

1 **Organic matter properties in soils afforested with**
2 ***Pinus radiata***

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20 **Abstract**

21 Aims. Afforestation causes important alterations in SOM content and composition that affect
22 the soil functions and C balance. The aim of this study was to identify the mechanisms that
23 determine the changes in SOM composition following afforestation of grasslands.

24 Methods. The study included 4 chronosequences and 5 paired plots comprising pastures and
25 land afforested with *Pinus radiata*. The SOM was characterized by ¹³C CP-MAS NMR
26 spectroscopy and differential scanning calorimetry.

27 Results. During the first 10-20 yrs after afforestation, the changes in SOM content varied
28 from slight gains to large losses (> 40 %). The analyses revealed that even SOM compounds
29 considered resistant to decomposition were degraded during this time. The SOM gains,
30 observed 20 yrs after stand establishment, were favoured by the higher recalcitrance of pine
31 litter and possibly by soil acidification. The concentrations of most SOM compounds,
32 particularly the stable compounds, were higher at the end of the rotation. The low degree of
33 protection, along with the favourable climatic conditions, may also explain the rapid
34 decomposition of SOM, including resistant compounds, in these soils. DSC analysis
35 complemented the information about SOM composition provided by other techniques.

36 Conclusions. The accumulation of stable SOM compounds at the end of the rotation suggests
37 a longer soil C turnover in these afforested soils, which may alleviate the gradual loss of SOC
38 in intensively managed forest soils.

39 *Key words: SOM, afforestation, pastures, ¹³C CP-MAS NMR, DSC, soil carbon sequestration*

40 **Introduction**

41 In Europe, North America and Russia, large areas of marginal arable and pasture land
42 are being transformed to forests, which may contribute to enhancing the C sink capacity of
43 terrestrial ecosystems and improving soil and water conservation. However, although
44 afforestation contributes to C sequestration in biomass, the amount of which is determined by
45 tree species and silvicultural management, the effects on soil organic matter (SOM) are not
46 necessarily positive. Thus, a great variety of soil responses have been observed, ranging from
47 SOM gains, mainly in arable land, to losses of SOM, mainly in pastureland (Marín-Spiotta et
48 al. 2009, Poeplau et al. 2011).

49 In humid temperate regions, afforestation of SOM-rich pasture soils usually leads to
50 large temporal decreases in SOC during the first years of the rotation, followed by subsequent
51 recovery. The initial SOM losses recorded after afforestation are attributed to reduced inputs
52 of labile organic compounds to the mineral soil, which creates an imbalance between C inputs
53 and C respired (e.g. Huang et al. 2011). However, despite this general pattern, such changes
54 in soil C stocks often display high levels of spatial variability, even in areas that appear
55 homogeneous with respect to bedrock or climate (Poeplau et al. 2011; Pérez-Cruzado et al.
56 2012a). This makes it difficult to predict changes in SOM at a regional level from differences
57 observed in a single or a few chronosequences. Some of the variability is due to the influence
58 of tree species and silvicultural treatments (Jandl et al. 2007). The different quality of the new
59 vegetation as plant litter is a major factor determining the SOC evolution after land
60 abandonment (Montané et al., 2010). Certain soil properties that determine SOM turnover,
61 such as initial SOM content and soil pH, may also be major factors in determining the
62 changes in SOM in response to afforestation. These soil properties are not usually considered
63 in models simulating SOM dynamics, which may explain the large discrepancies between
64 theoretical estimated amounts of SOM and the amounts measured in the field (Foereid et al.
65 2007; Pérez-Cruzado et al. 2012b). These aspects are crucial in highly managed forests. In
66 temperate and tropical regions, afforestation is usually carried out by intensive management
67 of fast growing tree species, which involves short rotations, clear cutting and collection of
68 logging residues. One of the main concerns regarding this type of afforestation is that the
69 initial SOM losses may not be totally compensated for throughout the stand rotation, which
70 would imply that the soil has a lower C sequestration potential (Wiesmeier et al. 2009; Nave
71 et al. 2010; Pérez-Cruzado et al. 2012a, b).

72 The effects of afforestation on SOM should not only be considered in terms of total C
73 stocks, but should also take into account changes in SOC structure, stability and functions
74 (Solomon et al. 2007), with the subsequent effects on soil and water conservation and
75 composition of the soil microbial community. Identification of the SOM fractions with
76 different residence times is of great interest for predicting the effects on soil properties and the
77 C balance. Although SOM composition and C balance are closely related, little is known
78 about how SOM composition affects changes in C following afforestation. Some studies have
79 been carried out in tropical (e.g. Marin-Spiotta et al. 2009; Wiesmeier et al. 2009) and boreal
80 (e.g. Cerli et al. 2008) climates. However, no studies have been carried out in humid
81 temperate regions, where large areas are affected by this type of land use change.

82 In comparison with forest vegetation, pasture soils usually contain a high proportion of
83 easily decomposable O-alkyl and lower amounts of aromatic C due to the high inputs of
84 carbohydrate-rich residues from turnover of grass litter (Mendham et al. 2002). Therefore, the
85 rapid loss of SOM after afforestation is probably due to the degradation of such compounds
86 along with the lack of inputs from grass litter (Huang et al. 2011; Pérez-Cruzado et al. 2012a).
87 Although some authors have suggested that this is a temporary effect, in which only the labile
88 C pool is exhausted (Cerli et al., 2008, Huang et al., 2011), recent findings indicate that
89 degradation of so-called recalcitrant SOM may also occur in the uppermost soil layers, where
90 the different mechanisms of SOM stabilization (spatial inaccessibility to decomposer
91 organisms and interactions with mineral surfaces and metal ions) appear to be less efficient
92 (von Lützow et al., 2006). The capacity of soils to stabilize SOM through physical protection
93 (by interaction with fine silt and clay) is limited, and it may reach its maximum as SOM
94 accumulates (Six et al. 2002). Therefore, in OM-rich soils, the mineral fraction of the soil
95 becomes saturated and additional C input will only accumulate in labile soil C pools with a
96 relatively rapid turnover rate (Gulde et al. 2008).

97 Forest litter contains large amounts of compounds that are resistant to decomposition,
98 mainly lipids (such as waxes, cutin and suberin) and terpenoids (Helfrich et al. 2006).
99 Following afforestation, the SOM may be enriched in chemically recalcitrant compounds,
100 resulting in a longer soil C turnover time (Marin-Spiotta et al. 2009). Furthermore,
101 acidification of the soil throughout the stand rotation (Berthrong et al. 2009) may also
102 contribute to SOM stabilization, although this has not yet been investigated. These aspects are
103 particularly important in forest plantations, in which the longer soil C turnover may mitigate
104 large SOM losses (Pérez-Cruzado et al. 2012b).

105 The identification of SOM fractions with different residence times is of great interest
106 for predicting how easily the SOC is mineralized in response to management and other types
107 of disturbance (e.g. von Lützow et al. 2006; Kleber et al., 2011). However, despite the interest
108 in characterizing the complex nature of SOM, there is still a high demand for quantitative
109 methods. Understanding and monitoring SOM dynamics requires simple, accurate and
110 reproducible techniques that can be applied to large numbers of samples (Solomon et al.
111 2007).

112 Thermal analysis techniques, such as differential scanning calorimetry (DSC) and
113 thermogravimetry (TG), are used to characterize the stability of organic compounds in soils,
114 plant tissues, compost and black carbon (e.g. Barros et al., 2011; Fernández et al. 2011).

115 These techniques are useful for obtaining information about the biogeochemical stability of
116 SOM. DSC enables calculation of the energy of SOM, which is one of the main factors
117 determining SOM degradability, and also quantification of SOM thermal stability, which is
118 associated with lower rates of SOM degradation (Rovira et al., 2008; Plante et al. 2011).
119 Enhancement of the energy of the substrate and SOM thermal stability are considered as
120 indicators of SOM stabilization. Thermal analysis offers advantages over other methods of
121 estimating SOM stability (incubation, extraction) as it is non-invasive, accurate, reproducible
122 and inexpensive, and it can therefore be used in studies involving large numbers of samples.

123 The main aim of this study was to obtain information about the mechanisms leading to
124 changes in SOM structure and stability following the afforestation of pasture soils with fast
125 growing trees species under humid temperate conditions. In previous studies in the same
126 region, we have described the SOM dynamics throughout the first rotation after afforestation
127 (Pérez-Cruzado et al. 2012a) and have developed models to describe the effect of forest
128 management in the long term (Pérez-Cruzado et al. 2012b). However, the influence of
129 afforestation on SOM structure and stability has not yet been investigated. To address this
130 question, the present study considers several examples of forest plantations located in
131 homogeneous conditions with respect to soil, climate and forest management. A set of
132 chronosequences and paired plots (pasture-mature plantation), in which different types of
133 changes in SOM occur in response to afforestation, were studied. The relationships between
134 the composition and stability of the SOM in these soils were analyzed by ^{13}C CP-MAS NMR
135 and DSC.

