SCAP
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Characterization of soil phosphorus in different land use over calcareous soils by chemical extraction methods and ^{31}P-NMR spectroscopy

Caracterización del fósforo edáfico por espectroscopía de ^{31}P-RMN en suelos calcáreos bajo diferentes sistemas de uso

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ABSTRACT

Many natural ecosystems around the world have been altered by human activities such as clearing for agriculture and pasture or timber harvesting. These land-use changes modify soil P status and dynamics. To evaluate the effects of land-use change on P chemical forms we used chemical fractionation and ^{31}P-NMR spectroscopy. Soil samples were collected in calcareous moors in Castilla y León region (Spain) at 4 different depths and in three different land-use systems: natural holm-oak forest, afforested *Pinus halepensis* forest, and agricultural land. Our results indicated that land-use change from holm-oak forest to cereal crops increased orthophosphate P concentrations and P availability due to phosphate fertilization. However, soil phosphorus distribution in *Pinus halepensis* forest was similar to that in native oak forest.

Keywords: Soil Phosphorus, P availability, Phosphorus pools, AEM-P, Land-use change

RESUMEN

Muchos ecosistemas naturales de todo el mundo han sido alterados por actividades humanas, como la tala para la agricultura y el pastoreo o la extracción de madera. Estos cambios en el uso del suelo modifican el estado y la dinámica del P del suelo. Para evaluar los efectos del cambio de uso del suelo en las formas químicas de P, utilizamos el fraccionamiento químico y la espectroscopia ^{31}P-NMR. Se recogieron muestras de suelo en páramos calcáreos de la región de Castilla y León (España) a 4 profundidades diferentes y en tres sistemas diferentes de uso del suelo: encinar natural, pinar de repoblación y suelo agrícola. Nuestros resultados indicaron que el cambio de uso del suelo del bosque de encina a cultivos de cereal aumentó las concentraciones de ortofosfato y la disponibilidad de P debido a la fertilización con fosfato. Sin embargo, la distribución de fósforo en el suelo en el bosque de pino fue similar a la del bosque nativo de encina.

Palabras clave: Fósforo edáfico, Disponibilidad de fósforo, Reservas de fósforo edáfico, AEM-P, Cambio de uso del suelo

INTRODUCTION

Phosphorus (P) is an essential element for all organisms and is usually a limiting nutrient for plant growth in many soils (McDowell & Stewart, 2006) because it can only be obtained by uptake from soil via plant roots as orthophosphate (Pierzynski *et al*., 2005).

Organic P (Po) can comprise 35–65% of soil P (Martín-Sanz *et al*., 2021) and is an important P source for plants in both natural and managed environments (Turrión *et al*., 2007). Despite the importance of soil Po, its chemical nature and dynamics remain poorly understood, partly due to analytical limitations. These difficulties are posed mainly by the broad compound classes in which soil Po forms are grouped (Condron *et al*., 2005). Full chemical speciation to identify specific P forms requires either extraction for individual forms or advanced spectroscopic techniques such as ^{31}P nuclear magnetic resonance (^{31}P-NMR) spectroscopy.

Over the past century, many natural ecosystems around the world have been modified from their original status by anthropogenic disturbances, including degradation practices such as poor logging, forest fires, or clearing for agriculture and pasture. This land-use change can greatly modify biogeochemical P cycle and status. Studies of the effects of land use change on the soil P forms in calcareous soils are scarce. However, calcareous soils are of particular interest because the high calcium saturation tends to keep them with low organic matter content (von Wandruzska, 2006) and very low P disponibility (Taalab *et al*., 2019).

In this context, our objective was to improve our understanding of soil P chemistry and the putative effect of land-use changes in its dynamics and forms. Specifically, we aim to characterize soil P forms in calcareous moor soils under three different land-use systems: native forest (*Quercus ilex subsp. ballota*), forest plantation (*Pinus halepensis*) and agricultural land (cereal crops).

