Influence of *Pinus pinaster* age on aluminium fractions in acidic soils

Influencia de la edad de Pinus pinaster *en las fracciones de aluminio de suelos ácidos*
Influência da idade de Pinus pinaster *nas frações de alumínio em solos ácidos*

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ABSTRACT

The influence of plantation age on the chemical properties of acidic soils was studied in 16 plots in adult *Pinus pinaster* stands established in Galicia (NW Spain). The Al fractions in the soil solid phase and the total Al in soil solution were determined in the upper soil layer (0-20 cm) and the lower soil layer (20-40 cm) in each plot. The pH, total C and N, exchangeable Ca, Mg, Na, K, and Al and Al saturation (% Al) were determined in the solid fraction. Aluminium was extracted from the solid phase with the following solutions: ammonium oxalate (Al_o), sodium pyrophosphate (Al_p), copper chloride (Al_{cu}) and ammonium chloride (Al_{NH_4}). The total Al in the liquid phase was also determined. All soil chemical parameters, except total N, C/N ratio and % Al, were significantly influenced by soil depth. The mean pH was lower in the upper than in the lower layer (4.57 vs. 4.97), but the opposite was observed for the organic C (77.2 vs. 50.4 g kg^{-1}), the effective cation exchange capacity (eCEC) (9.43 vs. 6.25 cmol$_{(+)}$ kg^{-1}), P (8.95 vs. 4.65 mg kg^{-1}) and the exchangeable cations. Organic matter, total N and eCEC were significantly and positively correlated with plantation age (r = 0.69 in the upper layer and r = 0.82 in the lower layer, p < 0.01; r = 0.62, p < 0.05 in the upper layer and r = 0.78, p < 0.01 in the lower layer; r = 0.77, p < 0.01 in the upper layer and r = 0.85, p < 0.0001 in the lower layer, respectively), and pH_{KCl} was negatively correlated with plantation age (r = -0.55 in the upper soil layer and r = -0.61 in the lower soil layer, p < 0.05). The concentrations of the different Al forms in all soils decreased in the order Al_p > Al_o > Al_{cu} > Al_{NH_4}. Highly stable organo-aluminium complexes (Al_{p-cu}) predominated over moderate and low stability complexes (Al_{cu}) in all soil plots. The highly stable organo-Al complexes were significantly more abundant in the lower layer, whereas the opposite was observed for the exchangeable Al and the total Al in soil solution. The concentrations of all Al forms (except Al_{p-cu}) were significantly and positively correlated with plantation age (Al_o r = 0.50, p < 0.05 for the upper layer and r = 0.67, p < 0.01 for the lower layer; Al_p r = 0.64, p < 0.01 for the lower layer; Al_{cu} r = 0.84 for the upper layer and r = 0.83 for the lower layer, p < 0.0001; Al_{cu-NH_4} r = 0.65 for the upper layer and r = 0.78 for the lower layer, p < 0.01; Al_{NH_4} r = 0.76, p < 0.01 for the upper layer and r = 0.84, p < 0.0001 for the lower layer; total Al in soil solution r = 0.61 for the upper layer and r = 0.60 for the lower layer, p < 0.05). Stepwise linear regression analysis showed that plantation age, pH and total C explained between 67% and 93% of the variance in the Al forms. In all regression models, plantation age was a significant predictor variable for the different Al fractions, except total soluble Al, which is an important variable to consider in the study of chemical properties in forest soils.

RESUMEN

La influencia de la edad de las plantaciones sobre las propiedades químicas de suelos ácidos se estudió en 16 parcelas de Pinus pinaster adulto establecidas en Galicia (Noroeste de España). Se determinaron las fracciones de Al en la fase sólida del suelo y el Al total de la disolución del suelo en la capa superior del suelo (0–20 cm) y en la capa inferior del suelo (20–40 cm) en cada parcela. El pH, el C y N total, Ca, Mg, Na, K y Al cambiables y la saturación de Al (% Al) se determinaron en la fracción sólida. El aluminio se extrajo de la fase sólida con las siguientes soluciones: oxalato amónico (Al_o), pirofosfato sódico (Al_p), cloruro de cobre (Al_{cu}) y cloruro amónico (Al_{NH_4}). También se determinó el Al total en la fase líquida. La profundidad del suelo tuvo un efecto significativo en todos los parámetros químicos del suelo, excepto la relación N total, C/N y % Al. Los valores medios de pH fueron más bajos en la capa superior (4,57 vs. 4,97), lo contrario se observó con el C orgánico (77,2 vs. 50,4 g kg^{-1}), con la capacidad de intercambio catiónico efectiva (CICe) (9,43 vs. 6,25 $cmol_{(+)}$ kg^{-1}), con el P (8,95 vs. 4,65 mg kg^{-1}) y los cationes cambiables. La edad de la plantación se correlacionó significativamente y positivamente con la materia orgánica, el N total y la CICe ($r = 0,69$ en la capa superior y $r = 0,82$ en la capa inferior, $p < 0,01$; $r = 0,62$, $p < 0,05$ en la capa superior y $r = 0,78$, $p < 0,01$ en la capa inferior; $r = 0,77$, $p < 0,01$ en la capa superior y $r = 0,85$, $p < 0,0001$ en la capa inferior, respectivamente), y negativamente con el pH_{KCl} ($r = -0,55$ en la capa superior y $r = -0,61$ en la capa inferior, $p < 0,05$). La concentración de las diferentes formas de Al en todos los suelos disminuyeron en el orden $Al_p > Al_o > Al_{cu} > Al_{NH_4}$. Los complejos organo–alumínicos de alta estabilidad (Al_{p-cu}) predominaron sobre los complejos de estabilidad media y baja (Al_{cu}) en todas las parcelas. Los complejos órgano–Al de alta estabilidad fueron significativamente más abundantes en la capa inferior, mientras se observó lo contrario con el Al cambiable y el Al total en disolución del suelo. La edad de las plantaciones se correlacionó significativa y positivamente con todas las formas de Al (excepto Al_{p-cu}) (Al_o $r = 0.50$, $p < 0.05$ en la capa superior y $r = 0,67$, $p < 0,01$ en la capa inferior; Al_p $r = 0,64$, $p < 0,01$ en la capa inferior; Al_{cu} $r = 0,84$ en la capa superior y $r = 0,83$ en la capa inferior, $p < 0,0001$; Al_{cu-NH_4} $r = 0,65$ en la capa superior y $r = 0,78$ en la capa inferior, $p < 0,01$; Al_{NH_4} $r = 0,76$, $p < 0,01$ en la capa superior y $r = 0,84$, $p < 0,0001$ en la capa inferior; Al total en la disolución del suelo $r = 0,61$ en la capa superior y $r = 0,60$ en la capa inferior, $p < 0,05$). El análisis de regresión lineal stepwise mostró que la edad de la plantación, el pH y el C total explicaron entre 67% y 93% de la varianza en las formas de Al. En todos los modelos de regresión, la edad de las plantaciones fue una variable predictiva para las diferentes fracciones de Al, excepto el Al total de la disolución, que es una variable importante a considerar en el estudio de las propiedades químicas en suelos forestales.