136 **Material and methods**

137 **Study site**

138 The study was carried out in Castro de Rey (Lugo, NW Spain, Fig.1a). The 20 year
139 annual average rainfall of the area is 900 mm, and the average temperature, 13.5 °C. The
140 wettest month is November, with an average rainfall of 139 mm, and the driest month is
141 August, with 45 mm. The lowest mean monthly temperature, 9.5 °C, occurs in February, and
142 the highest, 19.1 °C, occurs in August. The soil humidity and temperature regimes are Udic
143 (mean period with partial drought, 1 month) and Mesic (mean frost-free period, 10 months),
144 respectively.

145 The soils were developed from schist, and were classified as Alumi-humic Umbrisols
146 and Distric Cambisols (IUSS Working Group WRB 2006). The texture of the soils was loam
147 or sandy loam and the soils were well or moderately well drained.

148 **Stands selected and sampling**

149 To characterize the changes in SOM after afforestation, the following approach
150 including the combined use of chronosequences and paired-plots was used:

151 a) Four chronosequences comprising a pastureland and three-four *Pinus radiata* stands
152 of different ages established on the same land. These were designated as C1-C4.

153 b) Five paired plots, each pair comprising a pasture plot and a mature forest plantation
154 (29-41 yrs) plot of *Pinus radiata* established on the pasture. These were designated as P5-P9.

155 c) A mature *Pinus radiata* plantation (40 yrs old), not previously used for agriculture
156 or pasture, designated as MF (mature forest).

157 The study site was selected on the basis of a previous study involving the quantitative
158 evaluation of carbon in forest plantations established over former pastureland (Pérez-Cruzado
159 et al. 2012a). Measurement of the soil C concentration in 120 pasture plots enabled the spatial
160 variability for this variable to be determined. This information enabled identification of the
161 most homogeneous zone in terms of SOM distribution. Five paired plots studied by Pérez-
162 Cruzado et al. (2012a) in the studied area were re-sampled (P5-P9), and four chronosequences
163 (C1-C4) and a mature forest (MF) were established and sampled.

164 The sites were located no more than 3 km apart (Fig. 1a). In all cases, site preparation
165 prior to forest establishment consisted of ripping. Fertilization, tillage and weed control were
166 not carried out throughout the rotation. To ensure that all sites were similar in relation to soil
167 type and land use, selection of the study sites was based on direct observation of the terrain of
168 adjacent grasslands and consultations with local landowners. Information about prior land use
169 and transformation dates was obtained from reforestation subsidy reports (surplus reduction
170 program of the Common Agricultural Policy, EU).

171 The SOC contents of the 9 initial pastures varied widely (2.1-11.0 %). The three
172 pasture plots in which the SOC was higher than 8 % were managed with regimes based on
173 low intensity tillage. In three of the pasture plots, the SOC was initially lower than 4 %,
174 reflecting a more intensive management based on tillage every three years and intensive
175 rotation for silage. The SOC contents of other three of the pastures ranged from 4 to 8 %.

176 Although most of the soils were moderately acidic, the initial soil pH (in 0-5 cm depth)
177 ranged from 4.3 to 6.0. The texture of most of the soils was loam or sandy loam.

178 **Soil sampling, pH, C and texture of the mineral soils**

179 For soil sampling, a plot was established within each stand, at a distance of more than
180 30 m from the edge of the stand. In each plot, six samples were obtained from between tree
181 rows to minimize any influence from the site preparation in the stand establishment. Sampling
182 points were systematically established in a regular hexagonal frame, the position of which
183 was randomized in the stand (Fig. 1b).

184 For forest floor sampling, square frames (0.3 × 0.3 m) were collected at all six points.
185 Samples of forest floor were collected, avoiding removal of the mineral soil, and dried at 40
186 °C until constant weight. Senescent and decomposed litter were differentiated in this layer.
187 Samples of the mineral soil layer were collected with a steel corer, at depths of 0–5 cm, at the
188 same sampling points as the forest floor samples. These subsamples (senescent litter,
189 decomposed litter and mineral soil, separately) were combined to form six bulk samples per
190 plot for subsequent analysis. The samples were oven-dried at 40°C, homogenised for 1 hour at
191 200 rpm in a shaker and sieved (2 mm), before quantifying the stoniness. In the fine soil
192 fraction, total C and N were analyzed with a LECO Elemental analyzer, and soil particle
193 analysis (of the upper 5 cm) was performed by laser diffractometry, with a Mastersizer 2000
194 diffractometer.

195

196 **Thermal analysis**

197 Dry soil samples were analyzed by differential scanning calorimetry (DSC) (Q200, TA
198 Instruments). The analyses were performed with 10 mg samples of soil placed in open
199 aluminium pans under dry air (flow rate, 2.1 kg cm⁻² time⁻¹) at a scanning rate of 10 °C min⁻¹.
200 The temperature was varied between 50 and 600 °C. Samples of Indium (mp: 156.6 °C) were
201 used to calibrate the calorimeter. All samples were analyzed in triplicate.

202 The heat of combustion (Q, in J per gram) was determined by integrating the DSC
203 curves (in Wg⁻¹) over the exothermic region (150– 600 °C). Data recorded at <150 °C were
204 discarded, thus obviating weight losses and energy changes associated with moisture loss. The
205 Q values were divided by the mass loss in each measured sample in the DSC analysis (Q', in J
206 mg⁻¹ OM; Rovira et al. 2008) The areas under the DSC curves were divided into three groups

207 representing different degrees of resistance to thermal oxidation (Dell'Abate et al. 2002;
208 Fernández et al. 2011): labile organic matter, mainly comprising carbohydrates and other
209 aliphatic compounds (200-375 °C); recalcitrant organic matter, such as lignin or other
210 polyphenols (375-475 °C); and highly recalcitrant organic matter, such as polycondensed
211 aromatic forms (475-550 °C). The resulting partial heats of combustion were designated Q₁,
212 Q₂ and Q₃, respectively. The following were also determined: temperatures of maximum
213 combustion peaks in the DSC curves (T₁ and T₂), the temperature at which 50% of the
214 energy of SOM is released under the given conditions (T₅₀) and the temperature at which the
215 SOM combustion is complete (T_{end}).

216 The DSC curves may vary due to organo-mineral interactions, especially in soils with
217 low OM content (Rovira and Vallejo, 2000). In the present study, all soils were located within
218 a small area, and they were developed from the same bedrock and contained large amounts of
219 SOM. It was therefore assumed that the mineral interactions would be similar and that the
220 comparison of the DSC curves of bulk soil samples would reflect the changes in SOM. In a
221 preliminary study, DSC curves of bulk and demineralized samples were compared in four of
222 these same soils (data not shown). The results showed that both types of DSC curves were
223 useful for characterizing the changes in both thermolabile and resistant SOM fractions after
224 the afforestation

225

226 **Solid state ¹³C CP-MAS NMR**

227 Solid-state ¹³C NMR spectroscopy was applied to litter and forest floor of the mature
228 forest (MF) and mineral soil of two of the chronosequences (C1 and C3), showing different
229 initial SOM content and subsequent changes in SOM. Mineral soil samples were
230 demineralized five times with 10% (w-to-w) hydrofluoric acid (HF) for 12 h to remove
231 paramagnetic minerals, which cause widely ranging resonances and signal loss.

232 Solid NMR experiments were performed in an Agilent (Varian) VNMR5-500-WB
233 spectrometer (operated at a proton resonance frequency of 500 MHz and using a zirconia
234 rotor of volume 160µL). Carbon chemical shifts were referenced to the carbon methylene
235 signal of solid adamantane at 28.92 ppm. Cross Polarization Magic Angle Spinning (1D
236 CPMAS) analysis of the samples was carried out under the following conditions: contact time
237 1 ms, inter-scan delay 1 s (a proton T₁ experiment was performed to check the suitability of
238 this time for both litter and mineral soil samples), and MAS rate 12 kHz. The number of scans

239 was ca. 10000 - 35000. The cross polarization time was set to 1 ms. The NMR spectra
240 obtained were processed and the area of the signals was quantified using MestreNova
241 software 8.1.0 (Mestrelab Research Inc). Analysis including pure organic compounds (lignin)
242 revealed that the occurrence of spinning side bands was very low. Such bands only appeared
243 with carbonylic groups and usually accounted for at most ca. 1-2 % of the integral of the
244 central peak. For integration, the spectra were divided into four regions representing different
245 chemical environments of a ^{13}C nucleus: alkyl C (0–45 ppm), O-alkyl C (45–110 ppm),
246 aromatic C (110–160 ppm), and carbonyl C (160–210 ppm). For integration, the spectra were
247 divided into four regions representing different chemical environments of a ^{13}C nucleus: alkyl
248 C (0–45 ppm), O-alkyl C (45–110 ppm), aromatic C (110–160 ppm), and carbonyl C (160–
249 210 ppm).

