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Phosphorus Stock Depletion and Soil C:N:P Stoichiometry Under Annual Crop Rotations and Grassland Management Systems Over 13 Years

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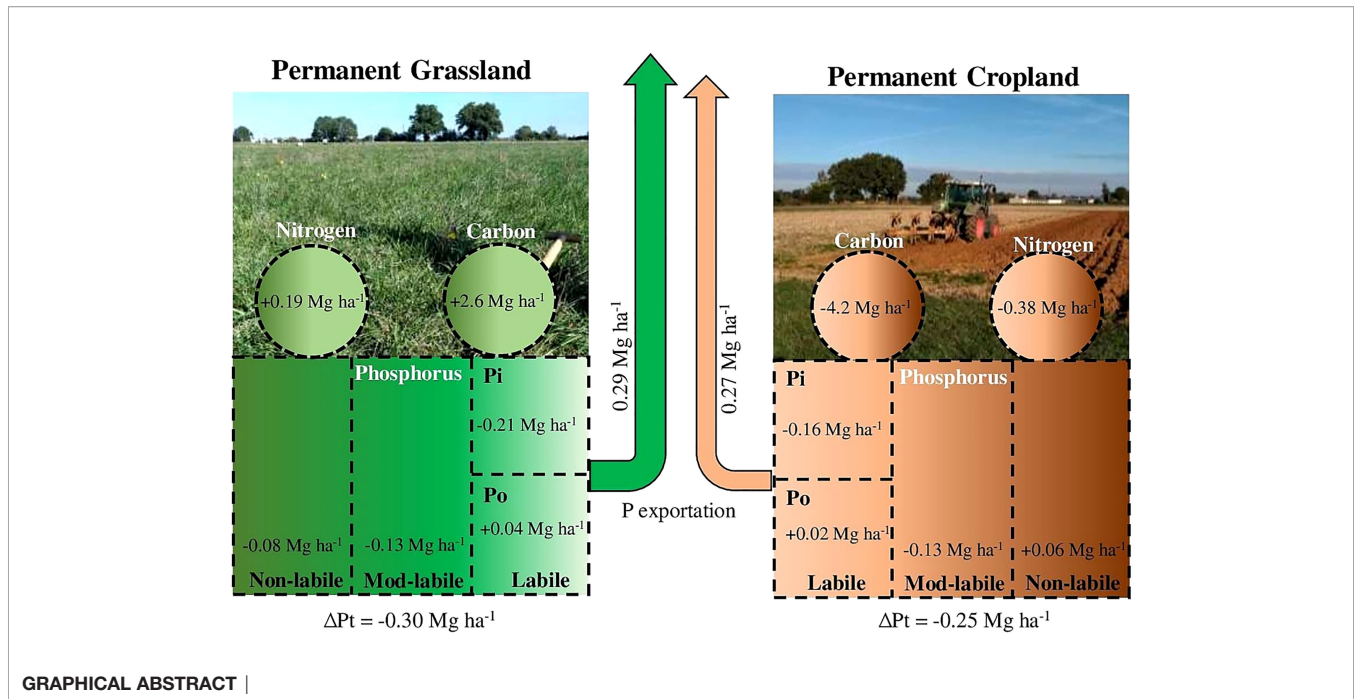
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Phosphorus (P) nutrition is essential to both plant yield and soil organic matter (SOM) input. However, continuous extraction of P by plants and biomass harvesting can lead to soil P stock depletion, a reduction in crop yields and ultimately a reduction in organic matter input to the soil. In this work, we analysed P, C and N stock trends in the 0-30 cm topsoil layer cultivated with permanent cropland (CC) and mowed permanent grassland (GG) for 13 years. In addition, we characterized the changes in P organic forms by using ³¹P-NMR. The results showed that the amount of P exported within 13 years was 10% greater in GG than in CC (295 and 268 kg ha⁻¹, respectively). The total P stocks decreased under both the CC and GG management systems (0.30 and 0.25 Mg ha⁻¹, respectively). This depletion was mainly observed in total Pi forms, which recorded reductions of 0.75 and 0.29 Mg ha⁻¹ in GG and CC, respectively. The total Po stock increased by 42.6% in GG; these results were consistent with the increase in C and N stocks in GG (2.6 and 0.19 Mg ha⁻¹, respectively) and their reduction in CC (-4.2 and -0.38 Mg ha⁻¹, respectively). Although P depletion mainly affected the P pool presenting the highest lability (labile P), this depletion did not have a negative effect on plant yield after 13 years due to the buffering capacity of P pools presenting less lability (moderately labile P). Mowing permanent grasslands led to a change in the pool of labile P from inorganic to organic forms and an increase in soil C and N stocks. Based on the ³¹P-NMR technique, permanent grasslands significantly reduced α -glycerophosphate and increased myo-IHP and adenosine monophosphate more than the permanent cropland. Although there was no significant decrease in productivity, the depletion of available P should be monitored over time, especially in mown permanent grassland crops, to prevent potential nutrient stress.