MATERIAL AND METHODS

Study area

This study was carried out at a high flat land (moor) of a calcareous geology in the Castilla y León region (Spain). Under semiarid climate, the native vegetation is holm-oak forest (*Quercus ilex subsp ballota*). However, during the nineteenth century most of these forests were converted into agricultural land, some of which remain until now. During the 1950s afforestation with *Pinus halepensis* was carried out on the less dense forest areas and in abandoned agricultural lands. Therefore, we selected these three types of land-use in these calcareous moors.

Soil sampling

Three plots were used. In each plot, three adjacent points of the three different land uses studied (agricultural, pine forest and natural oak forest) were selected. Four depth levels were considered (2–5 cm, 10–15 cm, 20–30 cm and 30–40 cm) and sampled at one sampling point randomly selected along the experimental area.

Soil analysis

Sieved soil samples (<2 mm) were analysed. Soil pH was measured in a 1:2.5 soil:water suspension using a glass electrode. Total organic C (TOC) and total N were determined using a LECO CHN 2000 Analyzer. Olsen P was also determined.

Phosphorus-31NMR was used to characterize the structural composition of alkali-soluble P (Turrión *et al*., 2010). Phosphorus was determined in all the extracts by the ammonium molybdate-ascorbic acid method (Murphy & Riley, 1962). The ^{31}P-NMR spectra were recorded with a Bruker DPX-300 spectrometer operating at 121.49 MHz using a 90-degree pulse length, an acquisition time of 0.96 s and a total accumulation time of 2 h and 3800 scans.

Statistical analysis

A two fixed-factor factorial experimental design was considered. The first factor was the land use effect on soil properties (agriculture, pine forest and holm oak forest), while the second factor was the soil depth effect, with four samplings along the profile: 2–5 cm, 5–10 cm, 20–30 cm and 30–40 cm, including also the interaction between factors. General linear mixed models were fit with PROC MIXED procedure in SAS software.

RESULTS AND DISCUSSION

Land use effect on soil properties

Significant effects of land use and depth were observed in all soil parameters considered, while land use by depth interaction was not significant for anyone (Table 1). Total organic carbon concentration (TOC) in soil decreased significantly with depth and was higher in the natural oak forest. Total nitrogen concentrations (N) were lower in the agricultural land and pine forest than in oak forest soils at all depths studied decreasing also with depth (data not shown). The pine forest soil showed significantly higher C/N ratio than soils from the other two land uses. Depth was also significant (Table 1), being this ratio lower on the surface than in deeper soil layers in all land uses.

Table 1 - ANOVA significance levels of the effects of land use, depth, and land use by depth interaction for the soil properties studied

	pH	TOC	N	C/N
ANOVA				
Use	**	**	*	***
Depth	*	*	**	*
Use x depth	ns	ns	ns	ns

****p*<0.001, ***p*<0.01, **p*<0.05, ns: not significant.

Soil P in the studied land-uses

Total P concentrations in the NaOH-EDTA extract of the agricultural soil ranged from 16.8 to 101.1 mg*kg^{-1}, whereas they ranged from 22.9 to 37.6 mg*kg^{-1} and from 9.42 to 34.03 mg*kg^{-1} in oak and pine forest soils, respectively. In the cultivated soil, total inorganic P represented around 75% of total P in the first layers of the profile, decreasing in the holm-oak and *Pinus halepensis f*orest soils to around 20%. However, total organic P mainly represented the total P of this alkaline extract in oak and pine forest soils, representing around 83% of total P in the first layers of those profiles, but just 30% of total P in the first layers of the agricultural soil.

Our results indicate that only a small amount of P remains in the soil after this sequential extraction, confirming previous works suggesting that most of the total P is recovered by EDTA–NaOH extraction for soils (Turner *et al.*, 2005). Labile P forms concentrations (AEM-P and Olsen-P) were significantly higher in the cultivated soil (data not shown), with the greatest effects of land use in the surface soil layers (2–15 cm).