RESUMO

A influência da idade das plantações nas propriedades químicas dos solos ácidos foi estudada em 16 parcelas de pinheiro–bravo (Pinus pinaster) adulto estabelecidas na Galiza (Noroeste de Espanha). A frações de Al na fase sólida do solo e o Al total na solução do solo foram determinadas, em cada parcela, na camada superficial do solo (0–20 cm) e na camada sub–superficial (20–40 cm). O pH, C e N totais, Ca, Mg, Na, K e Al de troca e a saturação de Al (% Al) foram determinados na fração sólida. O Al foi extraído da fração sólida com as seguintes soluções: oxalato de amónio (Al_o), pirofosfato de sódio (Al_p), cloreto de cobre (Al_{cu}) e cloreto de amónio (Al_{NH_4}). O Al total na fase líquida também foi determinado. A profundidade do solo teve um efeito significativo em todos os parâmetros químicos do solo, exceto no N total, relação C/N e % Al. Os valores médios de pH foram menores na camada superior (4,57 vs. 4,97), o contrário foi observado com o C orgânico (77,2 vs. 50,4 g kg^{-1}), a capacidade de troca catiónica efetiva (eCEC) (9,43 vs. 6,25 $cmol_{(+)}$ kg^{-1}), o P (8,95 vs. 4,65 mg kg^{-1}) e os catiões de troca. A idade da plantação correlacionou–se significativa e positivamente com a matéria orgânica, N total e eCEC ($r = 0,69$ na camada superior e $r = 0,82$ na camada inferior, $p < 0,01$; $r = 0,62$, $p < 0,05$ na camada superior e $r = 0,78$, $p < 0,01$ na camada inferior; $r = 0,77$, $p < 0,01$ na camada superior e $r = 0,85$, $p < 0,0001$ na camada inferior, respetivamente) e negativamente com pH_{KCl} ($r = -0,55$ na camada superior e $r = -0,61$ na camada inferior, $p < 0,05$). A concentração das diferentes formas de Al em todos os solos seguiram a ordem $Al_p > Al_o > Al_{cu} > Al_{NH_4}$. Os complexos organo–alumínicos de alta estabilidade (Al_{p-cu}) predominaram sobre os complexos de média e baixa estabilidade (Al_{cu}) em todas as parcelas. Os complexos de alta estabilidade foram significativamente mais abundantes na camada inferior, enquanto o oposto foi observado com o Al de troca e o Al total em solução. A idade das plantações correlacionou–se significativa e positivamente com todas as formas de Al (exceto Al_{p-cu}) (Al_o $r = 0,50$, $p < 0,05$ na camada superior e $r = 0,67$, $p < 0,01$ na camada inferior; Al_p $r = 0,64$, $p < 0,01$ na camada inferior; Al_{cu} $r = 0,84$ na camada superior e $r = 0,83$ na camada inferior, $p < 0,0001$; Al_{cu-NH_4} $r = 0,65$ na camada superior e $r = 0,78$ na camada inferior, $p < 0,01$; Al_{NH_4} $r = 0,76$, $p < 0,01$ na camada superior e $r = 0,84$, $p < 0,0001$ na camada inferior; Al total solúvel $r = 0,61$ na camada superior e $r = 0,60$ na camada inferior, $p < 0,05$). A analise de regressão linear stepwise mostrou que a idade das plantações, o pH e o C total explicavam entre 67% e 93% da variância nas formas de Al. Em todos os modelos de regressão, a idade da plantação foi uma variável preditiva significativa para as diferentes frações de Al, exceto o Al total solúvel, sendo uma variável importante a ser considerada no estudo das propriedades químicas em solos florestais.

1. Introduction

The soils in Galicia (NW Spain) are characterised by being mostly acidic. This is due to the predominance of acidic rocks and the existence of open and subtractive systems (in which precipitation exceeds evapotranspiration) in the region. At the initial stages of soil formation, the most unstable primary minerals are already partly weathered and neoformation of secondary minerals takes place while there is still an important pool of non-weathered primary minerals (Macías et al. 1982). At this stage, nutrient elements are available to plants and the soil productivity is close to optimum. The pool of weatherable minerals is gradually reduced, 1:1 minerals are formed and Fe/Al oxy-hydroxides and the base cations are retained in the system. This reduces the degree of base saturation of the exchange complex, with Al ions becoming the most important cations (Macías et al. 1982; Macías and Camps 2020). The acid conditions influence the concentration of nutrients in soil solution and their availability to plants. Thus, while the availability of N, P, K, S, Ca, Mg and Mo is greatly decreased, the solubility of Al and Mn increases becoming toxic to plants (Macías and Calvo 1992; Brennan et al. 2004). These conditions have important consequences for plant production, especially for calcophiles and eutrophiles, with reductions in root elongation, symbiotic N_2 fixation and nitrification and increases in phosphate fixation and aluminium mobilization (Macías et al. 1982; Macías and Camps 2020). Active aluminium species prevail in most Galician soils (García-Rodeja and Macías 1984), with high Al contents in both solid and liquid phases (Álvarez et al. 1992, 2002, 2005) that can cause toxicity and nutritional imbalances in forest stands.

