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Carbon and nitrogen in forest floor and mineral soil under four forest species in the Mediterranean region

Carbono y nitrógeno en horizontes orgánicos y minerales de suelos desarrollados bajo cuatro especies forestales en la región mediterránea
Carbono e nitrogénio nos horizontes orgânicos e minerais dos solos desenvolvidos sob quatro espécies florestais na região Mediterrânica

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ABSTRACT

The organic and mineral horizons of soils are of great importance in C and N storage in forest areas. However, knowledge of the effects of forest species on the stocks of these elements is still scarce, especially in Portugal. In order to contribute to this knowledge, a study was carried out in forest stands of *Pinus pinaster* Aiton (PP), *Pinus nigra* Arnold (PN), *Pseudotsuga menziesii* (PM) and *Castanea sativa* Miller (CS), installed in the 1950s in northern Portugal. Sampling areas with similar topography, lithology and climate were selected, in order to better identify hypothesized differences in C and N storage due to forest species effect. In each stand, 15 sites were selected randomly and the forest floor (organic layers) was collected in a 0.49 m^2 area. The layers H, L and F of the forest floor were identified and, for L and F, their components were separated in leaves, pine cones/chestnut husks and branches. At the same sites, soil samples were also collected at 0-10 and 10-20 cm depth. At these depths, undisturbed samples were also collected for bulk density determination. The concentrations of C and N were determined in forest floor and mineral components of the soil, and converted in mass per unit area. The quantity of C storage per unit area followed the sequence PN > PM > CS > PP, while for N the sequence was CS > PM > PN > PP, OM and PP keeping the same relative position in the sequence in both C and N concentrations. The PM and CS species store similar amounts of C and N, and about 90% of these elements is found in the upper 20 cm of the mineral soil. In PN and PP species, the contribution of forest floor to the storage of these elements is more expressive than in the other species, but lower than 30% in all cases.

RESUMEN

Los horizontes orgánicos y minerales de los suelos forestales tienen una gran importancia en el almacenamiento de C y N. Sin embargo, aún existe un escaso conocimiento sobre los efectos que las especies forestales pueden tener en la retención de estos elementos, particularmente en la región de montaña del Norte de Portugal. En este estudio se ha diseñado un muestreo aleatorio en parcelas situadas en la vertiente occidental de la Sierra Padrela, cerca de Vila Real, para las especies Castanea sativa *Miller (CS) y* Pinus pinaster *Aiton (PP), con una alta representación en el norte del país, y* Pseudotsuga menziesii *(Mirb.) Franco (PM) y* Pinus nigra *Arnold (PN), con menor representación pero con cierto interés forestal. En la selección de zonas de muestreo se ha buscado características similares atendiendo a la edad de la masa, topografía, litología y clima, con el fin de reducir el efecto de otras variables y poder identificar*

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mejor las posibles diferencias en el almacenamiento de C y N debido únicamente a la especie. Para cada especie se han seleccionado 15 puntos de medición al azar y se han recogido muestras de material orgánico (horizonte orgánico) en un área de 0,49 m² por punto. La recogida de esta muestra ha obedecido a criterios morfológicos y se han separado en las capas L, F y H. A su vez, en las capas L y F se han separado tres fracciones: hojas, conos o erizos y ramas. Además, en los mismos puntos se han recogido muestras de suelo a profundidades de 0-10 y 10-20 cm, así como muestras inalteradas para la determinación de la densidad aparente. Posteriormente se ha determinado la concentración de C y N en todas las muestras (horizontes orgánicos y minerales), transformándolas en masa por unidad de área. Los resultados obtenidos muestran que la cantidad de C por unidad de área sigue la secuencia PN > PM > CS > PP y CS > PM > PN > PP para el N. Las especies PM y CS almacenan cantidades idénticas de C y N, y alrededor de 90% de la cantidad de estos elementos se encuentran en el suelo. En el caso de las especies PN y PP la contribución de los horizontes orgánicos en la retención de estos elementos es más significativa que en el otras, pero siempre menor que 30%.

RESUMO

Os horizontes orgânicos e minerais dos solos florestais apresentam elevada importância no armazenamento de C e N. No entanto, o conhecimento dos efeitos das espécies florestais na retenção destes elementos é ainda escasso, principalmente em Portugal. Com o propósito de contribuir para este conhecimento realizou-se um estudo em povoamentos de quatro espécies florestais instalados na década de 50 do século XX na vertente poente da Serra da Padrela, próximo de Vila Pouca de Aguiar. Duas das espécies apresentam elevada representação na região Norte de Portugal, Castanea sativa *Miller (CS) e* Pinus pinaster *Aiton (PP) e duas, embora com menor representatividade, evidenciam interesse silvícola,* Pseudotsuga menziesii *(Mirb.) Franco (PM) e* Pinus nigra *Arnold (PN). Na selecção das áreas de amostragem, procuraram-se características semelhantes no que toca a topografia, litologia e clima, de modo a reduzir o efeito de outras variáveis e a melhor identificar as possíveis diferenças no armazenamento de C e N devidas à espécie florestal. Em cada povoamento, foram seleccionados 15 locais ao acaso e colhido o material orgânico (horizonte orgânico) numa área de 0,49 m² por local. A colheita do horizonte orgânico obedeceu a critérios morfológicos tendo sido separado nas camadas L, F e H. As camadas L e F, por sua vez, foram separadas em três fracções: agulhas ou folhas, pinhas ou ouriços e ramos. Nos mesmos locais foram ainda colhidas amostras de solo nas profundidades 0-10 e 10-20 cm. Nestas profundidades colheram-se também amostras não perturbadas para determinação da densidade aparente do solo. As concentrações de C e N foram determinadas em todas as amostras (horizontes orgânicos e minerais) e convertidas em massa por unidade de área. A massa de C por unidade de área segue a sequência PN > PM > CS > PP, mantendo as espécies PM e PP a mesma tendência para o N que segue a sequência CS > PM > PN > PP. As espécies PM e CS armazenam quantidades idênticas de C e N, sendo que cerca de 90% da quantidade destes elementos se encontra no solo. No caso das espécies PN e PP o contributo dos horizontes orgânicos na retenção destes elementos é mais expressivo do que nas restantes mas sempre inferior a 30%.*

KEYS WORDS
Northern Portugal, conifer species, broadleaved species, forest floor layers, C and N stocks.

PALABRAS CLAVE
Norte de Portugal, maderas blandas, maderas duras, capas orgánicas, contenido de C y N.

PALAVRAS-CHAVE
Norte de Portugal, resinosas, folhosas, camadas orgânicas, teor de C e N.