Keywords: forage, ³¹P-NMR, P export, P pools, C and N stocks, SOM, long-term



INTRODUCTION

Phosphorus (P) is a key element in all life cycles (1); it comprises approximately 0.2% of plant dry matter (2) and is a constituent of proteins and nucleic acids, in addition to participating in energy transfer in plants and animals (1, 3). Consequently, P nutrition is essential for enabling plants to incorporate carbon (C), produce dry matter and increase soil C stocks (i.e., soil organic matter (SOM)). P uptake occurs through the root system; therefore, satisfactory maintenance of soil P availability is essential for plant growth (4, 5). Soil P is found in inorganic (Pi) and organic (Po) forms. Inorganic P forms include the orthophosphates HPO_4^{2-} and H_2PO_4^- in soil solution. They bind to cations, such as calcium, and form geogenic or anthropogenic calcium phosphates. Furthermore, they are adsorbed by the inner sphere of functional groups of clay minerals and of Fe and Al oxyhydroxides (6, 7). Organic P binds to two different carbon atoms to primarily form orthophosphate monoesters (e.g., inositol phosphates and adenosine monophosphate) and orthophosphate diesters (e.g., nucleic acids and phospholipids) that constitute soil organic matter and soil microbial biomass (8–10).

Both P forms are simultaneously found in the soil, but they have different availabilities for plant uptake (P lability). P availability quantification is commonly assessed through sequential extractions with chemical reagents that have increasing extraction force (e.g., sequential extraction proposed by Hedley (11)) and through grouping into P pools, namely, labile P, moderately labile P and nonlabile P. Plants uptake P coming from the most available P pool (labile), which is buffered by that presenting less lability (12). In addition, the turnover of each P pool decreases as P lability decreases, which can take from

minutes to hundreds of years (13). Thus, soil P depletion due to agricultural activity is expected to be noticeable in most labile pools in the short term, and in the medium to long term in less labile pools. Available P buffering varies depending on the soil type and the adopted management system (14). Soils from tropical regions, which present high weathering intensity, may present high legacy P but low P availability to plants due to high P adsorption to the soil minerals (15, 16). However, P availability in temperate soils is facilitated by soil mineralogy resulting from lower pedogenic evolution compared to tropical soils.

Although P is mainly absorbed by plants in its orthophosphate ion form (2), the presence of Po in the soil is remarkably important to reduce P inner-sphere adsorption to clay minerals and oxides and to mobilize recalcitrant P (15, 17–19). Pi transformation into Po occurs when plants and microorganisms mobilize Pi into their tissues. Some plant species (e.g., *Lolium* sp. and *Lupinus* sp.) can mine and remobilize P by incorporating it into their tissues when grown in soil with a high P stock where most P is adsorbed to the inorganic constituent by inner-sphere complexation (20, 21). P returns to the soil after plant death, mainly when its organic form returns to SOM. Therefore, the presence of Pi or Po in the soil is determined by the SOM content, which can be changed depending on the adopted soil management system (22, 23). The cultivation of annual crops based on soil ploughing decreases SOM (24, 25), even with NPK fertilization (22, 26). However, varying the species of cultivated plants, such as the introduction of legume plants in the production system, favours SOM and coupling processes involving P, C and N cycles (22, 27, 28). Moreover, soils cultivated for pasture that are NPK fertilized can increase, or preserve, SOM stocks over time (29); consequently, they tend to preserve the Po content and the coupling of the C:N:P ratio. Changes in the stoichiometry C:N:P

can be indicative of nutrient restriction and the disbalance of the system equilibrium (30).