250 **Statistical analysis**

251 Despite the spatial variability in the SOC, the sampling procedure used in this study
252 ensures plot-level representativeness for the expected small scale SOC spatial variability
253 (Simón et al. 2012). The representativeness of the single combined plot-level bulk samples in
254 describing the pastures afforested with *P. radiata* plantations is guaranteed because of the
255 high replication and systematic establishment of samples at plot level, as well as the
256 homogenization process, which involves crushing the soil aggregates.

257 The significance of the changes in the studied parameters in afforested stands with age
258 since afforestation was evaluated by regression analysis, by using the lm function of the R
259 statistical package (R Core Team 2013). The null hypothesis $H_0: \beta_1=0$ was evaluated against
260 the alternative hypothesis $H_1: \beta_1 \neq 0$ in the regression $y=\beta_0+\beta_1x+e$, by using the statistic shown
261 in Eq. (1), which follows a t Student distribution with $n-2$ degrees of freedom.

$$\frac{\hat{\beta}_1 - \beta_1}{\sqrt{\frac{\sum_{i=1}^n (y_i - \hat{y}_i)^2}{(n-2)\sigma^2}}} \quad (1)$$

262
263 Differences between the pasture and afforested stands were evaluated by the
264 Wilcoxon-Man-Whitney nonparametric test, by using the wilcox.test function of the R
265 statistical package (R Core Team 2013). This test evaluates whether the median values of
266 both populations under study can be considered equal ($H_0: M_X=M_Y$; $H_0: M_X \neq M_Y$), by the
267 comparison statistic U , which is the sum of the negative ($D_{ij}=1; Y_j < X_i$) or positive differences

268 ($D_{ij}=0; Y_j \geq X_i$) of the values in Y with respect to the values in the X population, as shown in Eq.
269 (2). No normal distribution approximation because large samples was considered. Three
270 groups were considered in the pairwise comparisons: pasture, young and adult afforested
271 stands. The age threshold considered to distinguish young and adult afforested stands was 15
272 years, which was the age at which changes in soil parameters were detected in the previous
273 study (Pérez-Cruzado et al. 2012a).

$$U = \sum_{i=1}^m \sum_{j=1}^n D_{ij} \quad (2)$$

274

275 **Results**

276 **Litter layer development**

277 The changes in the litter layer throughout the rotation in the four chronosequences
278 studied (C1-C4) are shown in Fig. 2a. The amounts of litter C accumulated at the end of the
279 rotation in the four chronosequences (C1-C4) and in the 5 paired plots (P5-P9) are also shown
280 (Fig. 2a'). Significant accumulation of forest floor only could be distinguished after 8-10 yrs.
281 Except in chronosequence 4 (C4), forest floor accumulation appeared to stabilize 25 yrs after
282 plantation establishment. However, at the end of the rotation, there were large differences
283 between the plantations in the amount of forest floor accumulated, probably reflecting the
284 different tree growth rates. Most of the mature plantations showed accumulation between 40
285 and 50 Mg ha⁻¹.

286 **pH, SOC and C/N in the mineral soils**

287 The pH values for most of the pastures were rather low (< 5.0), reflecting the low
288 intensity of management (Fig. 2b). All afforested soils became gradually more acidic
289 throughout the rotation ($p=0.023$). Between 15 and 20 yrs after stand establishment, the soil
290 pH values in most of the plantations were lower than 4.0 and close to that in the semi-natural
291 forest (MF: pH 3.2), except in chronosequence C4, in which the soil pH was always slightly
292 higher.

293 The SOC contents of the pasture soils varied widely, from 2.1 to 11 % (Fig. 2c).
294 Carbon density in the organic layer increased significantly ($p<0.0001$) with age since
295 afforestation, up to values of 50 Mg ha⁻¹ at age 40 years. However, these different SOC

296 contents determined the subsequent changes in SOC throughout the rotation in the 4
297 chronosequences studied (Fig. 2c). In the chronosequences with initially low SOC contents
298 (C1 and C2), slight gradual increases in SOC were detected throughout the rotation. In
299 contrast, large losses of SOC occurred during 15-25 yrs after afforestation in the
300 chronosequences in which the initial SOC contents were higher (C3 and C4). In all of these
301 chronosequences, significant increases were observed in the last 10-20 yrs of the rotation. At
302 the end of the rotation (Fig. 2c'), the SOC in some afforested soils was higher than in the
303 pasture soils. However, in others, the SOC did not return to initial levels.

304 The C/N ratios were clearly lower in all of the pasture soils than in the semi-natural
305 forest soil (MF; Fig. 2d'). At the end of the rotation, the C/N values were significantly ($p=$
306 0.0012) higher (around 15) than in the adjacent pasture (average 11), although the final value
307 was never as high as in MF. Monitoring of this ratio in the four chronosequences (Fig. 2d)
308 revealed that these increases did not occur gradually throughout the rotation and only
309 occurred at the end of the rotation, coinciding with the gain in the SOC.

310 **OM in the organic horizon of the afforested soils**

311 The decomposition pattern of the new organic horizon and its influence on the SOM
312 were investigated by solid state ^{13}C NMR analysis of the senescent pine needles and the
313 humified litter layer (3-4 cm thick) of the forest floor. These data are useful for interpreting
314 the changes in OM composition in the mineral soils after afforestation. The resulting spectra
315 are shown in Fig. 3, and the relative content of the major C-types, calculated by integrating
316 the standard chemical shift ranges, are shown in Table 1.

317 The most prominent signal in the spectra of the senescent litter samples corresponded
318 to O-alkyl structures, mainly cellulose and hemicellulose (73 ppm and 105 ppm). The most
319 intense aromatic C signals occurred at 154 ppm, which corresponded to lignin. A prominent
320 lignin signal was also identified at 56 ppm (Kögel-Knabner, 2002). The alkyl region was
321 dominated by a peak at 30 ppm, attributed to polymethylene C (lipids, cutin). The peak at 145
322 ppm is attributed to tannins (Preston et al. 1997).

323 Comparison between senescent and humified litter revealed loss of alkyl-C
324 compounds, and to a lesser extent of O-Alkyl and aromatic compounds. The spectra of the
325 humified litter revealed a decrease at ca. 30 ppm (polyethylene C in lipids and lipid polymers)
326 and an increase at ca. 145 (tannins) and 115 ppm (lignin). The aromaticity and the ratio O-

327 alkyl C/aromatic C increased slightly during the humification process. On the contrary, the
328 ratio of alkyl -C /O-alkyl (aliphaticity level) increased (Table 1).

329 **Changes in OM in the mineral soil throughout the rotation: ¹³C CP-MAS NMR**

330 ¹³C CP-MAS NMR analysis was carried out to investigate the influence of
331 afforestation on the composition of the OM in the mineral soil. This technique was applied to
332 the two chronosequences with different initial SOM content in which different changes in
333 SOM occurred after afforestation (C1 and C3). It was also used in the mineral soil in the
334 mature (semi-natural) forest (MF) plots (Fig. 4 and Table 1).

335 In both pasture soils, the analyses revealed that the major C components were O-alkyl
336 C (49-52 % of total intensity, carbohydrates) and alkyl C (19 and 24 % of total intensity). In
337 both cases, a prominent signal, generated by cellulose and hemicelluloses, occurred at 73
338 ppm. Aromatic C was more abundant in the pasture of C1 than in C3 (23 % and 19 % of total
339 intensity, respectively), and carboxyl C represented about 5-10 % of the total intensity in both
340 soils. The aromaticity of pasture C1 was higher than that of C3, suggesting a higher degree of
341 humification in sample C1.

342 The MF soil contained fewer O-alkyl-C compounds than the pasture soils, whereas the
343 percentage of aromatic C was slightly higher. The spectrum of this soil includes a more
344 intense signal at 30 ppm, typical of methylene C from lipids and aliphatic biopolymers, such
345 as suberin and cutin, and intense signals at 128 ppm, assigned to lignin. The alkyl /O-alkyl
346 ratio and the aromaticity are also higher in this soil than in the pasture soils. These properties
347 suggest a more advanced stage of humification of the OM in this forest soil relative to that in
348 the pasture soils.

349 In both chronosequences, analysis of samples of the afforested soils of both
350 chronosequences revealed a gradual increase in the intensity of the signal at 30 ppm. In C3,
351 increases in the intensity of the lignin signal also occurred, as revealed by the more intense
352 signals at 126 ppm. To evaluate the extent of the changes in SOM, different ratios of various
353 organic C functionalities were considered in examining these spectra. The degree of
354 aliphaticity (alkyl C to O-alkyl C) increased in both chronosequences, whereas the
355 aromaticity only increased in C3.