Vegetation plays an important role in determining Al forms in the solid and liquid phases of soil (Álvarez et al. 2002, 2005). Thus, in forest soils, the type of organic matter is closely related to the forest species and strongly influences the formation of Al-humus complexes. The formation of these complexes in the soil solid phase plays a major role in regulating Al activity in soil solution (Mulder and Stein 1994; Takahashi et al. 1995). In addition, aluminium bound to organic matter in the soil solution is less toxic than inorganic Al species (Adams et al. 2000; Matús et al. 2006), which may explain the lack of negative effects of Al on the growth of tree species in acid soils.

Pinus pinaster is one of the most important tree species in Spain, both in terms of extent and wood production. This species is considered non-site-demanding and it is mainly planted for timber production and for restoration and landscaping purposes in northern Spain. In their natural range, pines appear to be particularly well adapted to marginal habitats, in which combinations of various related factors enable them to compete successfully with other tree species. The widespread use of *Pinus pinaster* in Galicia, where it covers 0.53 Mha of land in pure and mixed stands, is due to its extraordinary adaptation to poor, acidic, shallow sandy soils (Eimil-Fraga et al. 2014). Many processes associated with growth and nutrient cycling in pine strongly affect the underlying soils. These processes generally lead to nutrient depletion and consequently to soil acidification, which in turn provide pines with a competitive advantage against other plant species (Richardson 1998).

As a complement of a previous study that assessed Al-forms in the solid phase of soils developed on different parent materials under young plantations of *Pinus pinaster* (Eimil-Fraga et al. 2015), the aims of the present study are to investigate if the plantation age influences the chemical properties of acidic soils under adult *Pinus pinaster*. This study focuses on some Al fractions in the soil solid phase and on the total concentration of Al in soil solution, comparing the results obtained in two different soil layers.

2. Material and Methods

The study was carried out in a network of 16 plots established as a chronosequence in adult reforested plantations of *Pinus pinaster* located in Galicia (NW Spain). The plot size was 25 × 40 m and the age of the trees ranged between 27 and 58 years. This species is usually managed on rotations of 25-40 years.

The plot soils are characterised by different types of parent material: granite, acid metamorphic rock and sedimentary rock. In each plot, three soil samples were obtained at random from the upper layer (0-20 cm) and from the lower layer (20-40 cm). The three samples from each layer were combined to form a composite sample for

each depth. Composite soil samples were dried at 40 °C and sieved through a 2 mm mesh. The following parameters were determined in the soil solid fraction: pH in water and in 0.1 M KCl (Guitián and Carballas 1976); total C and N, by combustion in a CHNS LECO analyser (CHNS Truspec model); the exchangeable cations (Ca, Mg, Na, K and Al) displaced by 1 M NH_4Cl using the method proposed by Peech et al. (1947) and the effective cation exchange capacity (eCEC) as the sum of them (Kamprath 1970). The exchangeable cations were measured by atomic absorption (Ca, Mg, and Al) and atomic emission (Na and K) spectroscopy (Perkin Elmer, Optima 4300 DV model). Al saturation (% Al) was calculated as the ratio between exchangeable Al and the eCEC and was expressed as a percentage. The P concentration was measured by the Olsen method (Olsen and Sommers 1982).

Different forms of Al were extracted from the soil solid fraction by extraction with different reagents: the Al extracted with acid ammonium oxalate (Al_o) (ratio soil:extractant 1:100, shaking for 4 h in darkness) provided an estimate of total non-crystalline Al compounds (Blakemore 1978); the Al extracted with sodium pyrophosphate (Al_p) (ratio soil:extractant 1:100, with 16 h shaking) yielded an estimate of the total organically bound Al (Bascomb 1968); and the Al extracted with 0.5 M $CuCl_2$ (ratio soil:extractant 1:10, 30 min shaking) provided an estimate of organo-Al complexes of low and moderate stability (Al_{cu})

(Juo and Kamprath 1979). The Al extracted with unbuffered NH_4Cl was considered exchangeable Al (Al_{NH_4}) (Peech et al. 1947) (ratio soil:extractant 1:100 contact time, 12 h). Aluminium in the extracts was determined by atomic absorption spectroscopy (Perkin Elmer, Optima 4300 DV model). Subtraction of Al_{cu} from Al_p provided an estimate of the Al that forms highly stable complexes with organic matter (Al_{p-cu}), and subtraction of Al_{NH_4} from Al_{cu} provided an estimate of complexes of moderate and low stability (Al_{cu-NH_4}) (Urrutia et al. 1995).

The soil solution was prepared as aqueous extracts mixing soil with distilled water (soil: solution ratio, 1:10), with a contact time of 3 days. The extracts were filtered (0.45 μm) and total soluble Al was determined by visible spectrophotometry, with pyrocatechol violet (Dougan and Wilson 1974).

Forest plot characteristics included the tree diameter at breast height and total height of all trees in each plot, determined in 2012 when trees were between 27 and 58 years old. Site index (SI) was calculated as the dominant height of the stand (in metres) at a reference age of 20 years (Álvarez-González et al. 2005). Dominant height (H0) was calculated as the average total height of the 100 thickest trees per hectare. The stand basal area (G, m^2 ha^{-1}) and number of trees per hectare (N, trees ha^{-1}) were also calculated for each plot. The forest plot characteristics are summarised in **Table 1**.

Table 1. Values of individual tree and stand parameters in the plots under study

Variable	Mean	Minimum	Maximum	Standard deviation
Age of plantation (years)	38	27	58	9.345
Diameter at breast height (cm)	31.35	7.60	67.20	9.142
Total height (m)	20.08	7.90	34.20	3.635
G (basal area, m^2 ha^{-1})	44.99	21.63	74.28	163.9
N (number of trees, trees ha^{-1})	596	240	930	8.133
H0 (dominant height, m)	22.60	17.22	27.37	3.044
SI (site index, m)	15.08	12.00	18.20	2.025

2.1. Statistical Analysis

The data were analysed to determine mean values and ranges of variation. Duncan's test was used to classify the mean values in order to examine all possible differences in relation to soil depth. Pearson's correlation coefficients were calculated to assess the linear relationships between variables. Stepwise regression analysis was applied to the candidate variables for inclusion in predictive models of the different forms of Al. The variance inflation factors (VIF), which represent a measure of the inflation in the variances of the parameter estimates due to collinearity between (independent) variables, were calculated using the VIF option in the MODEL procedure. The data were analysed using the MEANS, CORR, REG and GLM procedures in the SAS statistical package (SAS Institute 2004).