1. Introduction

Forest species litter production and litter quality have a recognized importance in the accumulation, type and distribution of soil organic matter formed in the topsoil horizon. In turn, they determine a series of processes that influence soil properties, pedogenesis, and the productivity and sustainability of ecosystems (Oostra et al. 2006; Fisher and Binkley 2012). Forest species affect soil development both directly through the chemistry of the litter and indirectly through the effect of the litter on biological community composition (Reich et al. 2005; Kooch et al. 2017). The nutrient fluxes to the soil are related to litter quality, particularly the proportions of C and N (Fisher and Binkley 2012; Park 2015). Decomposition is an important component of the C and N cycles (Shaw and Harte 2001) and is closely related to the biological activity that, among several factors, depends on the climatic conditions, chemical and structural composition of organic residues and nutrient availability (Bargali et al. 2015). For similar climatic conditions, nature and quantity of litter have an important role in forest floor formation and energy transfer between plants and soil (Santa-Regina 2001; Shaw and Harte 2001; Marty et al. 2017). Also, the forest floor plays a crucial role in the hydrological processes and can influence considerable processes at an ecosystem level, due to their location at the interface of the atmosphere and the mineral soil (Keith et al. 2010).

The forest floor has a heterogeneous composition and can be divided into Litter layer (L), Fermentation layer (F) and Humus layer (H), corresponding to forest litter at various stages of decomposition (Wesemael 1993; Wardle 1993). Decomposition rates of plant residues can be slower or faster, depending on their nature. In general, it is accepted that organic residues from coniferous species decompose slower than broadleaved species, for example, due to the presence of non-hydrolyzable polyphenolic compounds in litter (Berg and McClaugherty 2003; Díaz-Pinés et al. 2011). On the other hand, fast-growing species would accumulate C faster than slow-growing species, but several studies have shown that replacement leads to a C loss, mainly in the mineral soil (Schroth et al. 2002; Wang and Wang 2007; Vallet et al. 2009). Owing to increasing emphasis on ecosystem services, the knowledge of the differences among forest species regarding C sequestration should be a decision support tool when introducing new forest species and can be used strategically to reach environmental goals (Oostra et al. 2006; Schulp et al. 2008; Vallet et al. 2009; Vesterdal et al. 2013).

The main objective of the present study was to quantify the effects of four forest species (*Pinus pinaster* Aiton (PP) and *Castanea sativa* Miller (CS), which are well represented in North Portugal; *Pinus nigra* Arnold (PN) and *Pseudotsuga menziesii* (Mirb.) Franco (PM), with lower coverage but with forestry interest) on C and N concentrations and stocks in forest floor and top mineral soil layers.

2. Material and methods

The experimental area was located in Serra da Padrela, North Portugal at 41º29'24''N, 7º36'43''W and an altitude ranging from 800 and 900 m (**Figure 1**). Mean annual temperature is 11.3 ºC, with monthly averages ranging from 4.0 ºC (December) to 21.9 ºC (August) and a mean annual precipitation of 1381 mm, with a typically Mediterranean seasonal distribution (INMG 1991). Soils are classified as Orthi-Umbric Cambisols developed on schists (IUSS Working Group 2015), with medium-texture, acid pH, very low P, moderate to high K contents and moderate organic matter content (Agroconsultores and Coba 1991; Martins et al. 2007).

The study was carried out in four 60 year old forest stands. Two of the tree species are well represented in the northern region of the country, *Castanea sativa* Miller (CS) and *Pinus pinaster* Aiton (PP), and two, although with lower coverage, have silvicultural interest, *Pseudotsuga menziesii* (Mirb.) Franco (PM) and *Pinus nigra* Arnold (PN). After 40 years, the CS stand was subject to a clearcut. Forest management continued in coppice regime from then onwards. As the PP stand is close to a village, some people collected forest floor litter (pine cones and leaves) for use as household fuel. In the selected sampling areas, topography, lithology and climate were similar, therefore allowing comparisons on soil C and N stocks between the tree species. Dendrometric characteristics of the tree species are shown in **Table 1**. Although tree density, dominant height and mean diameter are clearly different in stands with similar age (PP, PN and PM), characterizing these species stand at that age in such geographical setting, their basal area is around 50 m^2 ha^{-1}, while for CS it is half of that value. Data for CS corresponds to the coppice-period, meaning that the dendrometric characteristics represent those of a younger stand in the same ecological conditions.

In each stand, 15 sampling sites were randomly established. In each one of these, the forest floor was collected in spring in a 0.49 m^2 quadrat and divided, according to morphological criteria, into L-layer (Litter layer, composed by organic material recently fallen that is readily identifiable as

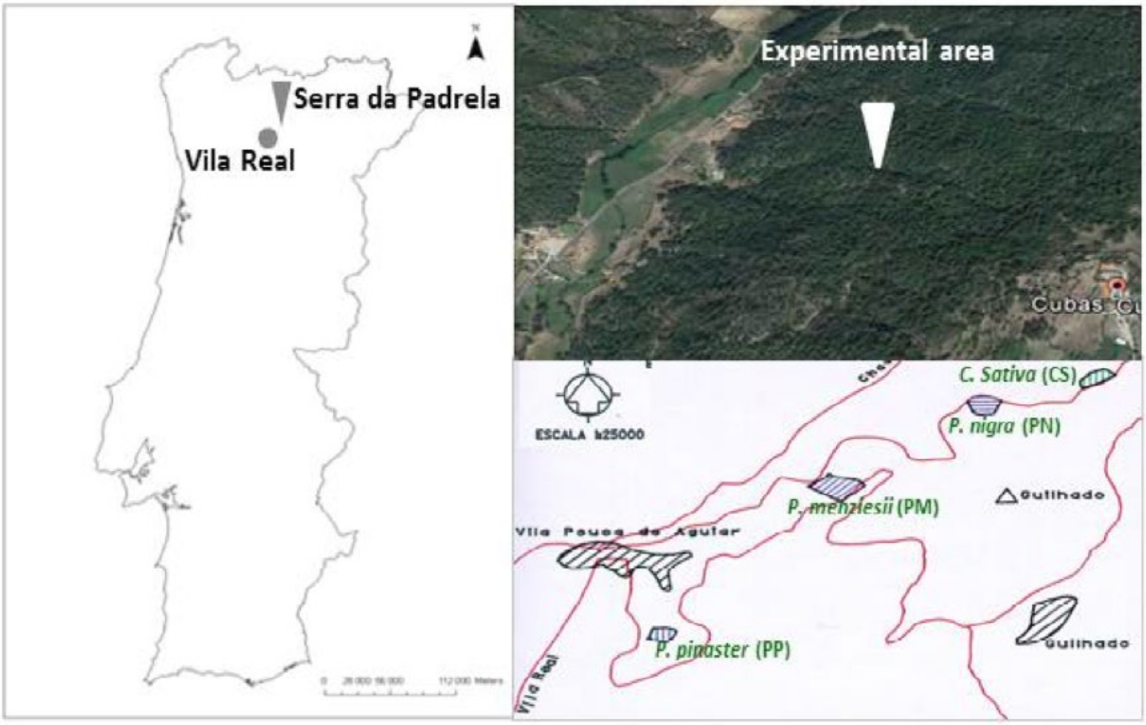

Figure 1. Location of the study area in Serra da Padrela, North of Portugal.