356 To study the changes in the different types of C throughout the rotation, the relative
357 intensities of the chemical shift regions in the spectrum were further normalized to the
358 organic-C content of the samples (Fig. 5). In C1, gradual increases in alkyl C and O-alkyl

359 contents occurred, whereas the increase in aromatic C was less important. In C3, large losses
360 of O-alkyl C, carbonyl C and alkyl C groups occurred (more than 50%) in the first 10-15 yrs
361 after afforestation. In the final years of the rotation (> 30 yrs), partial C gains were observed
362 in all organic compounds, reaching to values close to the MF soil.

363

364 **Changes in OM in the mineral soil throughout the rotation: DSC and thermal** 365 **properties**

366 The DSC curves for mineral soils from the four chronosequences (C1-C4) are shown in Fig.
367 6. They are shown in order of increasing SOC in pasture plots. In most soils, the DSC curves
368 showed a first combustion peak at around 300 °C and a shoulder at approximately 400 °C. The
369 soil from the 40 year-old stand in chronosequence C4 yielded two well defined exothermic
370 peaks at 325 °C and 425 °C. This data suggest an abundance of labile organic compounds and,
371 to a lesser extent, of recalcitrant compounds. Highly recalcitrant organic matter, which would
372 be found in the range 475-550 °C, was less abundant.

373 The four chronosequences displayed very different patterns of changes in the DSC
374 curves throughout the stand rotation (Fig. 6). In chronosequence C1, with the lowest initial
375 SOC content, the combustion heat (Q, the integration of the DSC curve) increased slightly
376 throughout the rotation, and corresponded with the increase in SOC. In this chronosequence,
377 the shapes of the exothermic curves did not change substantially, indicating that the Q gain in
378 the soil affected all thermal compounds in the same way. The partial heats of combustion (Q₁,
379 Q₂ and Q₃) changed slightly throughout the rotation, and the values always remained higher
380 than those corresponding to the pasture soil.

381 In chronosequences C3 and C4, in which the initial SOC contents in the pastures were
382 highest, Q values decreased greatly during the first years of the rotation, in accordance with
383 the large losses of SOC observed in both of these plots. In both chronosequences, Q₁ (Q < 375
384 °C) and Q₂ (375°C > Q < 475 °C) first decreased and subsequently increased at the end of the
385 rotation. The value of Q₃ (Q > 475 °C), which was much lower than the other values,
386 increased slightly at the end of the rotation. The changes in the DSC curves and partial heats
387 of combustion in chronosequence C2 were intermediate between the previous two patterns.

388 The changes in the selected thermal indices in the four chronosequences studied are
389 shown in Fig. 7. The Q/SOM ratio represents the energy available per unit of organic matter.
390 Calculation of this index can be influenced by the mineral matrix in soils with low C content.
391 However, since in the present study the soils are developed on the same parent material, the

392 changes in Q/SOM may be attributed to changes in the nature of the SOM. The Q/SOM
393 values correlated with C/N ($r=0.79$; $p < 0.01$) and followed a similar trend, decreasing during
394 the first years and increasing in the final years of the rotations. T_{50} (the temperature at which
395 50% of the energy of SOM is released under the given conditions) and T_{end} (the temperature
396 at which the SOM combustion is complete) increased slightly throughout the rotation. These
397 trends may reflect a gradual gain of more thermally stable fractions during the final years of
398 the stand rotation (Q_2+Q_3 , Fig. 7d). However, again no common pattern was distinguished in
399 the soils.

400 Selected parameters of DSC analysis, comparing the soils of the pasture and the oldest
401 stand in the 4 chronosequences and the 5 paired plots, are shown in Fig. 8. These results
402 provide information about the properties of the OM in afforested soils at the end of the first
403 rotation. In most cases, the heat of combustion (Q) varied in a similar way to the SOC
404 throughout the rotation (Fig. 2a), and in most cases it was higher in the forest soil. Increases
405 in both labile (Q_1 , $Q < 375^\circ\text{C}$) and recalcitrant (Q_2+Q_3 , $Q > 375^\circ\text{C}$) compounds were detected
406 in the soils. Certain thermal properties, such as T_{50} and T_{end} , increased in most cases,
407 suggesting a proportional gain in more stable compounds, although they did not reach the
408 values obtained in MF. The Q/SOM ratios in the soils from the mature plantations were
409 higher than those in the pasture soils. However, the values in the afforested soils were lower
410 than in MF.

411 Taking into consideration both sets of data (from chronosequences and paired plots),
412 increases in both labile and stable compounds were detected at the end of the stand rotation.
413 However, because of different rates of incorporation of organic compounds at this timescale,
414 large differences in the amount and composition of the SOM were observed at the end of the
415 first rotation in the different plots (Fig. 9). Although gains in recalcitrant compounds were
416 observed in all soils, important differences were found at the end of the first rotation, relative
417 to the mature forest soil, MF. The values of different thermal parameters (Q, T_2 , T_{50} , T_{end})
418 increased steadily from pasture to young and mature forest stands, but significant differences
419 were only detected between pasture and mature afforested stands ($p = 0.094-0.0276$). These
420 results suggest that that the changes occurred at the end of the rotations, as already observed
421 for the C/N ratio.

422 Discussion

423 Changes in SOC in response to afforestation

424 The present study was carried out in a homogeneous area with respect to soil type,
425 parent material and climate. The different SOC contents, C:N ratios and pH in the 9 pasture
426 soils under study can therefore be attributed to the different types of management. The high
427 SOC contents in some of the pastures (> 8 %) indicate the high potential of pasture soils for
428 sequestering C in humid temperate regions, mainly due to the large inputs of C via herbaceous
429 root litter (Guo et al., 2007) and the addition of organic amendments.

430 The results of the present study show that the initial SOC content was a major factor
431 influencing the changes in SOM after afforestation. When the initial SOC content was low (<
432 3 %), gradual increases in SOM were observed. On the contrary, in the soils with high OC
433 contents, large depletions in OM occurred during at least the first 10 yrs after plantation
434 establishment. However, in four of the nine cases, the SOC contents were not as high as in the
435 pasture soil at the end of the rotation. The magnitude of these changes and how they develop
436 throughout the forest rotation have been discussed in a previous study (Pérez-Cruzado et al.,
437 2012a). The large losses of SOM observed 10 yrs after afforestation can be attributed to the
438 combination of a low input of organic C and a high mineralization rate as a consequence of
439 the humid temperate climate. The present data also show that the moderate pH of the pasture
440 soil at the time of afforestation may have led to an increase in the SOM mineralization rate.
441 The longer period of SOM loss in chronosequence C4 may be attributed to the higher pH of
442 the soil (Fig. 2b, c). Liming can exert long-term effects on the decomposer community,
443 enzyme activities and toxicity of Al^{+3} (Spiegelberger et al. 2006).

444 The net gains in SOC from 10-15 yrs after plantation was related to accumulation of
445 the litter layer (> 30 Mg ha⁻¹), along with other factors such as the lower soil temperature after
446 forest canopy closure and gradual acidification, which may have reduced the rate of
447 mineralization in the mineral soil (Pérez-Cruzado et al., 2012a). Some studies have shown
448 that the incorporation of new organic compounds (15-20 g C m⁻² yr⁻¹; Fahey et al. 2011) may
449 be particularly rapid in systems characterized by high SOM mineralization rates
450 (Vancampenhout et al. 2009). The high litter decomposition rate in these Atlantic forests,
451 (greater than 30% for a one year period: Ouro et al., 2001) probably contributed to the
452 accumulation of OM in these mineral soils.

453

454 **Mechanisms of SOM loss after afforestation**

455 In addition to the climatic conditions and soil properties, the large losses of OM from
456 some soils may also be due to the initial composition of the OM in the pasture soils. This
457 aspect was addressed by the use of ^{13}C CP-MAS NMR and DSC as complementary
458 techniques to relate the nature and stability of the SOM. Although both techniques
459 complemented the information about the SOM, only weak relationships between variables
460 were observed. Thus, Q_1 was related to the alkyl C + O-alkyl C ($r= 0.6$, $p < 0.5$), whereas T_{50}
461 was related to the aromatic C ($r= 0.48$, $p < 0.05$), which is partly consistent with the findings
462 of other studies (Lopez-Capel et al. 2005). It is possible that the range of values was not wide
463 enough to distinguish any trend. However, assignment of thermal parameters to specific
464 chemical-shift regions of the NMR spectra is not simple, because the NMR chemical shift
465 regions include compounds of different stability. The latter technique detects functionalities
466 that can occur in different molecular structures and one molecular component usually has
467 several functionalities (e.g. lignin).

468 The ^{13}C CP-MAS NMR and DSC analyses both revealed that the pasture soils with
469 high OM contents were also rich in labile C. The NMR spectra indicated that carbohydrates
470 made up 50 % of the SOM. In the DSC analysis, the Q generated at temperatures lower than
471 $375\text{ }^\circ\text{C}$ (Q_1) accounted for approximately 75 % of the total Q. The abundance of labile C in
472 these pastures was possibly due to the high litter input through fine root biomass (Mendham
473 et al. 2002) and the frequent addition of organic amendments. Previous studies in Atlantic
474 pasture soils have reported large amounts of labile C (Fernández et al. 2012).