3. Results

3.1. General soil parameters

There were no significant differences in any of the assessed soil parameters between the parent materials. The mean values and standard deviations of the main chemical properties of soils in relation to soil depth are shown in **Table 2**. All soil chemical parameters, except total N, C/N ratio and Al saturation in the cation exchange complex (% Al), were significantly influenced by soil depth (**Table 2**).

The pH_{water} and pH_{KCl} were significantly higher in the lower soil layer (20-40 cm) than in the upper soil layer (0-20 cm). The concentration of exchangeable Ca reached values of between 0.22 and 0.56 $cmol_{(+)}$ kg^{-1} in both layers. The concentration of exchangeable Mg ranged between 0.10 and 0.21 $cmol_{(+)}$ kg^{-1} and the concentration of exchangeable K varied between 0.10 and 0.14 $cmol_{(+)}$ kg^{-1}. The concentrations of Ca, Mg and K were significantly higher in the upper soil layer than in the lower one. The concentration of P (8.95 vs. 4.65 mg kg^{-1}), the amount of organic matter (77.2 vs. 50.4 g kg^{-1}) and the eCEC (9.43 vs. 6.25 $cmol_{(+)}$ kg^{-1}) were also significantly higher in the upper than in the lower soil layer. The Al saturation was lower in the upper soil layer (82%) than in the lower layer (83%), although the difference was not significant (**Table 2**).

In both soil layers, C and pHwater were significantly and negatively correlated (r = -0.87, p < 0.0001 in the upper layer and r = -0.54, p = 0.0307 in the lower layer), as were C and pHKCl (r = -0.87, p < 0.0001 in the upper layer and r = -0.70, p = 0.0024 in the lower layer). The Al saturation was also negatively correlated with

Table 2. Mean values (and standard deviations) of general soil parameters in relation to soil depth, with the respective p values for the one factor ANOVA. Different letters indicate significant differences (p < 0.05) between layers (Duncan's test).

Variable	p value	0-20 cm layer	20-40 cm layer
pH_{water}	0.0001	4.57 (0.30) b	4.97 (0.22) a
pH_{KCl}	0.0017	3.88 (0.33) b	4.22 (0.21) a
Ca ($cmol_{(+)}$ kg^{-1})	0.0001	0.56 (0.31) a	0.22 (0.06) b
Mg ($cmol_{(+)}$ kg^{-1})	< 0.0001	0.21 (0.06) a	0.10 (0.03) b
K ($cmol_{(+)}$ kg^{-1})	0.0134	0.14 (0.05) a	0.10 (0.03) b
C (g kg^{-1})	0.0167	77.2 (3.41) a	50.4 (2.49) b
N (g kg^{-1})	-	0.33 (0.13)	0.25 (0.12)
C/N	-	23.3 (4.54)	20.4 (4.28)
eCEC ($cmol_{(+)}$ kg^{-1})	0.0129	9.43 (3.80) a	6.25 (2.95) b
% Al	-	81.7 (5.90)	83.2 (4.38)
P (mg kg^{-1})	0.0056	8.95 (4.89) a	4.65 (3.04) b

pH_{water} (r = -0.59, p = 0.0160 in the upper layer and r = -0.70, p = 0.0026 in the lower layer) and with pH_{KCl} (r = -0.69, p = 0.0033 in the upper layer and r = -0.80, p = 0.0002 in the lower layer).

Regarding the relationships between the general soil parameters and forest plot characteristics, the pH_{KCl} was negatively correlated with plantation age in the upper soil layer (r = -0.55, p = 0.0286) and in the lower cm soil layer (r = -0.61, p = 0.0118) (**Figure 1**). The amount of

organic matter in both soil layers was positively correlated with the age of plot (r = 0.69, p = 0.0034 in the upper layer and r = 0.82, p = 0.0001 in the lower layer) (**Figure 2**). The plantation age was also significantly and positively correlated with total N (r = 0.62, p = 0.0101 in the upper layer and r = 0.78, p = 0.0004 in the lower layer) and eCEC (r = 0.77, p = 0.0005 in the upper layer and r = 0.85, p < 0.0001 in the lower layer).
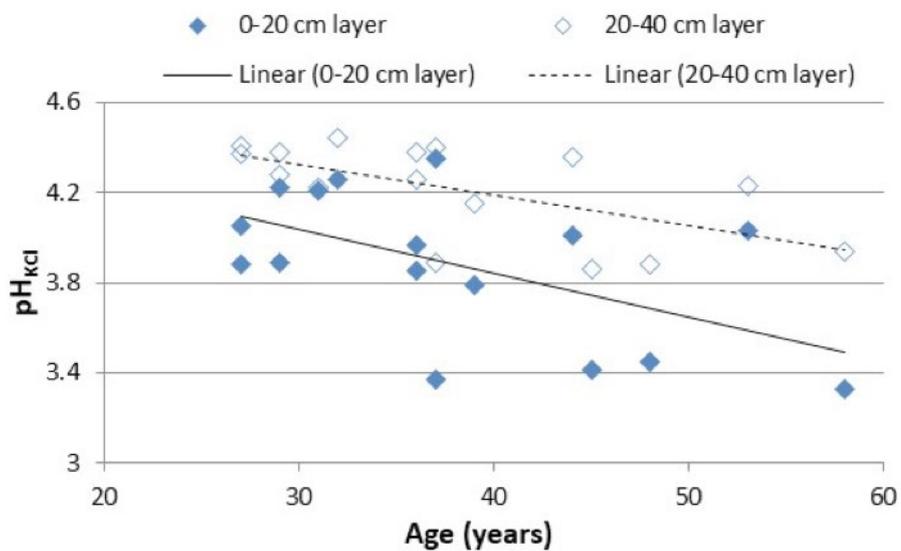
Figure 1. Relationship between plantation age and pH_{KCl} in the upper soil layer (0-20 cm) and the lower soil layer (20-40 cm).
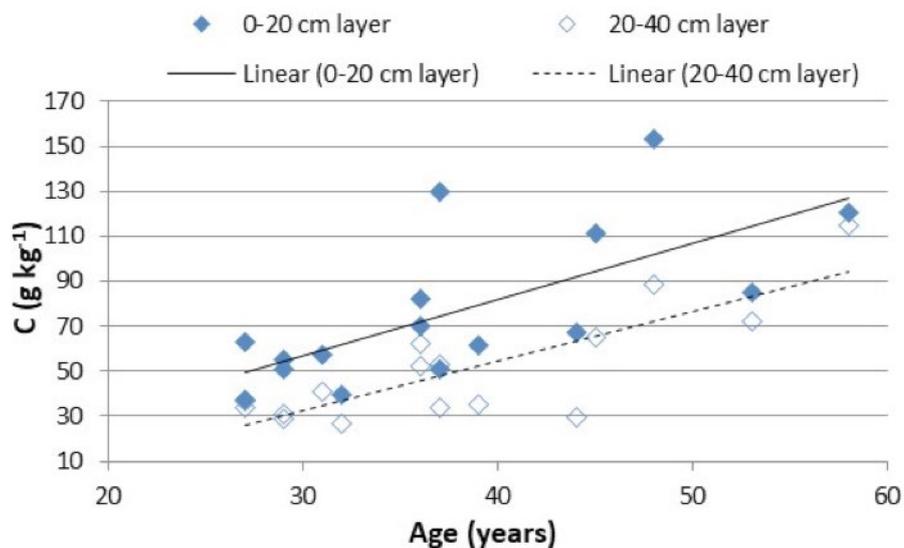
Figure 2. Relationship between plantation age and total C in the upper soil layer (0-20 cm) and the lower soil layer (20-40 cm).