Table 1. Mean stand characteristics of the studied plots for *Pinus pinaster* (PP), *Pinus nigra* (PN), *Pseudotsuga menziesii* (PM) and *Castanea sativa* (CS)

Species	*Number of stems (trees ha^{-1})*	*Age (years)*	*Dominant height (m)*	*Mean diameter (cm)*	*Basal area (m^2 ha^{-1})*
PP	988	58	16.2	25.4	49.1
PN	475	59	22.8	34.3	43.9
PM	313	59	34.8	46.8	53.3
CS*	1438	57	13.3	14.7	24.6

* For CS the characteristics correspond to the coppice period, 17 years old.

to origin), F-layer (Fermentation layer, comprised organic material partly decomposed, but yet recognizable as to origin) and H-layer (Humus layer, comprised well-decomposed organic material in which plant structures are generally not recognizable, containing considerable amount of mineral matter) (Wesemael 1993; van Delft et al. 2006). The L-layer and F-layer were separated in leaves, pine cones/chestnut husks and branches. Then, samples of each component of the L and F layers were grouped three to three, making five samples per component of each layer, resulting in a total of 15 samples for the L-layer, 15 samples for the F-layer and five samples for the H-layer. Forest floor samples were dried at 65 ºC for 72 h to determine dry mass. In the same 15 quadrats where the forest floor sampling was carried out, disturbed soil samples were collected in the depths 0-10 and 10-20 cm. Bulk density (BD) was measured at the same depths in undisturbed samples collected in 100 cm^3 cylinders. Soil samples were collected on mineral topsoil (0-20 cm), as this is the relevant depth for spatial survey of C storage, according to the

Kyoto Protocol requirements (Schulp et al. 2008; Vesterdal et al. 2008; Sil et al. 2017).

Soil samples were air dried and sieved to determine the coarse fraction (> 2 mm). All forest floor and mineral soil samples were analyzed for total C by dry combustion (ISO 1995) and for total N by Kjeldahl method. Soil samples were tested with an acid-drop but no carbonates were detected, thus the total soil C was assumed to be comparable to soil organic C. Forest floor mass values were converted to C (Mg ha^{-1}) and N (kg ha^{-1}) multiplying these values by the C and N concentrations in dry matter. Soil organic C (Mg ha^{-1}) and total N in soil (Mg ha^{-1}) were calculated multiplying C and N concentrations by bulk density and thickness of the mineral soil layer with a correction for the coarse element content. Statistical analysis of data comprised one-way ANOVA and multiple comparison of averages (Tukey, 5%), performed to assess the significance of species effects on results.

3. Results

3.1. Forest floor mass and concentrations of C and N

According to the results expressed in **Figure 2**, the forest floor mass was significantly higher for PN (59.3 Mg ha^{-1}), followed by PM (33.0 Mg ha^{-1}) and PP (23.5 Mg ha^{-1}) and finally the CS species (16.6 Mg ha^{-1}). Leaves were the main constituent of the forest floor in all species, but in PN the pine cones had an important mass (**Table 2**). The differences among species can be essentially related to the anthropogenic disturbances in PP (organic residues collection) and CS stands (clearcutting after 40 years), pine cones production (high for PN) and quality of organic material constituting the forest floor (**Tables 2 and 3**). Organic layer total mass increased from L to F and decreased to H (**Table 2**).

The organic components of the PM and CS tended to show higher concentrations of N and lower of C, relative to PP and PN, which was reflected in the C:N ratio and in the decomposition rate of the organic components (**Table 3**). The concentration of N tended to decrease from the leaves to the pine cones/chestnut husks and

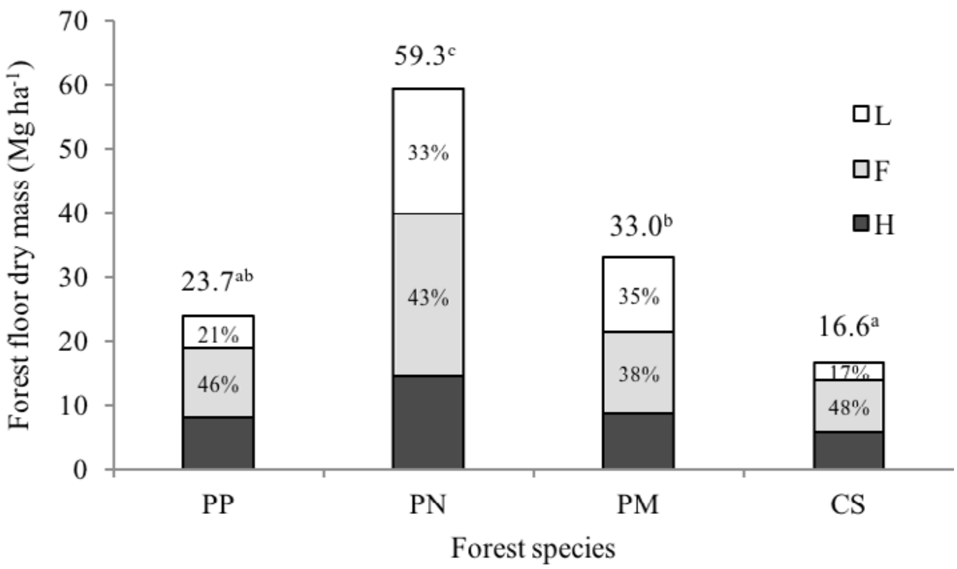

Figure 2. Total forest floor dry mass (Mg ha^{-1}) of L, F and H layers from species PP (*Pinus pinaster*), PN (*Pinus nigra*), PM (*Pseudotsuga menziesii*) and CS (*Castanea sativa*). Values followed by different letters are statistically different ($p < 0.05$).