475 The SOM analysis carried out in the four chronosequences revealed that most of the
476 losses that took place during the first 10 yrs after afforestation were due to the rapid
477 degradation of labile fractions. ^{13}C CP-MAS NMR analysis revealed large losses of O-alkyl
478 and aliphatic compounds, whereas DSC analysis revealed parallel decreases in Q_1 . Loss of
479 compounds from the labile fraction of the SOM has also been recorded in afforested pasture
480 land in New Zealand (Huang et al. 2011).

481 Moreover, the present data revealed that, under the specific environmental conditions
482 of the study, partial losses of alkyl C (0-45 ppm) and perhaps aromatic C (110-160 ppm),
483 which are considered to be relatively stable (Kögel-Knabner, 2002; Kleber et al., 2011), also
484 took place. In this respect, the DSC analysis revealed decreases in the heats of combustion at
485 between 375 and $475\text{ }^\circ\text{C}$ (Q_2) and $> 450\text{ }^\circ\text{C}$ (Q_3), also reflecting the loss of the resistant SOM
486 compounds.

487 Some studies have shown that even those compounds considered resistant to microbial
488 and enzymatic breakdown, such as lignin and lipids derived from cutans or suberans, are
489 altered relatively quickly and are not stabilized in the long term (Vancampenhout et al. 2009).
490 SOM compounds that are not stabilized by interactions with minerals or occluded in
491 aggregates decompose within 50 yrs and therefore cannot be considered recalcitrant (e.g.
492 Marschner et al. 2008). According to von Lützow et al. (2006), loss of such SOM compounds
493 is more common in surface mineral horizons because of the lack of protective mechanisms. In
494 the soils under study, the clay content is less than 20 % and the minerals are 1:1 type and Fe-
495 and Al-oxides, of low CEC and specific surface area. The mineral fraction therefore has a low
496 capacity to adsorb organic compounds. Considering the approach used by Six et al. (2002),
497 the protective capacity of most of these soils, based on the silt and clay contents, ranged from
498 74 to 100 g silt+clay C kg⁻¹ soil (data not shown). In accordance with these calculations, soils
499 with more than 7 % SOC (C3, C4, P8 and P9) were close to or exceeded the C saturation
500 point. This suggests that a large part of the SOM may constitute free light particles associated
501 with coarse silt and/or occluded in macro-aggregates (Stewart et al. 2009). A preliminary
502 study shows that in these soils a substantial fraction of mineral-associated SOM is bound
503 through weak bonds to inorganic components (López-Sangil and Rovira, 2013). Another
504 mechanism that may explain the rapid C mineralization may be the priming effect induced
505 by the availability of easily degradable organic substrates and nutrients (e.g. Kuzyakov,
506 2010). Thus, the abundance of labile C produced in the root exudates may have favoured the
507 degradation of SOM-resistant substances.

508

509 **Changes in SOM composition at the end of the stand rotation**

510 Analysis of the chronosequences by ¹³C CP-MAS NMR and DSC revealed gradual
511 gains in most of the SOM compounds throughout the rotation. However, despite the
512 homogeneous conditions of the sites, the rates of SOM gain and SOM composition differed in
513 the different chronosequences. As already mentioned, certain soil properties, such as pH and
514 degree of SOM protection, may affect the changes in SOM throughout the rotation. The
515 differences in the four chronosequences studied may also be due to differences in tree growth,
516 which determine how the forest floor develops. Moreover, differences in the development of
517 the understory, which was abundant in the first 10 yrs of the rotation, would contribute to the
518 differences.

519 The composition of the SOM in the mineral soil in mature stands can be determined
520 by the composition of the forest litter. The litter composition indicated by solid-state ^{13}C
521 NMR analysis is similar to previous reports (Parfitt and Newman, 2000; Almendros et al.,
522 2000) for the same species. The changes in the SOM compounds during litter decomposition
523 are similar to those described by the latter authors (i.e. loss of O-alkyl compounds, and
524 increases in alkyl and aromatic compounds). The alkyl / O-alkyl ratio increased during the
525 decomposition, which usually occurs in most tree species.

526 The higher Q_1 (heat combustion $< 375\text{ }^\circ\text{C}$) in the mineral soils reflects the gain in
527 labile organic compounds at the end of the rotation. Increases in the amounts of
528 polysaccharides (60-105 ppm) were also detected in the NMR spectra, especially in the
529 amounts of cellulose and hemicellulose (73 ppm). Gains in polysaccharides in afforested soils
530 have been also reported by Cerli et al. (2008). Mathers and Xu (2003) also observed increased
531 amounts of these compounds in an afforested (*Pinus radiata*) soil to which large amounts of
532 logging residues were added. The effect was also probably due to the direct input of plant
533 tissue (more than 50 % of the humified forest litter is made up of O-alkyl compounds, Table
534 1) and the degradation of OM by microorganisms. In this respect, although the signal for
535 tannins detected by ^{13}C CP-MAS NMR coincides with those of other groups, analysis of the
536 spectra appears to indicate the presence of these compounds in litter. Condensed tannin
537 structures in SOM under *Pinus radiata* have also been identified elsewhere (Parfitt and
538 Newman, 2000; Almendros et al. 2000; Mathers and Xu, 2003). Almendros et al. (2000)
539 attributed the preservation of O-alkyl structures during the process of litter decomposition in
540 this species to the protective effect of tannins. This effect may also explain the abundance of
541 polysaccharides and other compounds in mineral soils (Kraus et al. 2003).

542 At the end of the rotation, increases in T_{50} and the heat of combustion $> 375\text{ }^\circ\text{C}$ ($Q_2 +$
543 Q_3 %) were observed in most of the plots, which suggests some accumulation of stable SOM
544 compounds. The NMR analysis also revealed partial gains of alkyl and/or aromatic
545 compounds. Higher C/N ratios were also observed at the end of the rotation. The increase in
546 the abundance of aromatic compounds in the mineral soil at the end of the rotation may be
547 due to the translocation of waxes, suberin and lignin from the coniferous litter, as also
548 suggested by Helfrich et al. (2006) and Otto and Simpson (2006). Aromatic compounds can
549 also be produced during biosynthesis by soil microbes (Cerli et al. 2008; Marín-Spiotta et al.
550 2009; Wiesmeier et al. 2009; Huang et al. 2011), and aliphatic compounds can be formed by
551 polymerization (de Leeuw et al. 2006). These findings suggest that the end-of-rotation gains

552 in SOC may be favoured by the higher recalcitrance of pine litter relative to grass litter, in
553 addition to the higher C input. A similar mechanism has also been suggested by Montané et
554 al. (2010) to explain the SOM gain after shrub encroachment into grasslands.

555 The increase in recalcitrant compounds, together with the soil acidification, may lead
556 to a longer turnover of SOM in these afforested soils. This effect is important in highly
557 managed plantations because it may partly alleviate the gradual loss of SOC that takes place
558 as a result of the usual perturbations in plant cover and soil after each rotation (Ussiri and
559 Johnson, 2007; Nave et al. 2010).

560

561 **Conclusions**

562 The changes in SOC following the afforestation of pastureland were mainly
563 determined by the decrease in the input of C and the initial high amount of labile organic
564 compounds in the soil. The large losses of SOC from the SOM-rich pasture soils during the
565 first years after afforestation were favoured by other factors affecting SOM decomposition,
566 such as the humid temperate climate, the moderate soil acidity brought about by the previous
567 liming and the lack of physical protection of the SOM. The data suggest that even stable SOM
568 compounds are decomposed during the early stages of afforestation. The low capacity of the
569 soil to adsorb organic compounds due to low clay content is another possible explanation for
570 the loss of SOC. The subsequent gains in SOM observed 10-20 yrs after stand establishment
571 did not always ensure recovery of the initial values by the end of the stand rotation. Thus
572 SOM may be lost in successive rotations, especially in plantations managed in short rotations
573 or when logging residues are removed. The incorporation of recalcitrant compounds from
574 coniferous litter and other factors, such as acidification, lower temperature and possibly the
575 protective effect of tannins (derived from the litter), may have prolonged the SOM turnover
576 and therefore the SOC gains at the end of the forest rotation. The changes in SOM
577 composition after afforestation may imply changes in soil functions.

578 The highly variable changes in SOM following afforestation emphasize the difficulty
579 in translating the observed differences from one or a few chronosequences into predictions at
580 a regional level, even under rather homogeneous environmental conditions. This high
581 variability is due to the large number of factors involved, such as certain soil properties
582 (mainly SOM content and pH), tree growth rate and understory development. Over the
583 timescale considered, these variables determine differences in the amount and type of organic

584 compounds incorporated. As a consequence, differences in the amount and composition of the
585 SOM were found at the end of this first rotation in the different plots. Although there was a
586 trend towards a proportional increase in recalcitrant compounds in the final years of the
587 rotation, the final SOM composition depended on the specific development of the plantation.
588

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594

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Table 1. Relative intensities of different signals in the solid-state ^{13}C CPMAS NMR spectra (%) of the soils studied.