Inorganic orthophosphate represented around 62% (at 2–5 cm) of total P extracted by NaOH-EDTA in the cultivated land soil and around 20% (at 2–5 cm) in pine and holm-oak forests soils. Phosphate monoesters was the main functional class of Po in soils from all land uses, representing 32%, 68%, and 64% of Po in the surface samples in the agricultural land, pine forest and oak forest, respectively. Moreover, monoesters concentrations decreased significantly with depth in all the land uses (*P<0.001*). Phosphate diesters include nucleic acids (DNA and RNA), phospholipids, and teichoic acids, and they represented between 5 to 12% of the total P extracted by NaOH-EDTA in the studied soils. Phosphate diesters usually constitute less than 10% of soil Po, but higher proportions have been detected in some forest soils (Cade-Menun et al., 2000). Diester P is more labile and accessible to microbial and enzymatic attack compared with monoester P (Zhu *et al.*, 2013) and also, diester dominates Po inputs to soil from plants and microbes (Turner *et al.*, 2005). Both reasons can explain the lower amount of diaester P observed in all the studied land use systems comparing to the monoester P forms. DNA-P concentrations were similar and low in all depths studied and in all the land uses. It is widely accepted that an increase or decrease in the proportion of diester P in soils is associated with corresponding changes in microbial P compounds (Cade-Menun *et al.*, 2017). In contrast, easily mineralizable phosphate diesters in soils can accumulate when conditions for microbial decomposition become limited

Table 2 - Concentration of different soil P forms measured by $_{31}$P-NMR in three distinct uses. Relative contribution (%) of each P form to total P in brackets

	Orthophos.-P	Monoesters-P	Phosphol.-P	DNA	Pyrophos.-P
			(mg kg^{-1})		
Cultivated land					
2-5 cm	62.74 (62)	32.04 (32)	2.61 (3)	1.91 (2)	1.81 (2)
10-15 cm	43.44 (47)	40.09 (44)	3.44 (4)	3.18 (4)	1.99 (2)
20-25 cm	21.80 (47)	20.63 (45)	1.62 (4)	1.83 (4)	0.42 (1)
30-40 cm	1.90 (11)	12.55 (75)	1.40 (8)	0.60 (4)	0.35 (2)
***Pinus halepensis* reforestation**					
2-5 cm	7.01 (21)	23.12 (68)	1.00 (3)	1.70 (5)	1.20 (3)
10-15 cm	3.61 (18)	14.87 (74)	0.65 (3)	0.68 (3)	0.40 (2)
20-25 cm	2.54 (16)	12.12 (76)	0.50 (3)	0.56 (4)	0.16 (1)
30-40 cm	1.04 (11)	7.55 (81)	0.29 (3)	0.51 (5)	0.03 (0)
Holm-oak natural forest					
2-5 cm	7.60 (20)	24.11 (64)	1.52 (4)	1.28 (3)	3.06 (8)
10-15 cm	5.14 (20)	18.41 (70)	0.78 (3)	1.03 (4)	0.93 (3)
20-25 cm	1.81 (12)	11.93 (77)	0.65 (4)	0.47 (3)	0.60 (4)
30-40 cm	7.94 (35)	13.07 (57)	0.88 (4)	0.79 (3)	0.22 (1)

by climate (Makarov *et al*., 2002), or by acid soil pH and high content of free Al (Turrión *et al*., 2000), but this is not the case of the studied soils. Pyrophosphate-P was also very similar among land uses (not significant), but significant differences were observed with depth (*P<0.05*) (Table 2).

CONCLUSIONS

Land use change from holm oak forest to cereal crops in the calcareous moor studied affected soil P pools increasing orthophosphate P concentrations and P availability, as expected, due to the phosphate fertilization. However, afforestation with *Pinus halepensis* throughout the last 60 years has resulted to a phosphorus distribution like the existing under the native oak forest vegetation. The low concentration of diester P in all the studied land systems further reveals that in these calcareous soils the microbial activity is high.

REFERENCES

Cade-Menun, B.J. (2017) - Characterizing phosphorus forms in cropland soils with solution 31P-NMR: past studies and future research needs. *Chemical and Biological Technologies in Agriculture*, vol. 4, art. 19. https://doi.org/10.1186/s40538-017-0098-4

Condron, L.M.; Turner, B.L. & Cade-Menun, B.J. (2005) - Chemistry and dynamics of soil organic phosphorus. *In*: Sims, J.T. & Sharpley, A.N. (Eds.) - *Phosphorus, agriculture and the environment*, vol. 46, p. 87–121. Madison: Soil Science Society of America.