SOM dynamics are a key factor driving cycles and balancing C, N and P and are directly dependent on the adopted soil management system. The Po mineralization step before P uptake by plants is necessary, but this process changes depending on the recalcitrance of the organic compound. Accordingly, orthophosphate monoesters have lower turnover than orthophosphate diesters due to their chemical bonding to soil colloids (19, 31). This behaviour may induce higher accumulation of the Po form of monoesters than the diesters form in SOM. Quantifying the organic natural P is not possible using the common P fractionation methods (i.e., Hedley method). For this purpose, the use of the ^{31}P -NMR technique is necessary and allows an analysis of the potential of soil P organic compound degradation.

Overall, soil with a high P stock due to fertilization input or soil parent material may fulfil plant demand and help the plants maintain their yields for longer periods, even without P fertilization. However, a lack of phosphate fertilization in association with constant P export leads to a negative soil P balance, leading to a decrease in P stocks and availability (32, 33). This process can cause a significant reduction in plant yield; consequently, it affects SOM accumulation and maintenance on a time scale that depends on P export and the level of initial P stock availability in plants.

Thus, understanding P depletion in soil subjected to different management systems (grassland or cropland systems) is one of the main strategies for avoiding the negative effects of P deficiency on plant growth and yield. The main scientific questions of the present study are as follows: How does the soil P stock change over time in forage and annual crop soil management systems, without any P fertilization, in a temperate climate agroecosystem? What are the changes observed in the relationships between P, C and N cycles and stocks under such a management system? On the basis of these questions, we hypothesized that: 1) soil under mowed permanent grassland would show higher P stock depletion than soil cultivated under crop rotation due to P export through dry matter and; 2) there would be a significant soil organic P pool increase in permanent grassland because it couples with C and N in SOM. Thus, we analysed a 13-year long-term field trial carried out based on contrasting soil management systems for SOM and P dynamics.

MATERIALS AND METHODS

Experimental Field

The experimental site is part of the long-term Agro-ecosystems, Biogeochemical Cycles and Biodiversity Observatory on Environmental Research (SOERE ACBB), which is managed by the French National Institute of Agricultural Research and Environment (INRAE), in Lusignan, France (46°25'12.91" N; 0°07'29.35" E) (Figure 1). The area was under managed grassland, grain cropping or ley arable rotations for at least 70 years before the experimental site was implemented in 2005

(INRAE archive). The climate in the region is characterized by a mean annual temperature of 12°C and by a mean annual rainfall of 750–800 mm. The soil profile is characterized by two main horizons: upper soil horizons, characterized by loamy texture and classified as Cambisol, and lower soil horizons, characterized by rubified clayey horizons rich in kaolinite and iron oxides and classified as Paleo-Ferralsol (34–36).

The experiment started in 2005 and followed a completely randomized block design with four repetitions. The treatments analysed included permanent cropland (CC) with maize–wheat–barley rotation and permanent grassland (GG) (Figure 1). *Zea mays* L. (maize) was sown at a density of 8.5 seeds m^{-2} in the permanent cropland, *Triticum aestivum* L. (wheat) was sown at a density of 150 seeds m^{-2} , and *Hordeum vulgare* L. (barley) was sown at a density of 165 seeds m^{-2} . A mix of *Dactylis glomerata* L. (cocksfoot), *Festuca arundinacea* Schreb (tall fescue) and *Lolium perenne* L. (ryegrass) was used in the permanent grassland.

The soil was conventionally ploughed and subsequently planted in CC treatments after each crop harvest. No P and K fertilization was applied over time, and N application followed plant demand and the growing season (37). Grasslands (GG) were mowed 3 to 5 times a year with a tractor mower in GG treatments, depending on climatic conditions; all aboveground plant biomass was removed. No animal grazing was carried out on the mowed grassland since the experiment was implemented in 2005. No P or K fertilization was applied over time. N fertilization was only carried out based on the amount of shoot biomass N exported at each mowing event. In addition, the timing and rate of N fertilization were guided by the nitrogen nutrition index (NNI) – between 0.9 and 1.0 – to provide the lowest N limits for potential plant production (38).