The site index, a parameter related to forest production, was negatively correlated with the percentage of Al in the cation exchange complex in the upper layer ($r = -0.62$, $p = 0.0101$) and positively correlated with the pH_{KCl} in the lower soil layer ($r = 0.60$, $p = 0.0133$).

3.2. Al fractions in the solid phase and total Al in the soil solution

The concentrations of non-crystalline aluminium (Al_o), which varied between 4.67 and 5.73 g kg^{-1}, were higher in the lower than in the upper soil layer, but the difference was not significant. The concentration of organically bound aluminium (Al_p) reached values of between 5.97 and 7.44 g kg^{-1} and was also higher in the lower layer, although again the difference was not significant

(**Table 3**). The concentration of Al_o in both soil layers was significantly and positively correlated with plantation age ($r = 0.50$, $p = 0.0468$ for the upper layer and $r = 0.67$, $p = 0.0041$ for the lower layer), whereas for Al_p, correlation with plantation age ($r = 0.64$, $p = 0.0080$) only occurred in the lower soil layer.

The concentration of organo-aluminium complexes of low and moderate stability (Al_{cu}) varied between 1.66 and 1.89 g kg^{-1} and were higher in the upper than in the lower layer. The Al extracted by $CuCl_2$ did not differ significantly in the two layers (**Table 3**). The concentration of Al_{cu} in both layers was positively and significantly correlated with stand age ($r = 0.84$, $p < 0.0001$ for the upper layer and $r = 0.83$, $p < 0.0001$ for the lower layer) (**Figure 3**).

Table 3. Mean values (and standard deviations) for Al extracted by acid ammonium oxalate (Alo), sodium pyrophosphate (Al_p), $CuCl_2$ (Al_{cu}), NH_4Cl (Al_{NH_4}), $Al_{p-cu} = Al_p-Al_{cu}$, $Al_{cu-NH_4} = Al_{cu}-Al_{NH_4}$ and total Al in soil solution in relation to soil depth. The values of p account for the one factor ANOVA. Different letters indicate significant differences ($p < 0.05$) between layers according to Duncan's test.

Variable	p value	0-20 cm soil layer	20-40 cm soil layer
Al_o (g kg^{-1})	-	4.67 (1.21)	5.73 (2.18)
Al_p (g kg^{-1})	-	5.97 (1.11)	7.44 (2.19)
Al_{cu} (g kg^{-1})	-	1.89 (0.52)	1.66 (0.97)
Al_{NH_4} (g kg^{-1})	0.0353	0.70 (0.32) a	0.48 (0.26) b
Al_{p-cu} (g kg^{-1})	0.0110	4.08 (0.99) b	5.78 (2.30) a
Al_{cu-NH_4} (g kg^{-1})	-	1.18 (0.29)	1.18 (0.75)
Total Al (mg L^{-1})	<0.0001	14.7 (5.93) a	5.20 (3.05) b
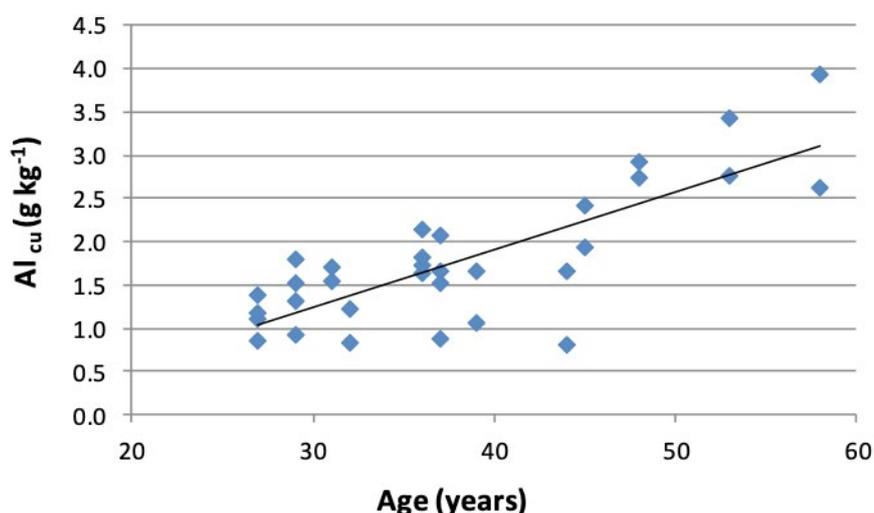
Figure 3. Relationship between plantation age and organo-aluminium complexes of low and moderate stability.

