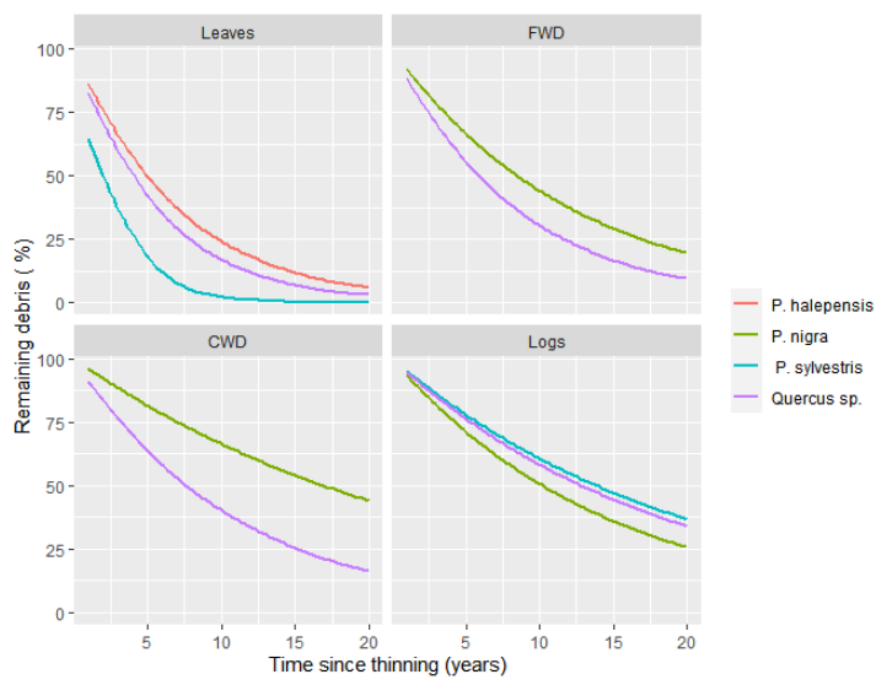


Appendix 2. Figure 1. Demonstration set-up of the C contribution from decomposing wood of different qualities to soil C stocks



Appendix 2. Figure 2. Demonstration set-up of the C contribution from decomposing wood of different qualities to soil C stocks.

Appendix 3. Decomposition k -Olson parameters for different debris types and tree species



Appendix 3. Figure 1. Simulation of remaining debris over years after treatment per different debris types and species resulting from applying the Olson decomposition k values shown in the Table 7.