Phosphorus and Vegetation Outputs

Plant biomass was assessed shortly before harvest (once a year for cereals and 3–5 times a year for grasslands) in an area of 7.5 m^2 . Biomass was oven-dried at 60°C for dry matter quantification. Thereafter, the P concentration was determined according to the procedure described by Masson et al. (39). Briefly, 0.5 g of plant powder dry matter was calcined at 450°C for 4 h. Then, the ash was heated in 5 ml of concentrated HNO_3 (at 65%) on a hot plate until near evaporation. The ash was then filtered through P-free filter paper and placed in 50 ml vials, and total P was determined by a radial targeting inductively coupled plasma emission spectrometer (ICP–AES). To ensure the quality of the results, the introduction of blanks and 3 replicates of certified plant powder samples in the analytical run was applied. P export was computed based on the product of the element concentration and dry matter production.

Soil Sampling

Soil samples were collected in 2005, 2011, 2014, and 2017, when maize was present in the system (i.e., at the beginning of the experiment and 6, 9, and 12 years later, respectively) from the 0–30 cm layer to quantify C and N. For the P analysis, samples from 2005, 2011, 2014 and 2018 (i.e., at the beginning of the experiment and 6, 9 and 13 years later) were taken in a 0–30 cm topsoil layer. Five samples were randomly collected per