The highly stable complexes formed with organic matter (Al_{p-cu}) varied between 4.08 and 5.78 g kg^{-1} and were significantly higher in the 20-40 cm layer than in the 0-20 cm layer.

The concentration of complexes of moderate and low stability (Al_{cu-NH_4}) was 1.18 g kg^{-1} in both soil layers (**Table 3**). The Al_{cu-NH_4} was significantly and positively correlated with plantation age in both layers (r = 0.65, p = 0.0068 for the upper layer, and r = 0.78, p = 0.0004 for the lower layer) (**Figure 4**).

Aluminium extracted by NH_4Cl ranged between 0.48 and 0.70 g kg^{-1}. The concentration of Al_{NH_4} was significantly higher in the upper soil layer (p = 0.0353) (**Table 3**). The concentration of AlNH4 in both soil layers was significantly and positively correlated with plantation age (r = 0.76, p = 0.0006 for the upper layer and r = 0.84, p < 0.0001 for the lower layer) (**Figure 5**)

The concentration of total Al in soil solution varied from 5.2 to 14.7 mg L^{-1} and was significantly
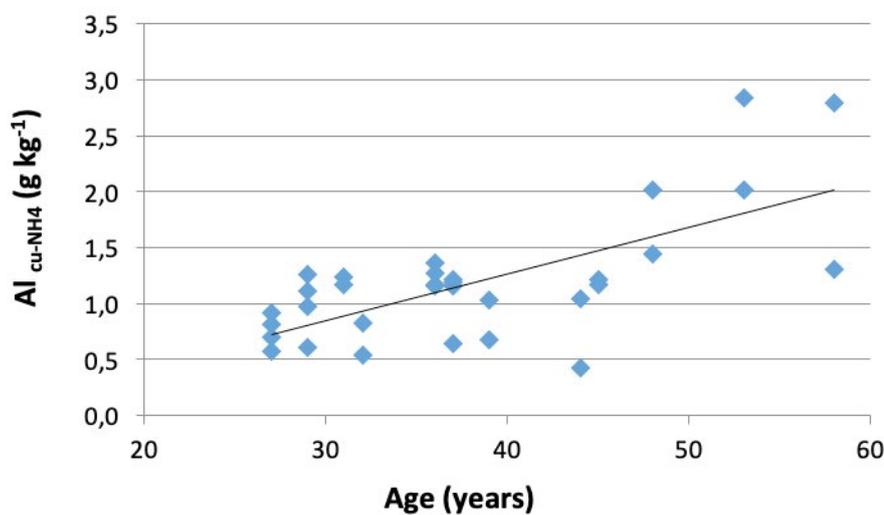
Figure 4. Relationship between plantation age and concentration of medium and low stability complexes.
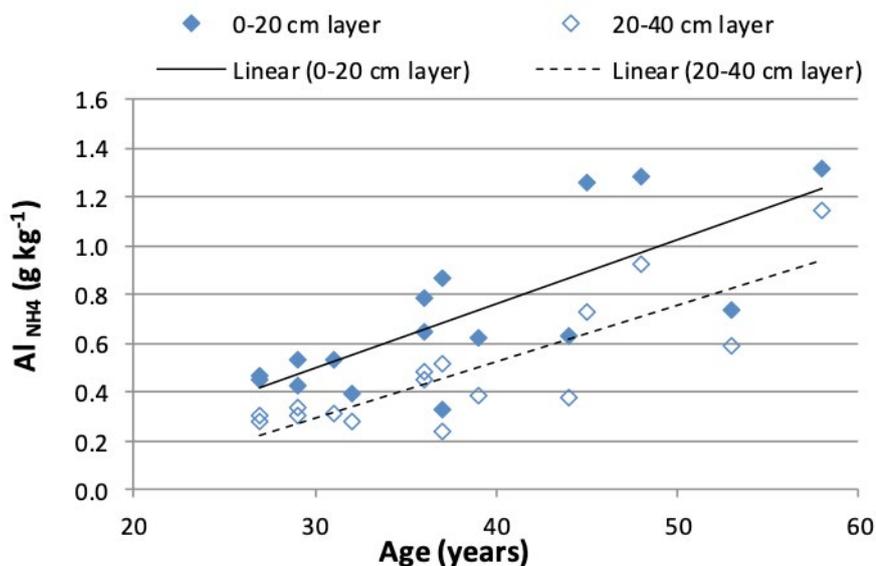
Figure 5. Relationship between plantation age and concentration of exchangeable Al in the upper soil layer (0-20 cm) and in the lower soil layer (20-40 cm).

higher in the 0-20 cm layer, coinciding with the low pH of this layer (**Tables 2 and 3**). The total soluble Al in both soil layers was positively and significantly correlated with stand age (r = 0.61, p = 0.0113 for the upper layer and r = 0.60, p = 0.0142 for the lower layer) (**Figure 6**).
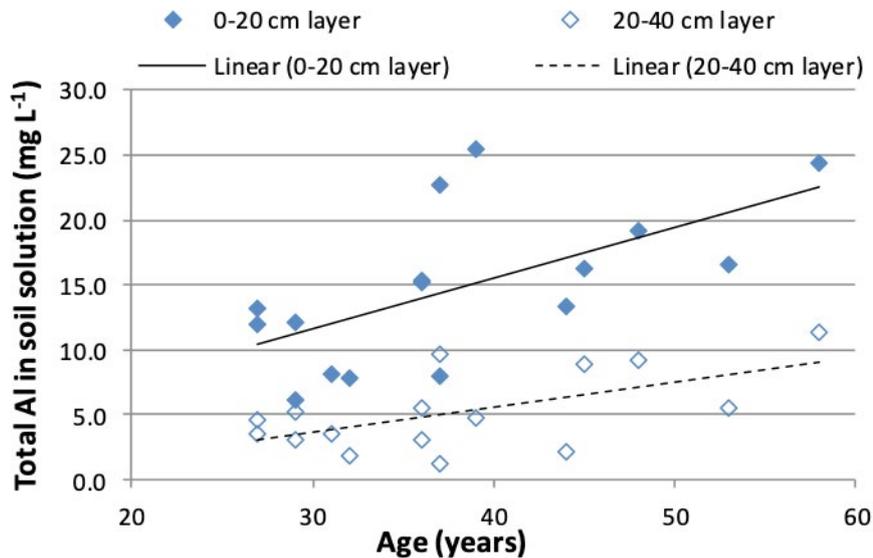
Figure 6. Relationship between plantation age and total Al concentration in soil solution for the upper layer (0-20 cm) and the lower layer (20-40 cm).