Makarov, M.I.; Malysheva, T.I.; Vladychenskii, A.S. & Tseikh, V. (2002) - Phosphorus Compounds in Primitive Soils on Mantle Loam under Various Phytocenoses. *Eurasian Soil Science*, vol. 35, n. 9, p. 934–938.

Martín-Sanz, R.C.; Pando, V.; Bueis, T. & Turrión, M.B. (2021) - Influence of Soil Properties on P Pools and Its Effect on Forest Productivity in Mediterranean Calcareous Soils. *Forests*, vol. 12, n. 10, art. 1398. https://doi.org/10.3390/f12101398

McDowell, R.W. & Stewart, I. (2006) - The phosphorus composition of contrasting soils in pastoral, native and forest management in Otago, New Zealand: sequential extraction and 31PNMR. *Geoderma*, vol. 130, n. 1-2, p. 176–189. https://doi.org/10.1016/j.geoderma.2005.01.020

Murphy, J. & Riley, J.P. (1962) - A modified single solution method for the determination of phosphate in natural water. *Analytica Chimica Acta*, vol. 27, p. 31–36. https://doi.org/10.1016/S0003-2670(00)88444-5

Pierzynski, G.M.; McDowell, R.W. & Sims, J.T. (2005) - Chemistry, cycling and potential movement of inorganic phosphorus in soils. *In*: Sims, T. & Sharpley, A.W. (Eds.) - *Phosphorus: Agriculture and the Environment*, vol. 46, p. 53–86. Madison: Soil Science Society of America.

Taalab, A.S.; Ageeb, G.W.; Siam, H.S. & Mahmoud, S.A. (2019) - Some Characteristics of Calcareous soils. A review. *Middle East Journal of Agriculture Research*, vol. 8, n. 1, p. 96-105.

Turner, B.L.; Cade-Menun, B.J.; Condron, L.M. & Newman, S. (2005) - Extraction of soil organic phosphorus. *Talanta*, vol. 66, n. 2, p. 294–306. https://doi.org/10.1016/j.talanta.2004.11.012

Turrión, M.B.; Glaser, B.; Solomon, D.; Ni, A. & Zech, W. (2000) - Effects of deforestation on phosphorus pools in mountain soils of the Alay range, Khyrgyzia. *Biology and Fertility of Soils*, vol. 31, p. 134-142. https://doi.org/10.1007/s003740050636

Turrión, M.B.; Lafuente, F.; Aroca, M.J.; López, O.; Mulas, R. & Ruipérez, C. (2010) - Characterization of soil phosphorus in a fire-affected forest Cambisol by chemical extractions and $_{31}$P-NMR spectroscopy analysis. *Science of the Total Environment*, vol. 408, n. 16, p. 3342-3348. https://doi.org/10.1016/j.scitotenv.2010.03.035

Turrión, M.B.; López, O.; Lafuente, F.; Mulas, R.; Ruipérez, C. & Puyo, A. (2007) - Soil phosphorus forms as quality indicators of soils under different vegetation covers. *Science of the Total Environment*, vol. 378, n. 1-2, p. 195-198. https://doi.org/10.1016/j.scitotenv.2007.01.037

von Wandruszka, R. (2006) - Phosphorus retention in calcareous soils and the effect of organic matter on its mobility. *Geochemical Transactions*, vol. 7, art. 6. https://doi.org/10.1186/1467-4866-7-6

Zhu, R.; Evans, T.W.; Wörmer, L.; Lin, Y.-S.; Zhu, C. & Hinrichs, K.-U. (2013) - Improved sensitivity of sedimentary phospholipid analysis resulting from a novel extract cleanup strategy. *Organic Geochemistry*, vol. 65, p. 46– 52. https://doi.org/10.1016/j.orggeochem.2013.10.002