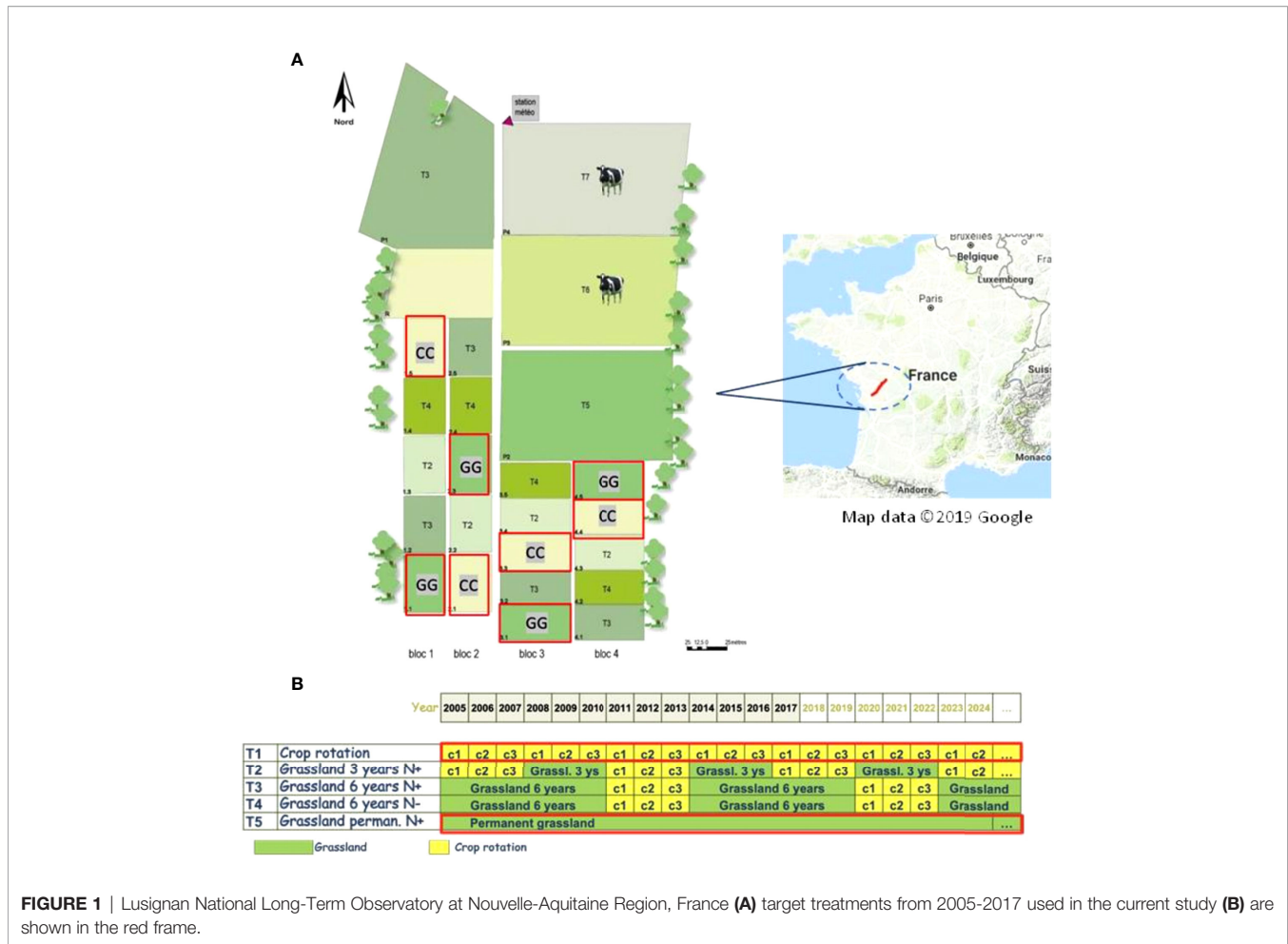


FIGURE 1 | Lusignan National Long-Term Observatory at Nouvelle-Aquitaine Region, France **(A)** target treatments from 2005-2017 used in the current study **(B)** are shown in the red frame.

field repetition; they were mixed to determine the composite sample representative of the field per plot. In total, four truly representative field replications were utilized. Samples were oven-dried at 60°C, sieved to 2 mm, and stored for chemical analysis after soil sampling.

The bulk density of the topsoil (0-30 cm) was determined in 2005, 2010 and 2016 through soil sampling with the aid of a cylinder of known volume. The soil was weighed after drying at 105°C for 24 h, and the density was determined based on the soil mass:cylinder volume ratio.

Soil Chemical Analyses

Total P (Pt) was determined through the ignition method at 550°C for 2 h (40), followed by extraction with 1 mol L⁻¹ H₂SO₄ for 24 h. EnviroMAT SS-2 standard soil (No. 140-025-002) was used as a reference for the method, and 96% recovery of total P was found. Total organic P (Po) was measured as follows: two subsamples were weighed; one subsample was ignited at 550°C for 2 h; and the other subsample was unignited. P was extracted into both subsamples with 10 mL of 0.5 mol L⁻¹ H₂SO₄ after the subsample was stirred for 24 h. Po represents the difference in P concentration in the two subsamples. Total inorganic P (Pi) was quantified as the difference between Pt and Po.

P-lability was assessed through five-step sequential extraction (11) modified by (41). Briefly, 0.5 g of soil was weighed in a Falcon tube, and P was sequentially extracted by a) an anion exchange resin (AER) membrane (AR 103 Plate 434 QDP); b) 10 mL of 0.5 mol L⁻¹ NaHCO₃ through shaking for 16 h; c) 10 mL of 0.1 mol L⁻¹ NaOH through shaking for 16 h; d) 10 mL of 1 mol L⁻¹ HCl through shaking for 16 h; and e) 10 mL of 0.5 mol L⁻¹ NaOH through shaking for 16 h. Pi was quantified by means of the molybdate colorimetry method at 700 nm in alkaline extracts (NaHCO₃ and NaOH) (42), and Pt was determined after mineralization with ammonium persulfate (7.5%) + H₂SO₄ (1:1; v/v) in an autoclave at 121°C for two hours (43). Po was determined based on the difference between Pt and Pi. The Pt of mineralized alkaline extractions and Pi in acid reagents were measured by means of colorimetry at 882 nm after acid phosphomolybdate complex formation (44). P lability analyses considered include:

$$\text{Labile P} = \text{P}_{\text{AER}} + \text{P}_{\text{NaHCO}_3} \text{ and } \text{P}_{\text{NaHCO}_3} \quad (1)$$

$$\begin{aligned} \text{Moderately labile P (mod - labile - P)} \\ = \text{P}_{\text{NaOH}0.1} \text{ and } \text{P}_{\text{NaOH}0.1} + \text{P}_{\text{HCl}} \quad (2) \end{aligned}$$

$$\text{Nonlabile P} = \text{P}_{\text{NaOH}0.5} \text{ and } \text{P}_{\text{NaOH}0.5} \quad (3)$$