Table 2. Forest floor dry mass (Mg ha^{-1}) of L, F and H layers (mean ± standard deviation). Leaves, pine cones/chestnut husks (pc/ch) and branches from species PP (*Pinus pinaster*), PN (*Pinus nigra*), PM (*Pseudotsuga menziesii*) and CS (*Castanea sativa*). For components or layers, values followed by different letters are statistically different ($p < 0.05$)

Species	***Forest floor layers***	***Component***			***Overall***
		leaves	*pc/ch*	*branches*	
PP	L	3.5 ± 1.8	0.5 ± 0.8	0.9 ± 0.7	4.9 ± 1.0^{a}
	F	9.9 ± 2.7	0.1 ± 0.2	0.9 ± 0.9	10.9 ± 1.5^{b}
	H				7.9 ± 0.5^{b}
	Overall	13.4 ± 2.3^{b}	0.6 ± 0.5^{a}	1.8 ± 0.8^{a}	
PN	L	3.6 ± 1.3	10.7 ± 8.5	5.3 ± 7.8	19.6 ± 4.3^{b}
	F	17.1 ± 4.1	5.7 ± 4.9	2.6 ± 2.5	25.4 ± 3.8^{b}
	H				14.3 ± 1.0^{a}
	Overall	20.7 ± 2.6^{c}	16.4 ± 6.2^{b}	7.9 ± 3.9^{a}	
PM	L	4.6 ± 3.3	0.5 ± 0.5	6.6 ± 3.8	11.7 ± 2.7^{a}
	F	12.0 ± 5.1	0.1 ± 0.2	0.6 ± 0.6	12.7 ± 1.7^{a}
	H				8.6 ± 0.6^{a}
	Overall	16.6 ± 3.2^{c}	0.6 ± 0.3^{a}	7.2 ± 1.8^{b}	
CS	L	2.0 ± 1.0	0.2 ± 0.2	0.7 ± 0.4	2.9 ± 0.5^{a}
	F	7.2 ± 2.4	0.2 ± 0.2	0.6 ± 0.4	8.0 ± 1.6^{b}
	H				5.7 ± 0.4^{b}
	Overall	9.2 ± 1.3^{b}	0.4 ± 0.3^{a}	1.3 ± 0.4^{a}	

Table 3. Carbon and N concentrations and C:N ratio of L, F and H forest floor layers, expressed as mean and standard deviation. Leaves, pine cones/chestnut husks (pc/ch) and branches from species PP (*Pinus pinaster*), PN (*Pinus nigra*), PM (*Pseudotsuga menziesii*) and CS (*Castanea sativa*). For the same component, values followed by different letters in columns are statistically different ($p < 0.05$)

Species	***Forest floor layers***						
	L			***F***			***H***
	leaves	**pc/ch**	**branches**	**leaves**	**pc/ch**	**branches**	
C concentration (g kg^{-1})							
PP	568 ± 5^{b}	574 ± 3^{a}	567 ± 6^{a}	447 ± 22^{b}	559 ± 2^{a}	563 ± 7^{a}	213 ± 29^{ab}
PN	572 ± 2^{b}	576 ± 2^{a}	575 ± 3^{a}	459 ± 67^{b}	564 ± 6^{a}	566 ± 6^{a}	251 ± 65^{b}
PM	526 ± 15^{a}	562 ± 12^{a}	571 ± 3^{a}	344 ± 31^{a}	541 ± 20^{a}	562 ± 7^{a}	129 ± 49^{a}
CS	560 ± 3^{b}	554 ± 38^{a}	569 ± 4^{a}	365 ± 23^{a}	527 ± 25^{a}	554 ± 5^{a}	143 ± 40^{a}
N concentration (g kg^{-1})							
PP	5.5 ± 0.3^{a}	7.6 ± 3.7^{ab}	5.6 ± 3.3^{ab}	14.0 ± 4.8^{a}	8.7 ± 4.4^{a}	5.4 ± 0.4^{a}	12.0 ± 4.1^{a}
PN	7.9 ± 4.4^{a}	4.9 ± 4.3^{a}	3.3 ± 0.5^{a}	13.3 ± 1.5^{a}	5.4 ± 0.7^{a}	4.5 ± 0.9^{a}	14.5 ± 3.2^{ab}
PM	14.3 ± 2.0^{b}	5.3 ± 1.5^{a}	4.9 ± 0.5^{ab}	18.5 ± 4.9^{ab}	7.5 ± 6.4^{a}	6.3 ± 1.9^{a}	19.2 ± 4.1^{ab}
CS	15.1 ± 0.6^{b}	13.0 ± 1.1^{b}	8.6 ± 2.0^{b}	22.8 ± 2.3^{b}	16.7 ± 1.1^{b}	11.3 ± 2.4^{b}	22.8 ± 6.7^{b}
C:N ratio							
PP	94 ± 7^{b}	103 ± 28^{b}	118 ± 46^{b}	38 ± 20^{b}	49 ± 31^{b}	111 ± 10^{b}	18 ± 4^{b}
PN	85 ± 29^{b}	168 ± 72^{c}	175 ± 31^{c}	34 ± 3^{b}	107 ± 15^{c}	132 ± 28^{c}	17 ± 2^{b}
PM	38 ± 6^{a}	120 ± 43^{b}	117 ± 13^{b}	20 ± 6^{a}	42 ± 2^{b}	91 ± 32^{b}	7 ± 2^{a}
CS	38 ± 1^{a}	53 ± 14^{a}	76 ± 13^{a}	16 ± 2^{a}	26 ± 27^{a}	49 ± 13^{a}	7 ± 2^{a}

from these to the branches, showing an inverse trend for C, but with some variations of this pattern in the L-layer. As expected, the C concentration decreased slightly from the L-layer to F-layer and more markedly to the H-layer, while N concentration increased. The decrease of C was more evident for leaves, since the C:N ratio was lower for these components (**Table 3**). The increased N from the L-layer to H-layer was related with the process of humification. The C:N ratio varied between 143:1 (L-layer of PN) and 7:1 (H-layer of PM and CS). In the L and F layers the maximum was reached in branches of PN (175:1 and 132:1, respectively) and the minimum in leaves of CS (38:1 and 16:1, respectively) (**Table 3; Figure 6**).

3.2. Carbon and nitrogen storage in forest floor

The C storage was significantly higher in the F-layer for PP and CS, with the other species (PN and PM) showing values similar to those of the L-layer. The H-layer was, in all species, the one with the lowest values. The amount of N stored follows a pattern slightly different from that recorded for C as the three organic layers differ significantly from each other in all species, following the sequence F-layer > H-layer > L-layer. For PP and CS approximately 70% of the C was stored in leaves. In the PM and PN this value decreased to 54% and 36% respectively, with the pine cones representing a high percentage in the case of the PN (34%) and the branches in the case of PM (33%) (**Table 4**).