3.3. Regression analysis: Al forms

The stepwise linear regression showed that the plantation age, pH_{KCl} and total C explained 70% of variance in total non-crystalline Al (Al_o), all with positive coefficient in the equation (**Table 4**). For the organically bound Al (Al_p), plantation age, pHwater and total C explained 67% of variance, all with a positive influence (**Table 4**). Plantation age explained 62% of the variance in organo-Al complexes of low and moderate stability (Al_{cu}), reaching 74% when total C was included in the stepwise linear regression and 81% when age, C and pH_{water} were included. All variables had a positive effect on the regression (**Table 4**). Total C was the main parameter explaining the variance in exchangeable Al (88%), whereas age contributed to an additional 2% and the pH_{KCl} a 3% more with the last one entering negatively in the equation (**Table 4**). Most of the variation in total Al in soil solution was explained by pH_{water} (64%), with a negative influence, increasing to 67% when total C was included. Plantation age was included in all equations as an explanatory variable for the different Al forms, except total soluble Al in soil solution (**Table 4**).

4. Discussion

The soils under study were acidic and rich in organic matter. The concentrations of Ca, Mg and K (Ca < 0.3 $cmol_{(+)}$ kg^{-1}, Mg < 0.15 $cmol_{(+)}$ kg^{-1}, K < 0.12 $cmol_{(+)}$ kg^{-1}) in the lower layer can be considered deficient for forest plantations, and the concentrations in the upper layer intermediate (Ca 0.3-1.0 $cmol_{(+)}$ kg^{-1}, Mg 0.15-0.25 $cmol_{(+)}$ kg^{-1}, K 0.12-0.20 $cmol_{(+)}$ kg^{-1}) according to Bonneau (1995). The soils in all plots were "alic" (%Al > 60%) and the Al saturation was always higher than 80% in both layers (Buol et al. 1975), indicating that these soils would suffer stress due to excess aluminium as base saturation is less than 15% (Cronan and Grigal 1995).

The organic matter content increased with plantation age due to continuous deposition of plant debris. The positive correlation between eCEC and plantation age may be related to the increase in organic matter in the soil, thus explaining the strong correlation observed between C and eCEC (r = 0.88, p < 0.0001 in

Table 4. Regressions between different Al forms, plantation age and soil parameters

Dependent variable*		Independent variable				
		Variable	Estimate	p level	R2	VIF
Al_o (mg kg^{-1})	-29339 (p < 0.0001)	Age	94.8	0.0021	0.3162	1.89
		pH_{KCl}	6780.8	< 0.0001	0.5279	3.85
Al_p (mg kg^{-1})	-39312 (p < 0.0001)	C	548.5	0.0006	0.7057	5.35
		Age	73.3	0.0778	0.2590	2.10
		pH_{water}	8136.6	< 0.0001	0.4984	3.27
Al_{cu} (mg kg^{-1})	-6144.6 (p = 0.0015)	C	699.7	0.0005	0.6759	5.09
		Age	27.5	0.0099	0.6203	2.10
		C	228.2	< 0.0001	0.7427	5.09
Al_{NH_4} (mg kg^{-1})	1265.3 (p = 0.0056)	pH_{water}	1136.1	0.0027	0.8146	3.27
		C	46.4	0.0002	0.8800	5.35
		Age	8.6	0.0009	0.9020	1.89
Al_t (mg L^{-1})	61.48 (p = 0.0032)	pH_{KCl}	-320.1	0.0020	0.9307	3.85
		pH_{water}	-11.59	0.0032	0.6417	2.78
		C	0.60	0.1161	0.6714	2.78

* Aluminium forms extracted by acid ammonium oxalate (Al_o), sodium pyrophosphate (Al_p), $CuCl_2$ (Al_{cu}) and NH_4Cl (Al_{NH_4}) and total Al in soil solution (Al_t). Age: plantation age. C: Carbon. VIF: variance inflation factor.

the upper layer and r = 0.96, p < 0.0001 in the lower layer). Although the decrease in pH with age would lead to a diminution in the eCEC due to a decrease in the negative charge of organic matter, the increase in organic C supply appears to have a greater effect on the eCEC value. Although the pH of the older plots is lower, the value reached is still high enough to allow the deprotonation of the carboxylic groups of organic matter as it increases when pH raise above 4, according to Marschner et al. (2005).

The concentrations of the different Al forms in all soils followed the order $Al_p> Al_o> Al_{cu}> Al_{NH_4}$. The same order of abundance has also been observed in other Galician soils with *Pinus pinaster* (Eimil-Fraga et al. 2015) and in acidic soils with below *Pinus massoniana* and *Cunninghamia lanceolate* (Larssen et al. 1999). The greater extraction efficiency of Al by sodium pyrophosphate than by ammonium oxalate has frequently been observed in organic matter rich soils (Camps Arbestain et al. 2003; García-Rodeja et al. 2007; Ferro-Vázquez et al. 2014; Eimil et al. 2015), possibly related to the predominance of organo-aluminium complexes over inorganic compounds of low crystallinity

or/and to the extraction of inorganic forms of low crystallinity by pyrophosphate (Kononova and Belchikova 1970; Kaiser and Zech 1996). Alo and Alp were significantly correlated with organic matter content in the lower soil layer (r = 0.77, p = 0.0004 and r = 0.66 p = 0.0051 respectively), indicating that both fractions (Al_o and Al_p) are associated with the soil organic matter, and highlights the important role of the organic matter in preventing the evolution of non-crystalline Al towards more crystalline forms (García-Rodeja et al. 1987; Álvarez et al. 2002). The concentrations of Alo and Al_p were much lower than those obtained by Eimil-Fraga et al (2015) in soils from young plantations of the same species (*Pinius pinaster*), as well as in other soils also developed on acidic rocks (granites and slates).