The total soil C and N contents were determined through elementary analyses carried out in an elemental analyser (CHN NA 1500, Carlo Erba) after milling the soil samples at 100 μm . All C was considered organic C since there was no carbonate in the soil (25).

P, C and N were analysed as soil stocks; they were determined by considering the concentration and bulk density of the 0-30 cm soil layer. The following soil atomic ratios (C:Po and N:Po) were determined through an equation described by Xue et al. (45):

$$\text{Atomic Ratio} = \frac{\text{Mass Ratio}}{\text{Atomic Weight Ratio}} \quad (4)$$

based on the following atomic weights: C: 12.0107, N: 14.0067 and P: 30.9738 g mol^{-1} .

³¹P Nuclear Magnetic Resonance (³¹P NMR)

One composite sample per treatment was used to perform ³¹P-NMR spectroscopy to investigate the structure of the alkali-soluble P form in samples from 2005 (initial state) and 2018, i.e., 13 years after the experiments were installed. To do so, suspensions with 1 g of soil from each repetition of treatments and 10 mL of 0.25 mol L^{-1} NaOH + 0.05 mol L^{-1} EDTA were prepared in centrifuge tubes. These tubes were shaken for 4 hours and centrifuged at 3,000 \times g for 15 min. The extracts of the four repetitions were homogenized; Pt, Po and Pi contents in the extract were quantified based on the aforementioned methods and freeze-dried. Lyophilized extracts were redissolved in 2.7 mL 0.25 mol L^{-1} NaOH + 0.05 mol L^{-1} EDTA and 0.3 mL D₂O and stirred in a vortex for 5 min to perform the ³¹P-NMR analyses. The supernatant was separated by centrifugation (3000 \times g for 15 min) after 120 min of contact, filtered (<45 μm) and transferred into a 10 mm NMR tube. The ³¹P-NMR spectrum was determined utilizing a Bruker Advance DPX 400 spectrometer operating at a frequency of 162 MHz with proton decoupling, a pulse angle of 90°, a temperature of 20°C, an acquisition time of 0.5 seconds and a relaxation time of 2 seconds. The number of scans ranged from 27,000 to 32,000. Peak area integration was performed electronically at a baseline of 10 Hz in Bruker TopSpin 3.6.2 software. The following phosphate compounds were identified: orthophosphate (7.10 ppm), monoester (6.33 to 5.09 ppm), diester (2.00 to -1.00 ppm), pyrophosphate (-3.14 to -3.58 ppm) and adenosine diphosphate (-9.36 to -9.41 ppm). The hydrolysis of diester compounds was not considered in the peak area integration. The monoester compounds were separated into *myo*-inositol hexakisphosphate (*myo*-IHP), *scyllo*-inositol hexakisphosphate (*scyllo*-IHP), α -glycerophosphate, β -glycerophosphate and adenosine monophosphate (46–49).

Statistical Analyses

The variables were analysed based on a randomized complete block design, with split plots in time and four repetitions. Soil management was taken as the main factor (plot), and management time was used as the secondary factor (split plot).

The adopted statistical model was described through Equation 5.

$$Y_{ijk} = \mu + B_i + T_j + \text{error } a(j, i) + R_k + \text{error } b(i, k) + (TR)_{ij} + \text{error } c(i, j, k) \quad (5)$$

where Y is the observation; μ is the overall experimental average; B is the blocks ($i = 1, 2, 3, 4$); T is the soil management ($j = 1$ and 2); R is the management time ($k = 1, 2, 3, 4$); and *error* is the experimental error.