	Alkyl C (0-45 ppm)	O-alkyl (45-110 ppm)	Aromatic C (100-160 ppm)	Carboxyl C (160-210 ppm)	Alkyl-C /O-Alkyl	Aromaticity	O-alkyl-C /Aromatic C
FOREST FLOOR							
Senescent litter	15	59	21	5	0.25	0.22	2.8
Decomposed litter	18	55	22	5	0.33	0.23	2.5
MINERAL SOIL							
Mature forest soil	26	40	25	9	0.65	0.27	1.6
Chronosequence 1 (C1)							
Pasture	19	49	23	9	0.39	0.25	2.13
Affor (22 yr)	22	47	23	8	0.47	0.25	2.04
Affor (30 yr)	24	47	21	7	0.51	0.23	2.24
Chronosequence 3 (C3)							
Pasture	24	52	19	5	0.46	0.20	2.7
Affor (10 yr)	22	44	25	9	0.50	0.27	1.8
Affor (30 yr)	26	38	27	9	0.68	0.30	1.4

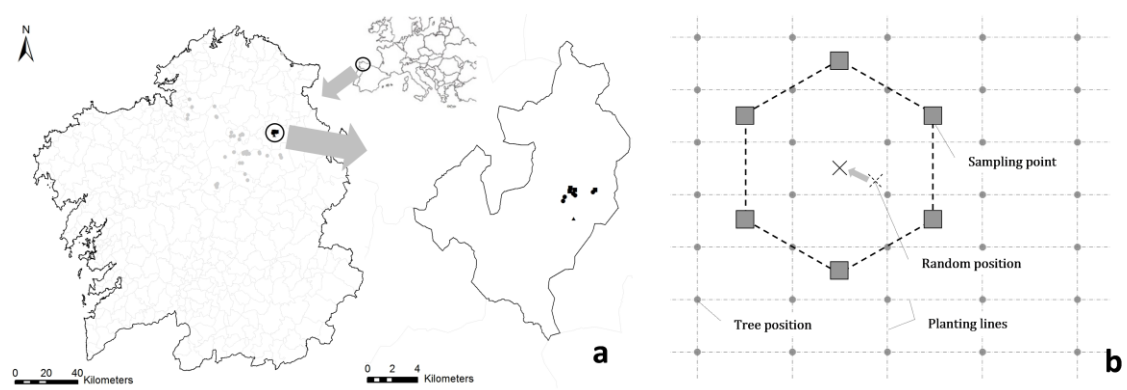


Fig. 1. a) Location of the sample plots. *P. radiata* stands studied by Pérez-Cruzado et al. (2012a) (grey and black circles); chronosequences C1-C4 (black squares); paired plots P5-P9 (black circles); mature forest MF (black triangle). b) Sampling design in the study plot.

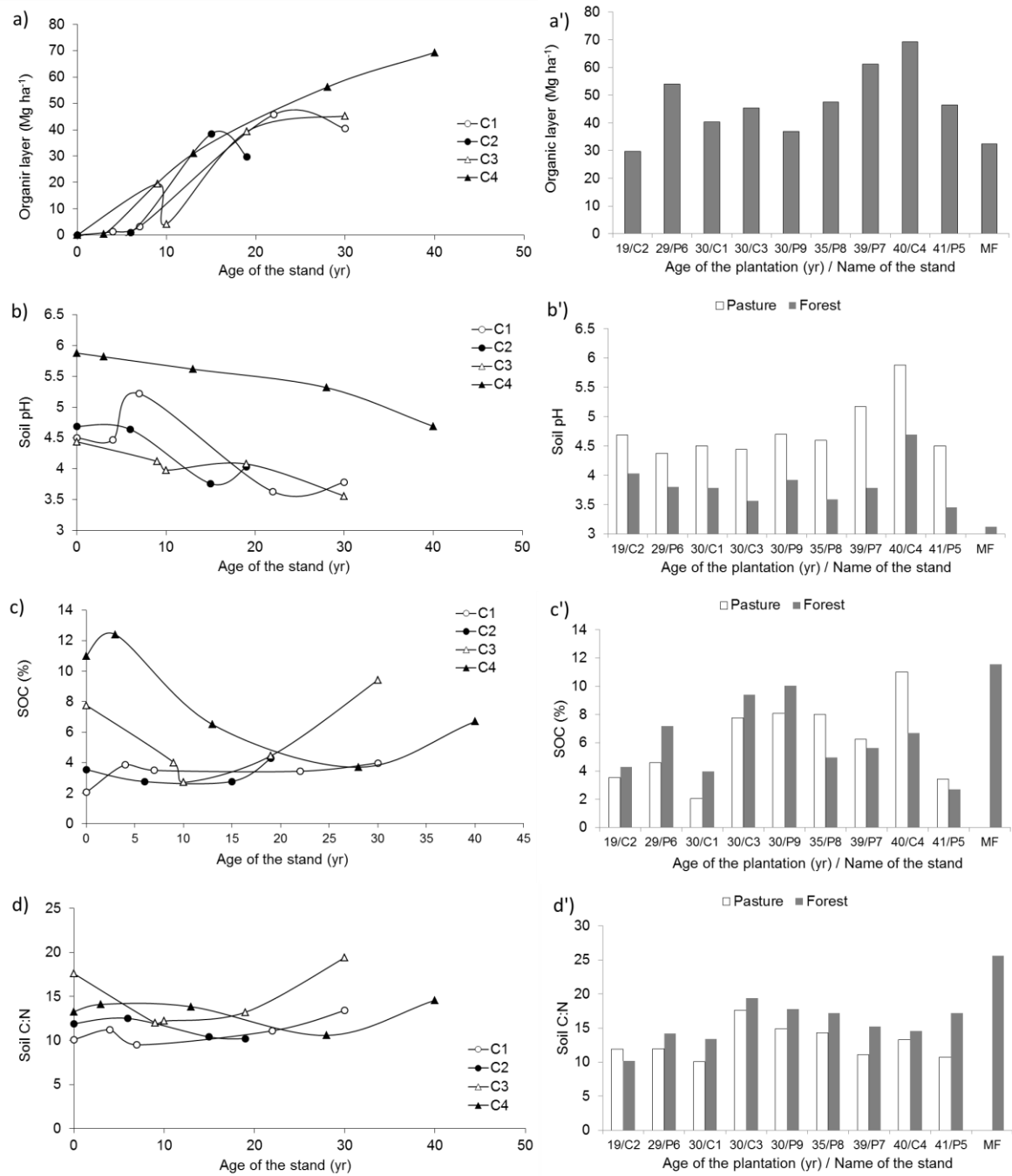


Fig. 2. Litter layer and soil mineral pH, SOC and C/N (0-5 cm depth). The changes in these parameters throughout the rotation are shown in the pictures of the left column (a-d), which include the four chronosequences selected (C1-C4, stands of different ages established in the same pasture). The comparison between the pastures and the mature stands established on them are shown in the figures of the right column (a'-d'), which include the four chronosequences (C1-C4) and the five paired plots (P5-P9, each made up by the former pasture land and a mature plantation) selected.

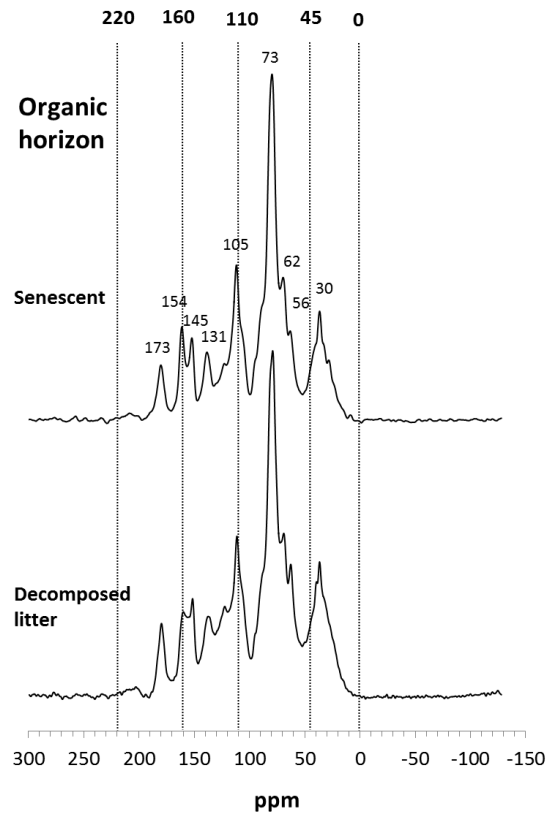


Fig. 3. Solid-state ^{13}C CP MAS NMR spectra of the forest floor in a plot afforested with *Pinus radiata*.