Table 4. Carbon (Mg ha^{-1}) and N stocks (Kg ha^{-1}) in L, F and H forest floor layers, expressed as mean and standard deviation. Leaves, pine cones/chestnut husks (pc/ch) and branches from species PP (*Pinus pinaster*), PN (*Pinus nigra*), PM (*Pseudotsuga menziesii*) and CS (*Castanea sativa*). For components or layers, values followed by different letters are statistically different ($p < 0.05$)

Species	*Layer*	*Component*			*Overall*	*Component*			*Overall*
		leaves	*pc/ch*	*branches*		*leaves*	*pc/ch*	*branches*	
		C storage (Mg ha^{-1})				**N storage (kg ha^{-1})**			
PP	L	2.0 ± 1.0	0.3 ± 0.5	0.5 ± 0.4	2.8 ± 0.5^a	19.0 ± 6.0	3.0 ± 3.2	3.8 ± 1.3	25.8 ± 3.2^a
	F	4.6 ± 1.1	0.1 ± 0.1	0.5 ± 0.5	5.2 ± 0.6^b	142.7 ± 50.2	1.7 ± 1,9	4.9 ± 2.4	149.3 ± 16.1^c
	H				1.8 ± 0.3^a				107.0 ± 1.2^b
	Overall	6.6 ± 1.0^b	0.4 ± 0.4^a	1.0 ± 0.5^a		161.7 ± 33.8^b	4.7 ± 2.6^a	8.7 ± 1.5^a	
PN	L	2.0 ± 0.7	6.2 ± 4.9	3.0 ± 4.5	11.2 ± 3.2^b	28.3 ± 16.2	57.9 ± 64.2	16.0 ± 14.3	102.2 ± 28.1^a
	F	7.8 ± 2.2	3.2 ± 2.8	1.4 ± 1.4	12.4 ± 1.8^b	228.9 ± 47.3	29.9 ± 8.3	11.0 ± 3.0	269.8 ± 9.7^c
	H				3.7 ± 0.9^a				212.9 ± 15.7^b
	Overall	9.8 ± 1.7^b	9.4 ± 3.3^b	4.4 ± 2.9^a		257.2 ± 39.1^c	87.8 ± 10.7^b	27.0 ± 5.3^a	
PM	L	2.4 ± 1.8	0.3 ± 0.3	3.8 ± 2.2	6.5 ± 1.1^b	65.0 ± 22.6	2.8 ± 1.4	32.4 ± 14.8	100.2 ± 13.9^a
	F	4.2 ± 1.9	0.1 ± 0.2	0.3 ± 0.3	4.6 ± 0.5^b	230.0 ± 85.7	3.3 ± 1.7	3.9 ± 2.4	237.2 ± 10.3^c
	H				1.2 ± 0.4^a				167.7 ± 7.5^b
	Overall	6.6 ± 1.2^b	0.4 ± 0.2^a	4.1 ± 1.3^b		295.0 ± 66.4^c	6.1 ± 1.5^a	36.3 ± 6.2^b	
CS	L	1.1 ± 0.5	0.1 ± 0.1	0.4 ± 0.7	1.6 ± 0.4^b	30.1 ± 9.3	2.9 ± 1.7	5.2 ± 3.8	38.2 ± 2.9^a
	F	2.7 ± 1.0	0.1 ± 0.1	0.3 ± 0.2	3.1 ± 0.2^c	166.4 ± 57.0	3.4 ± 3.0	6.4 ± 1.0	176.2 ± 13.4^c
	H				0.8 ± 0.2^a				131.0 ± 7.5^b
	Overall	3.8 ± 0.9^b	0.2 ± 0.1^a	0.7 ± 0.5^a		196.5 ± 42.1^b	6.3 ± 3.2^a	11.6 ± 2.9^a	

Total forest floor C and N stocks varied significantly among species. The PN had the largest forest floor C stocks (27.3 Mg ha^{-1}), significantly different from the other three species (**Figure 3**). For this species, the C stored in leaves (9.8 Mg ha^{-1}), pine cones (9.4 Mg ha^{-1}) and branches (4.4 Mg ha^{-1}) of the L and F layer, presents more equitable proportions than the remaining ones. In contrast, a significantly lower amount of C is stored in the forest floor of CS (5.5 Mg ha^{-1}), with the leaves storing 3.8 Mg ha^{-1}, meaning about 70% of the total C stocks (Table 4). A similar pattern was found for N, as the highest amounts of N stored were recorded for PN (5.8 kg ha^{-1}) and PM (5.1 kg ha^{-1}), whereas PP (2.9 kg ha^{-1}) and CS (3.5 kg ha^{-1}) had significantly lower amounts. In general, the leaf component represented more than 50% of the N stored in the L and F layers of the forest floor (Table 4, Figure 3).

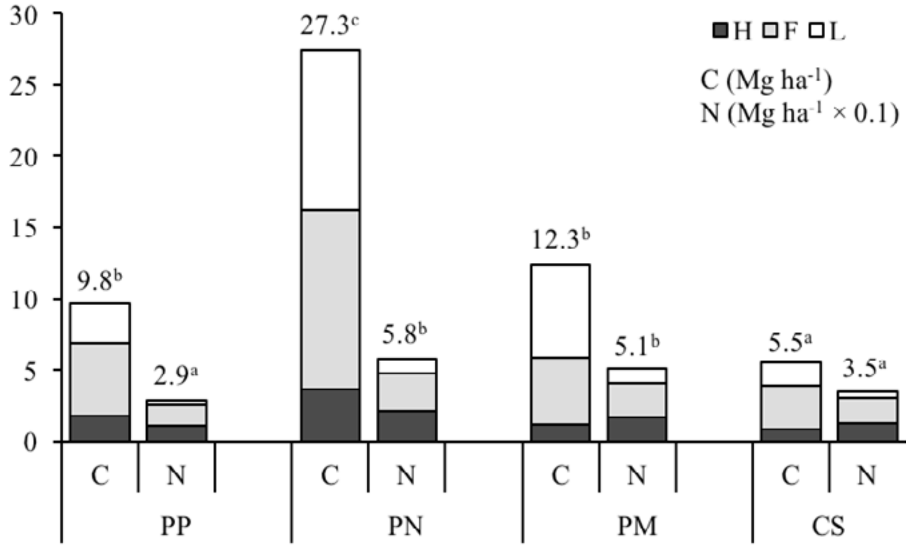

Figure 3. Carbon and N stocks of L, F and H forest floor layers from species PP (*Pinus pinaster*), PN (*Pinus nigra*), PM (*Pseudotsuga menziesii*) and CS (*Castanea sativa*). For C or N, values followed by different letters are statistically different ($p < 0.05$).