Highly stable organo-aluminium complexes (Al_{p-cu}) predominated over moderate and low stability complexes (Al_{cu}) in all plots (73.5% Al_{p-cu} and 26.5% Al_{cu}). Moderate and low stability complexes were more abundant in the plots with older trees, reflected by the strong correlations between Alcu and plantation age (r = 0.84) and Al_{cu-NH_4} and plantation age (r = 0.65).

The Al_{NH_4} was also higher in the plots with older trees (r = 0.76, p = 0.0006 for the upper layer and r = 0.84, p < 0.0001 for the lower layer) (**Figure 5**). The results obtained for exchangeable Al in terms of amount are consistent with those reported by Boruvka et al. (2005) for forest species. However, in the present study, most of the Al forms in the solid phase were more abundant than those observed in other studies in forest soils from other parts of Europe (Sweden, Poland) possibly related to different soil characteristics, derived from very different geology and climatic conditions (Berggren and Mulder 1995; Walna et al. 2005; Frankowski et al. 2013). The total Al in the soil solution was also more abundant in the plots with older trees (**Figure 6**) and the concentration was higher than the values reported in other forest soils in Galicia with pine presence (Fernández-Sanjurjo et al. 1998; Camps-Arbestain et al. 2004; Álvarez et al. 2005; Eimil-Fraga et al. 2015). The values obtained in the present study were also higher than those previously observed for different types of soils and forest species (Boudot et al. 2000; Dlouhá et al. 2009; Tejnecky et al. 2010; Collignon et al. 2012). These comparisons show that *Pinus pinaster* has a great capacity to resist high Al concentrations in the soil solid phase and in soil solution.

We previously studied the Al forms in soils under young *Pinus pinaster* plantations and developed on the same type of rock (Eimil-Fraga et al. 2015). The difference in the content of the Al forms and in the stability of the organo-aluminium complexes observed in both studies may be explained by differences in the plantation age. In the previous study (op.cit.), the plantations were all between 11 and 13 years old, while in the present study they are between 27 and 58 years old. In both young and old plots, there were clear differences in the C/N ratio (15 and 23, respectively) and in the pH value, which was slightly higher in the younger plantations (4.85 vs 4.57). The effect of the C/N ratio on Al forms is associated with the degree of humification of the organic matter, which is lower when the C/N ratio is high. The higher pH and the higher degree of humification of organic matter (lower C/N) favour the formation of highly stable organo-aluminium complexes. Therefore, it seems that the greater reactivity of organic matter in young plantations favours the formation of organo-aluminium

complexes in the solid phase of the soil and that these complexes are more stable.

Focusing on plantations between 27 and 58 years in the present study showed that Al_o, Al_p and Al_{cu} increased with plantation age. These results can be explained by taking into account that the influence of the plantation age is not the same in all Al forms. Thus, while plantation age explains 31% and 26% of the variance in Al_o and Al_p respectively, it explains up to 62% of the variance in complexes of moderate and low stability (Al_{cu-NH_4}). The pH and the organic matter content are the other parameters contributing to explaining the concentrations of these Al forms. We observed a significant increase in the organic matter content and a decrease in soil pH in the soils as plantations become older, while the C/N ratio did not vary significantly. The greater amount of organic matter may explain the increase in non-crystalline Al (Al_o and Al_p) in the soils in older plantations in this age range, but the higher acidity of the plots in these plantations would cause a decrease in the reactivity of the organic matter. The higher acidity and the lower reactivity favour the formation of more labile Al-organic matter complexes, at the expense of more stable complexes (Eimil-Fraga et al. 2015), and they also favour an increase in exchangeable Al and Al in solution that can be observed in older plantations. Thus, at lower soil pH, as in the older plantations, the solubility of Al increases and some of the dissolved Al can interact with the organic matter in the soil to form labile complexes, some will be exchangeable Al and some may remain in soil solution (Mulder et al. 1989).

On the other hand, the more acidic pH and the higher C/N ratio of the upper soil layer (**Table 2**) would explain the lower presence of highly stable organo-aluminium complexes and the higher concentration of exchangeable Al and Al in soil solution in relation to the lower soil layer.

The regression models improved our previous findings in four young *Pinus pinaster* plots (Eimil-Fraga et al. 2015). In the present study, the equations were fitted with a larger set of stands, yielding more robust findings regarding prediction of Al forms. The R^2 values were higher in all linear regressions, and the input variables were very similar to the equations for

young plots, and the difference in plantation age explained an important part of the variability in Al forms in the present study.

The study findings showed a clear increase in all Al fractions with plantation age. Most previous studies involving Al fractionation in forest soils have not considered the age of the tree species. Thus, although Álvarez et al. (2002), Walna et al. (2005) and Collignon et al. (2012) reported the stand age, they did not evaluate the relationships between this parameter and the different Al forms. The present findings highlight the need to adapt pinewood management by avoiding excessive lengthening of rotations or by promoting the natural regeneration of broadleaf species as the pine stands reach a mature stage of development.

The results of the present study cannot be fully compared with those of other studies because of the lack of previous studies relating plantation age and soil properties, in particular, Al fractions in the solid phase and the total Al in soil solution.

5. Conclusions

The study findings clearly show a relationship between soil aluminium chemistry and plantation age and thus contribute to a better understanding of the growth of *Pinus pinaster* in acidic soils. Older plots of *Pinus pinaster* may have a higher risk of Al toxicity, because they are more acidic and because of an increase in the low stability of the organo-aluminium complexes and the exchangeable Al, which could cause an increase in Al in soil solution. The high yields of young and adult plantations of *Pinus pinaster* in these poor and acidic soils confirms the resistance of this species to high Al concentrations, even in adult plantations, in which Al concentrations are particularly high.

6. Acknowledgements

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