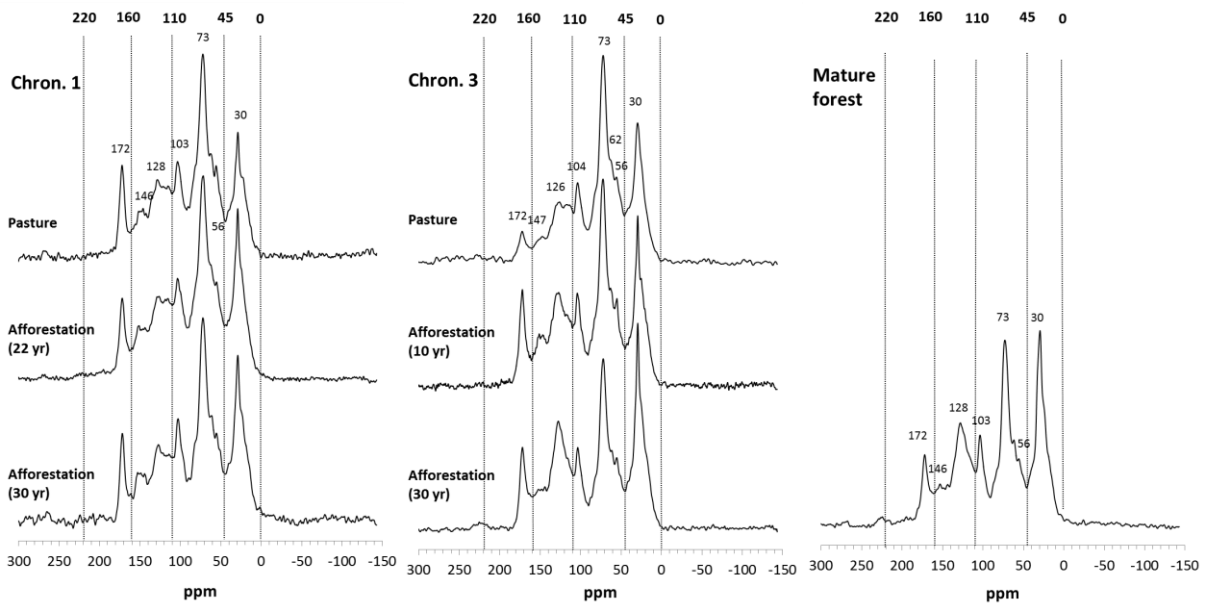


Fig. 4. Solid-state ^{13}C CP MAS NMR spectra of the mineral soil samples (0-5cm) from the two chronosequences studied (C1 and C3).

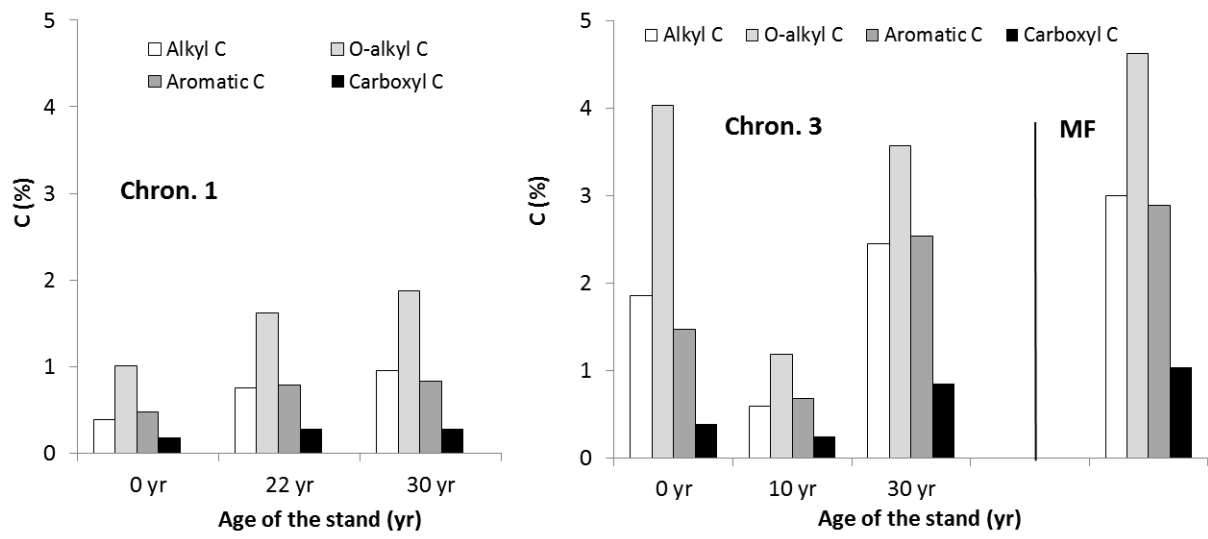


Fig. 5. C distribution of various chemical-shift regions revealed by the solid-state ^{13}C CPMAS NMR in soil samples of the two chronosequences selected.

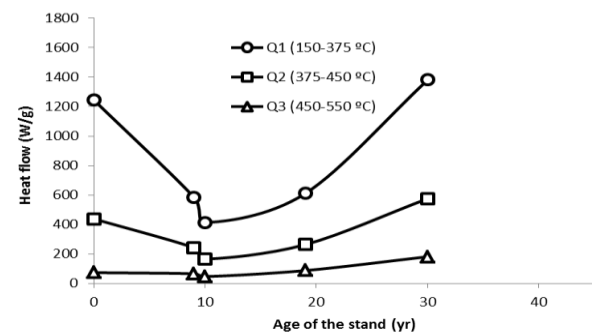
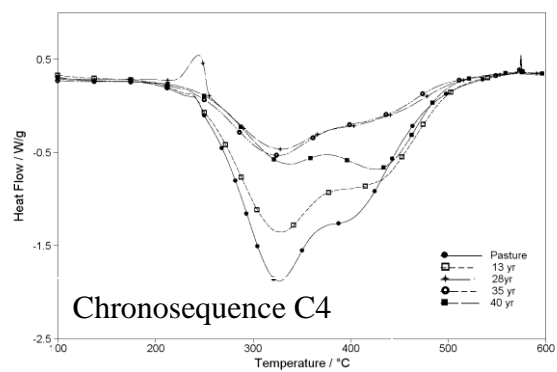
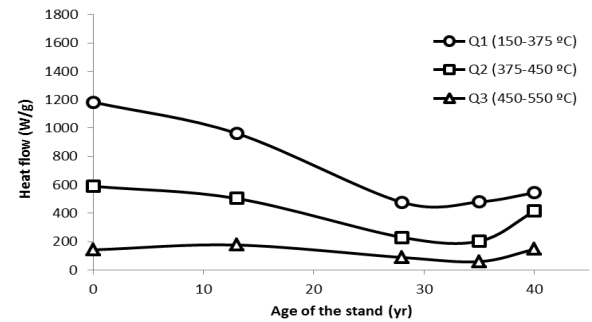
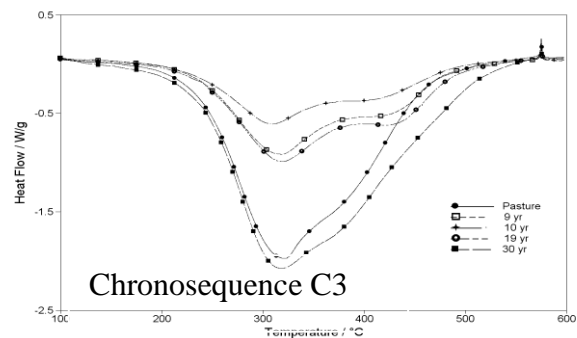
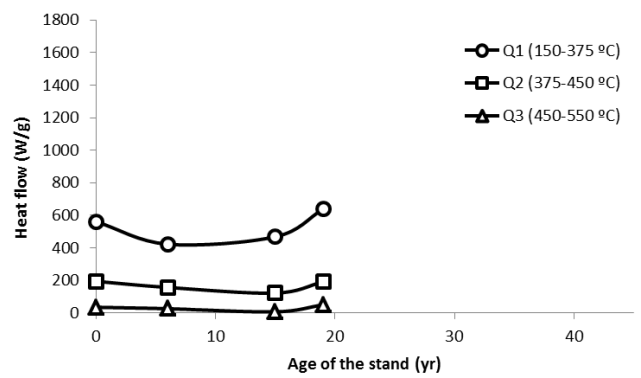
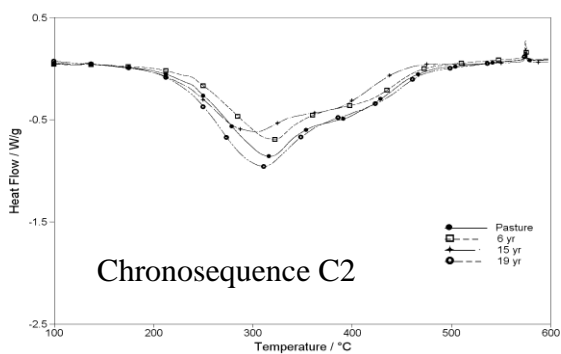
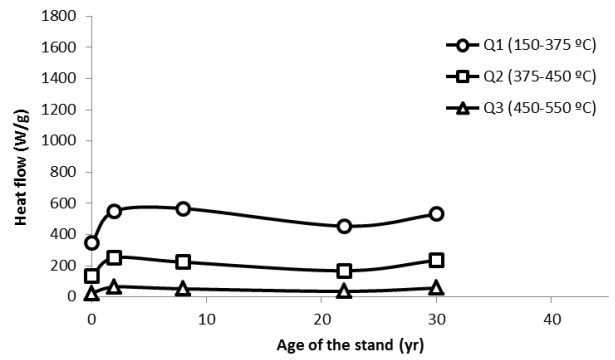
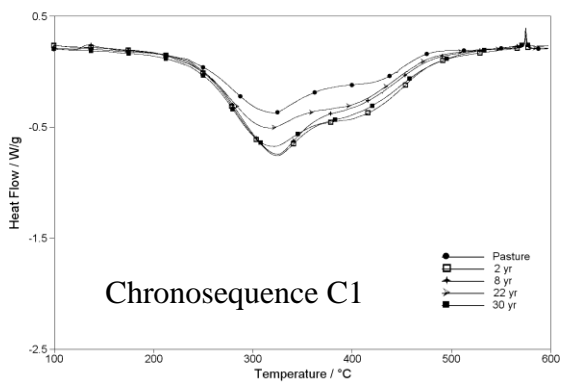