Table 5. Carbon and N concentration (g kg^{-1}), soil bulk density (g cm^{-3}) and coarse element content (%) in mineral soil layers for species PP (*Pinus pinaster*), PN (*Pinus nigra*), PM (*Pseudotsuga menziesii*) and CS (*Castanea sativa*), expressed as mean and standard deviation. Different letters in the rows indicate significant differences among species ($p < 0.05$)

Depth (cm)	*Species*			
	PP	**PN**	**PM**	**CS**
SOC concentration (g kg^{-1})				
0-10	21.0 ± 12.9a	40.8 ± 3.0b	33,1 ± 3.0ab	38.0 ± 7.0ab
10-20	14.6 ± 9.5a	34.5 ± 6.8ab	28.9 ± 3.3ab	35.2 ± 7.8b
Total N concentration (g kg^{-1})				
0-10	1.5 ± 1.0a	2.9 ± 0.3ab	2.9 ± 0.6ab	3.4 ± 0.8b
10-20	1.1 ± 1.0a	2.0 ± 0.4ab	2.7 ± 0.6b	3.0 ± 0.3b
Bulk density (g cm^{-3})				
0-10	1.13 ± 0.03a	0.98 ± 0.01a	1.03 ± 0.06a	0.96 ± 0.03a
10-20	1.16 ± 0.02a	1.07 ± 0.03a	1.08 ± 0.03a	1.02 ± 0.03a
Coarse element content (%)				
0-10	42.8 ± 6.1a	32,1 ± 4.5a	35,4 ± 5.3a	34.1 ± 3.2a
10-20	40.5 ± 5.6a	33.2 ± 2.9a	40.8 ± 5.1a	44.1 ± 4,3a

SOC: soil organic carbon

3.3. Carbon and nitrogen storage in mineral soil

The C and N concentrations in soils decreased with increasing depth in all species, showing values below 41 g kg^{-1} for C and 3.5 g kg^{-1} for N. Overall, PP and CS showed the lowest and highest values, respectively (**Table 5**). In the soil surface layer (0-10 cm) C concentration ranged from 40.8 g kg^{-1} (PN) to 21.0 g kg^{-1} (PP) and N concentration ranged from 3.4 g kg^{-1} (CS) to 1.5 g kg^{-1} (PP), in both cases revealing significant differences. Soil coarse elements content and bulk density do not differ between species, showing PP the highest values for both variables (**Table 5**).

The 0-20 cm soil depth contained a total of 64.6, 58.3, 52.1 and 30.3 Mg ha^{-1} of C in the PN, CS, PM and PP, respectively (**Figure 4**). The N stocks followed the sequence CS (5.1 Mg ha^{-1}) > PM (4.6 Mg ha^{-1}) > PN (4.2 Mg ha^{-1}) > PP (2.2 Mg ha^{-1}) (**Figure 5**). The distribution of C and N in the two soil layers (0-10 and 10-20 cm) is quite similar, presenting a slightly higher amount in the uppermost soil layer (slightly above 50%) (**Figures 4 and 5**). Soil C:N ratio range from 11:1 (CS, PM) to 17:1 (PN). As expected, the C:N ratio drops considerably from the L and F layers to the H layer and the mineral soil layers (**Figure 6**).

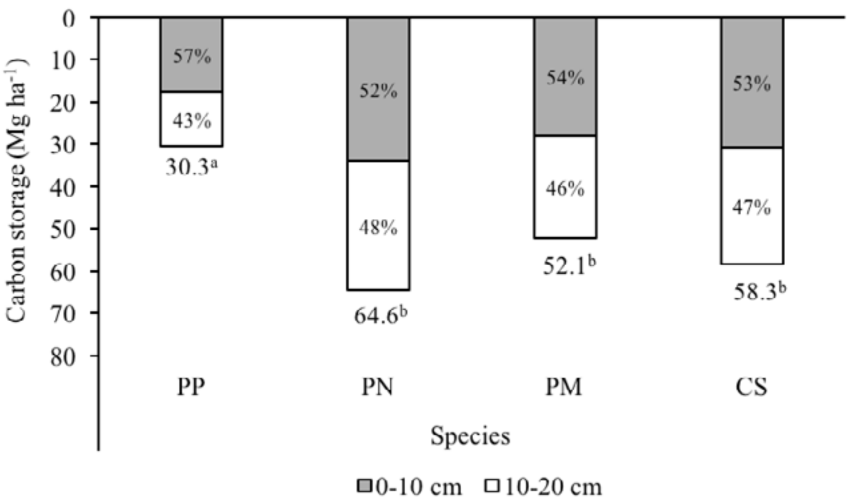

Figure 4. Total soil C storage from species PP (*Pinus pinaster*), PN (*Pinus nigra*), PM (*Pseudotsuga menziesii*) and CS (*Castanea sativa*). Values followed by different letters are statistically different ($p < 0.05$).

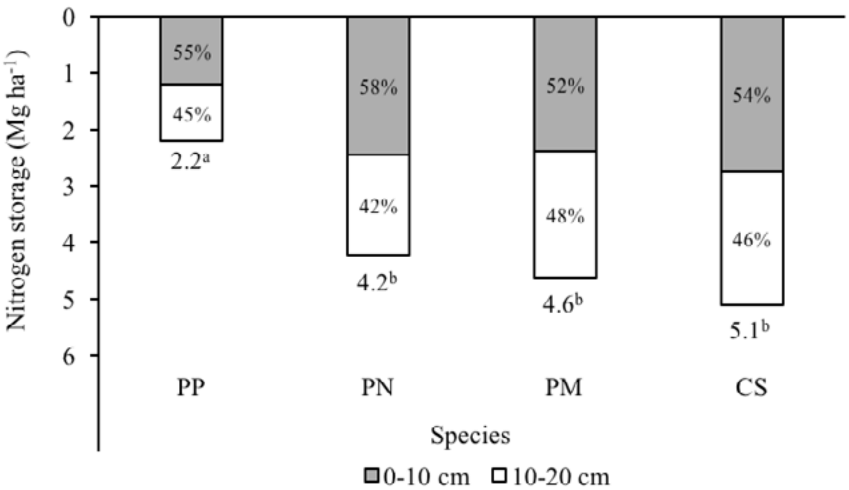

Figure 5. Total soil N storage from species PP (*Pinus pinaster*), PN (*Pinus nigra*), PM (*Pseudotsuga menziesii*) and CS (*Castanea sativa*). Values followed by different letters are statistically different (p < 0.05).

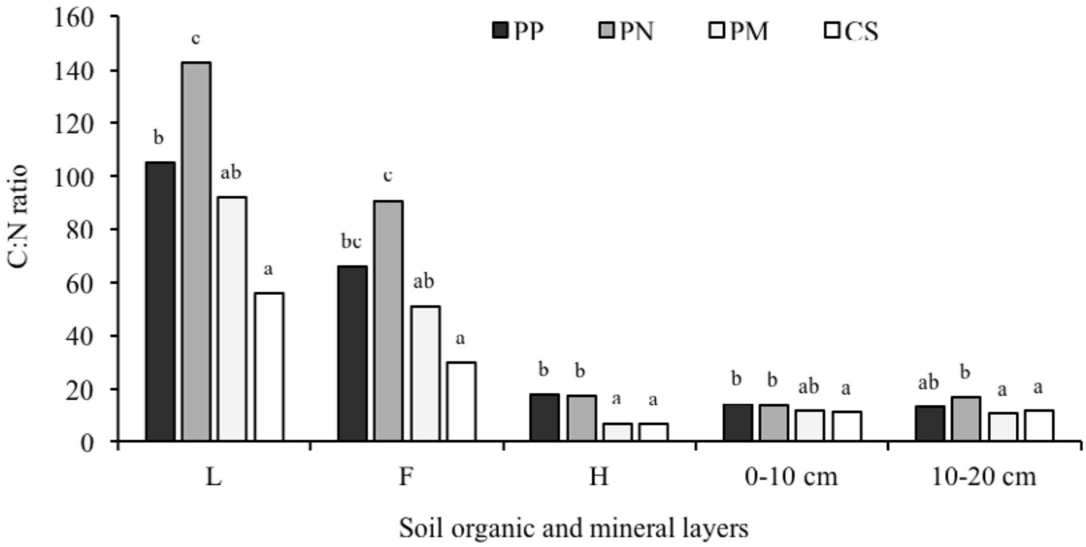

Figure 6. The C:N ratio of L, F and H forest floor layers and soil mineral layers (0-10 and 10-20 cm) from species PP (*Pinus pinaster*), PN (*Pinus nigra*), PM (*Pseudotsuga menziesii*) and CS (*Castanea sativa*). For each layer, values followed by different letters are statistically different ($p < 0.05$).

3.4. Total carbon and nitrogen storage in forest floor and mineral soil

After 60 years the effect of forest species and management practices on C and N storage was visible both in the forest floor and the mineral soil (**Figure 7**). Total C storage was significantly higher in PN (92.1 Mg ha^{-1}) and significantly lower in PP (40.0 Mg ha^{-1}), the two remainder species showing intermediate values, practically identical (64.4 Mg ha^{-1} for PM and 63.9 Mg ha^{-1} for CS). Overall, the forest floor in CS contained less C and N than forest floor in conifer stands (PP, PN and PM), but the mineral soil of the deciduous tree species (CS) recorded amounts of N higher than in the three evergreen species and C stocks very similar to PN, the forest species with the highest amount of C in the mineral soil. The soil was the largest C and N pool representing percent values that ranged from 70 (PN) to 91 (CS) for C and from 88 (PP and PN) to 94 (CS) for N (**Figure 7**).

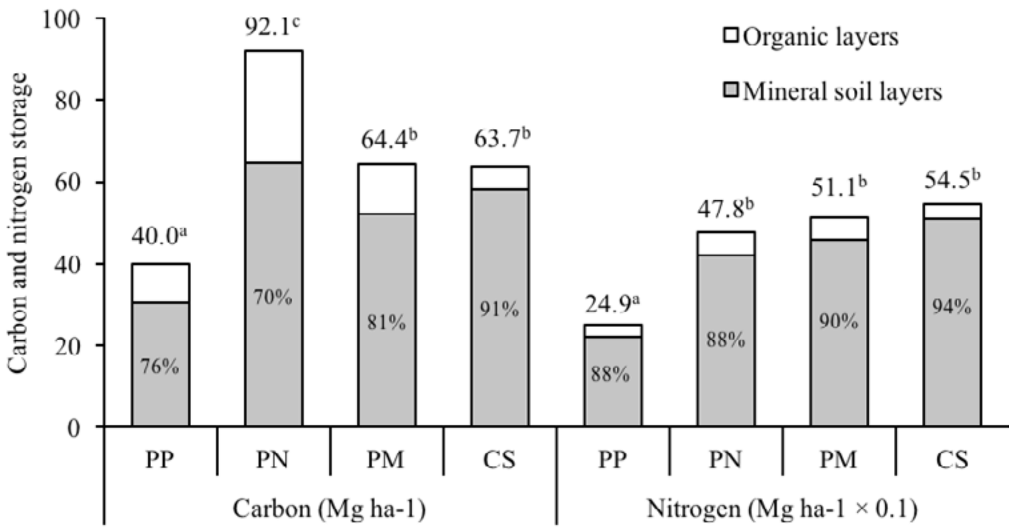

Figure 7. Total C and N stored in soil organic and mineral layers from species PP (*Pinus pinaster*), PN (*Pinus nigra*), PM (*Pseudotsuga menziesii*) and CS (*Castanea sativa*). For C or N, values followed by different letters are statistically different ($p < 0.05$).

4. Discussion

The overall forest floor mass in conifer stands (PP, PN and PM) was higher in all organic layers (L, F and H) than in broadleaf stands (CS). Under the coniferous species there was a huge quantity of organic materials poorly decomposed, while under broadleaved species (CS) there was the biggest transformation of forest floor and incorporation in mineral soil, because litter chemistry was a determinant factor controlling the decomposition process (Hobbie 1996; Shaw and Harte 2001; Mueller et al. 2012; Guendehou et al. 2014; Kooch et al. 2017). These observations were in accordance of decomposition litter study conducted in these stands, which recorded annual losses of 15.3, 18.3, 29.7 and 41.7% for PN, PP, PM and CS, respectively (unpublished data). Identical results were reported by other authors (Rapp 1984; Schulp et al. 2008; Fonseca and Figueiredo 2010; Bargali et al. 2015). The lowest value found beneath the CS stand could be explained by the highest decomposition rate associated with a lower C:N ratio of the forest floor layers (Thomsen et al. 2008; Trum et al. 2011; Guendehou et al. 2014; Cools et al. 2014). Secondly, this stand was clearcut after 40 years, which had important consequences at soil level, namely alteration in the quantity and quality of biomass production, modification of the spontaneous vegetation and of microbial community composition (Merzouki et al. 1989; Prescott and Grayston 2013; Bargali et al. 2015). In addition, the export of plant material modifies the microclimatic and pedoclimatic conditions and the gains by the soil of organic material, resulting in a greater mineralization of the organic residues deposited on the soil surface. The clearcut was followed by a coppice regime, which implied the export of young plant material, rich in mineral components, and might result in the reduction of soil fertility in long term. The low forest floor mass of the PP species is explained by the litter transferred (leaves and pine cones) for domestic fuel and by the lower density of canopy per hectare. The values of PN and PM are mainly explained by size of leaves, being the main difference between these two species in pine cones production (very high for the PN) and in the decomposition rate (highest for PM).