Fig. 6. DSC curves for the four chronosequences (left column) and variations in the three heats of combustion throughout the rotation (right column).

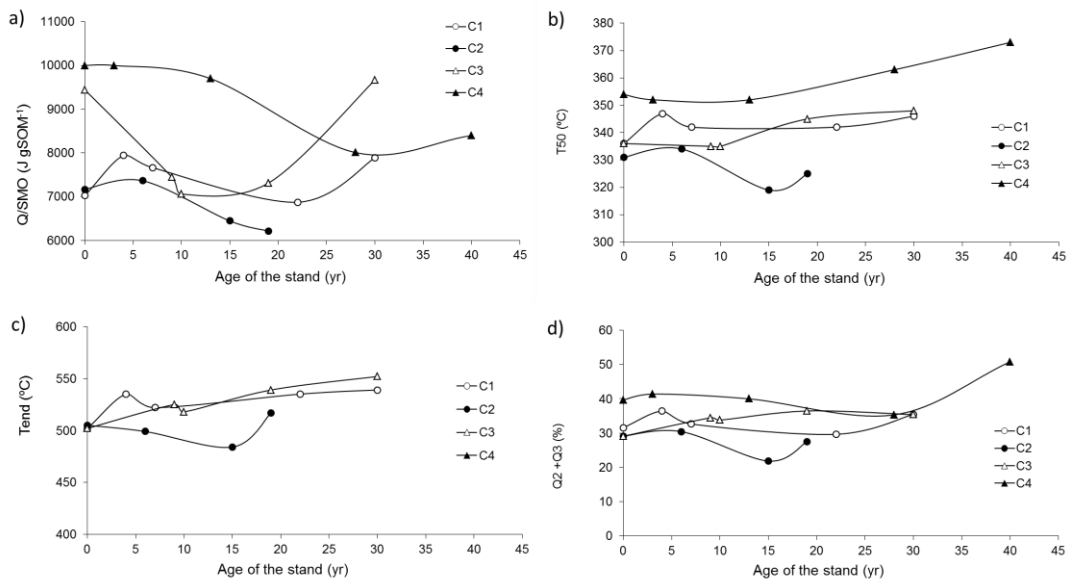


Fig. 7. Changes in some thermal indices obtained by DSC analysis of the four chronosequences studied.

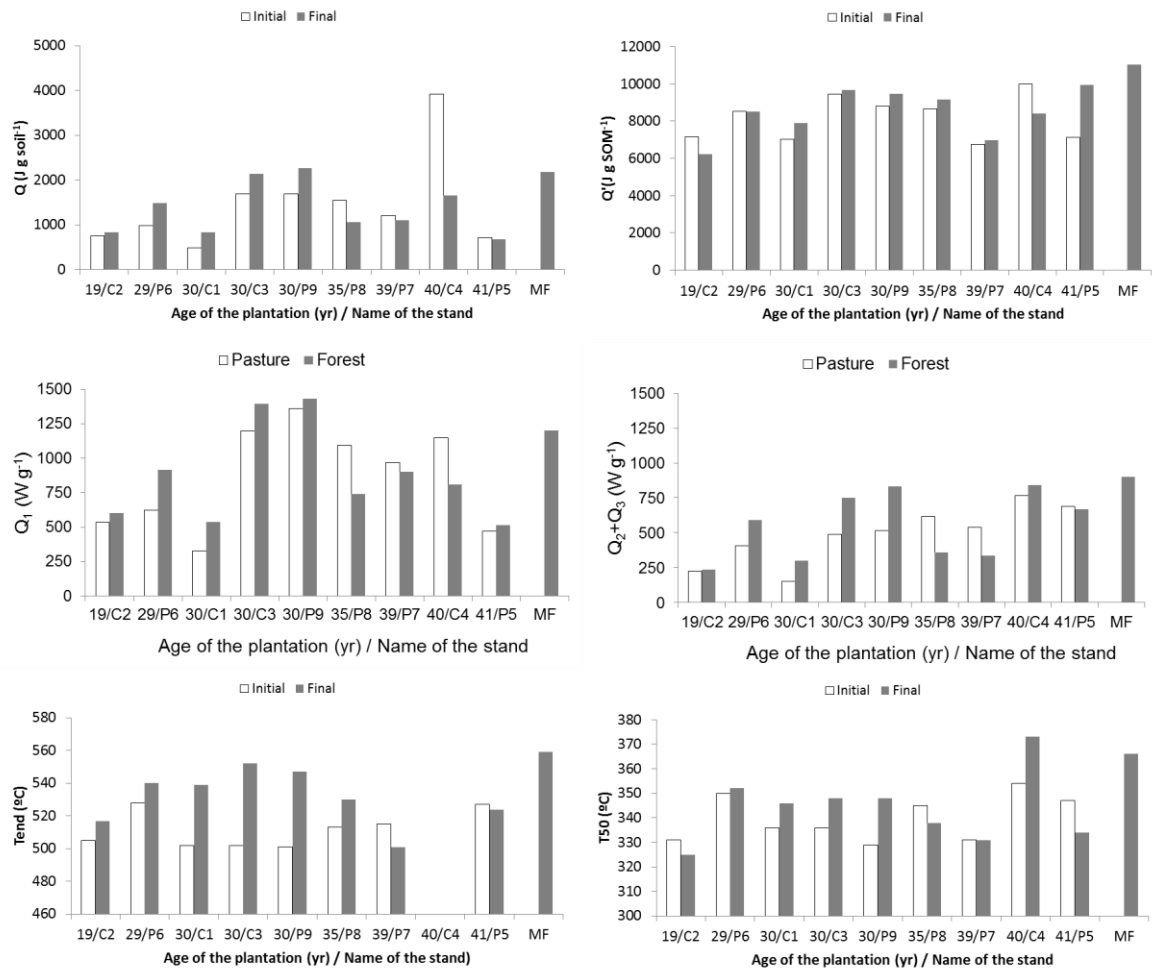


Fig. 8. Thermal indices for the pastures and the mature plantations established on them. The figures include the four chronosequences (C1-C4) and the five paired plots (P5-P9) (comprising the former pasture land and a mature plantation) selected for study.

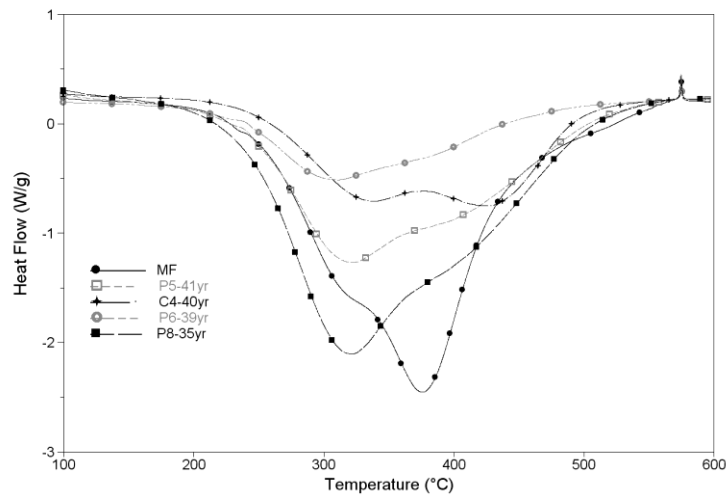


Fig. 9. DSC curves for the soils of the oldest chronosequences and the mature forest (MF) soil.