Forest floor quality differs among species, with the highest N concentration and lowest organic C under CS species. The C concentration and C:N ratio decreased from the L to the H layer and the N concentration reached its maximum in H-Layer, which is in agreement with other authors (Trum et al. 2011; Cools et al. 2014). The decrease of C concentration is associated with the increase of the humification degree (Wardle 1993), with the mineral matter addition by biological activity and with the high porosity of the organic layers that allow fast exchanges with the atmosphere (Fernández et al. 1993). This reduction was more evident for leaves, with a higher decomposition rate and lower C:N ratio (Yamashita et al. 2006). Forest floor C:N ratio under CS was the lowest and comparable with PM. Cremer et al. (2016) reported similar results when compared *Pseudotsuga menziesii* and *Fagus sylvatica* species. According to Kaspari et al. (2008), the decomposition process is affected by the initial N and P concentrations and the C:N ratio of litter. The C:N ratio decreased with decomposition, that is, from L-layer to H-layer (Cools et al. 2014). The evolution of the C concentration in the organic layers was similar between coniferous and broadleaved species, which is in agreement with other studies (e.g. Fernández et al. 1993; Trum et al. 2011). The increase in N concentration during decomposition (from L to H layers) is a generally occurring phenomenon (Polglase et al. 1992; Shaw and Harte 2001; Trum et al. 2011; Bargali et al. 2015). Morphological and chemical characteristics of the organic residues may explain the highest N concentrations observed in CS (Fyles et al. 1991). Nitrogen deficiencies are frequent in coniferous stands where this element is immobilized in the organic layers accumulated on the surface soil. In these stands, residue accumulation is favored (Mahendrappa et al. 1986), due to litter quality produced, which in turn affects the decomposition rate and chemical elements release to the soil (Martins 2009). In summary, C and N concentrations in the forest floor layers were related to the nature of the forest species that affect the decomposition rate and the turnover of these elements in the soil (Martins et al. 2009; Fonseca et al. 2012; Kooch et al. 2017).

The C and N storage differs in forest floor and top mineral soil (0-20 cm) under different tree species (e.g. Alban 1982; Reich et al. 2005; Díaz-Pinés et al. 2011; Trum et al. 2011; Kooch et al. 2017). The C and N stocks in the forest floor highlight the effect of the human disturbances that occurred in the PP and CS stands and the combined effects of the quantity and quality biomass produced by different species (Alban 1982; Thomsen et al. 2008; Fonseca and Figueiredo 2012). This may have contributed to the lower values found for these species (PP and CS). In forest floor, C stocks were higher under conifer species (PN, 27.3 Mg ha^{-1} > PM, 12.3 Mg ha^{-1}; PP, 9.8 Mg ha^{-1} > CS, 5.5 Mg ha^{-1}) and N stocks showed a slightly different behavior (PN, 5.8 Mg ha^{-1}; PM, 5.1 Mg ha^{-1} > CS, 3.5 Mg ha^{-1}; PP, 2.9 Mg ha^{-1}). Overall, N storage was more representative in the F-layer, C storage was quite similar in the L and F layers, especially in PN and PM species. Herrero et al. (2016) reported similar results for C stocks in forest floor, with higher values in *Pinus* spp. (13.6 Mg ha^{-1}) than in *Quercus pyrenaica* (5.4 Mg ha^{-1}). Average soil C stocks (0-20 cm) were lower under PP (30.3 Mg ha^{-1}) than PN (64.6 Mg ha^{-1}1), PM (52.1 Mg ha^{-1}) and CS (58.3 Mg ha^{-1}), with significant differences between the first and the former species. Trends in soil N stocks were comparable to those referred to C stocks. Soil C stocks under PP stand were lower than those found by Nunes et al. (2010) until 30 cm of depth in soils developed beneath the same species in NW Portugal, where the values varied from 90.2 to 123.9 Mg ha^{-1}. As stressed before, the human disturbances in the PP stand considerably affected the contents of C and N both in the forest floor and mineral soil. Several studies that focused on the species effects on mineral soil C pool found no significant influence; nevertheless occasionally there was a tendency of more C in soils developed under some broadleaved species when compared with conifers (Oostra et al. 2006; Vesterdal et al. 2008, 2013). A similar trend was also observed for CS (broadleaved species) in this study. Usually, the species with low C stocks on the forest floor tend to have more C in the mineral soil (Vesterdal et al. 2013), however only CS species showed this trend. The C:N ratio in soil depths 0-10 and 10-20 cm was variable beneath the four species (PP, PN, PM and CS), which is seemingly associated with litter quality (Neirynck et al. 2000; Cools et al. 2014), besides other factors influencing the soil C:N ratio such as management practices (Zhang et al. 2016), soil properties (Yamashita et al. 2006; Cools et al. 2014), forest species (e.g. Fonseca et al. 2012; Vesterdal et al. 2013; Cools et al. 2014; Zhang et al. 2016) and climate (Fonseca and Figueiredo 2012; Marty et al. 2017). The C:N ratio reflects the stability of soil organic matter and relative decomposition stage (Thomsen et al. 2008). Accordingly, the soil C and N stability was higher in PN and PP stands (higher values of C:N ratio) and smaller in CS and PM stands (lower values of C:N ratio).

Total C and N stored in soil horizons (forest floor + mineral soil) were affected by species (e. g. Reich et al. 2005; Díaz-Pinés et al. 2011; Fonseca et al. 2012; Gurmesa et al. 2013). The PN stand presented the largest amount of C (92.1 Mg ha^{-1}) and CS stand of N (54.5 Mg ha^{-1}). The mineral soil top layer was the major C and N pool in all species, representing above 70% for C and exceeding 88% for N. Despite its minor contribution to C and N stocks, the forest floor is an important component of the C and N biogeochemical cycles (Ordóñez et al. 2008), as it is the boundary between vegetation and soil.

5. Conclusions

Sixty years after installation of the stands, C and N concentrations and stocks in both forest floor and mineral soil are visibly different in areas covered with different forest species, under similar topographic, lithologic and climatic conditions. The H-layer showed the highest concentrations in N and the lowest in C, leaves in the L and F layers being the component with higher concentrations in both cases. On the other hand, the F-layer provides the larger contribution for C and N storage and N, and, as also found in the H layer, leaves were the main pool of these elements. In general, the conifer species (PP, PN and PM) showed higher C and N stocks in the forest floor layers compared to the broadleaved species (CS). Stocks of C and N in the topsoil (0-20 cm) were lower in PP species than in other species (PN, PM and CS, all with similar values).

Total C storage (forest floor + mineral soil till 20 cm depth) was higher in PN, intermediate in PM and CS and lower in PP. In contrast, N storage was similar in CS, PM, PN, and significantly lower in PP. Mineral soil had a major contribution to C and N storage, representing more than 70% and 88% of the totals stored down to 20 cm soil depth, respectively. Scientifically grounded improvements on current knowledge about forest species effects in C and N storage can be very important for decision-making on species selection for stand installation; in view of ecosystem services provision by forest areas.

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