The soil management effects were tested by the Scott–Knott test ($p \leq 0.05$) based on complex variance. Variables were subjected to regression analysis to test the time effects ($p \leq 0.05$). The statistical analyses were carried out in SISVAR 5.6 software.

RESULTS

Yield and Nutrient Export

The mean dry matter export reached 8.0 and 7.9 $\text{Mg ha}^{-1} \text{ year}^{-1}$ in CC and GG, respectively. This led to cumulative exports of 112 and 110 Mg ha^{-1} (for CC and GG, respectively) at the end of the assessed period (2005-2018).

The dry matter exported by GG during the early experimental years (2005-2008) was 40% higher than that recorded for CC in the same period. However, after this time, it was possible to observe a shift in the weight of the exported dry matter. A yield decrease was observed in both treatments, mainly in GG, over time (Figure 2). The mean P exportation rates were 19.1 and 21.0 $\text{kg ha}^{-1} \text{ yr}^{-1}$ for CC and GG, respectively. Consequently, the accumulated amount of exported P was 10% higher in GG than in CC (295 and 268 kg ha^{-1} , respectively) (Figure 2). However, there was no trend of P concentration reduction in the dry matter; the mean P concentration reached 0.23% and 0.28% in CC and GG, respectively.

Total Carbon, Nitrogen and Phosphorus Stocks

The temporal pattern observed for the C and N stocks in GG and CC can be seen in Figure 3. GG enhanced the C stock by 5.5% (an increase of 2.6 Mg ha^{-1} within 12 years), whereas CC promoted a reduction of 8.5% in the C stock (a decrease of 4.2 Mg ha^{-1} within 13 years). GG increased N by 3.7% (0.19 Mg ha^{-1}), and CC reduced N by 7.4% (0.38 Mg ha^{-1}) in the stocks.

There was a total P stock decrease in both CC and GG. A linear decrease of 0.0217 and 0.0159 $\text{Mg ha}^{-1} \text{ yr}^{-1}$ was identified in GG and CC, respectively (Figure 4). The accumulated depletion of the total soil P stock reached 0.30 Mg ha^{-1} (9.4%) and 0.25 Mg ha^{-1} (8.0%) in GG and CC, respectively, after 13 years. In addition, the total Pi stock was more aggressively (2.3 times) depleted in GG than in CC (Figure 4). This depletion represented -0.75 and -0.29 Mg ha^{-1} in both treatments, respectively, at the end of 13 years. However, the Po stock increased by 42.6% in GG at a rate of 32 $\text{kg ha}^{-1} \text{ yr}^{-1}$; it did not change in CC (Figure 4).

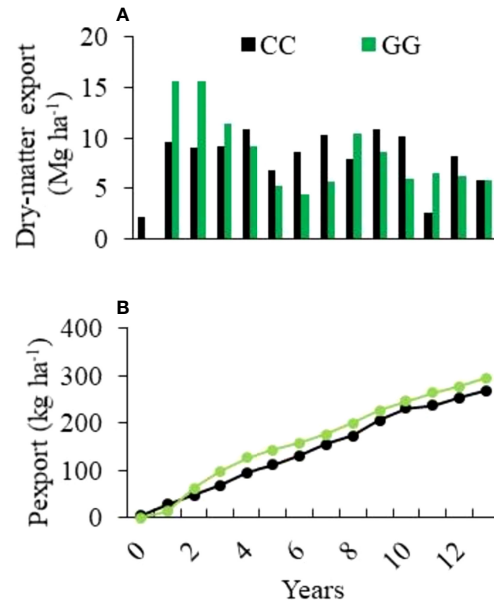


FIGURE 2 | Exportation of (A) annual dry matter and (B) accumulated phosphorus from the experimental field over 13 years of cultivation. CC-permanent conventional crop rotations and GG-permanent grassland.

Phosphorus Lability

A significant reduction in the labile P fraction was observed in both GG and CC over time (Figure 5). However, the moderately labile P (Figure 5) and nonlabile P (Figure 5) fractions were not

changed by the adopted soil management systems. Moderately labile P represented 44% of the total P stock in the 0-30 cm topsoil layer, on average, although it was not statistically significant. There was a mean of 0.13 Mg ha⁻¹ decrease in both

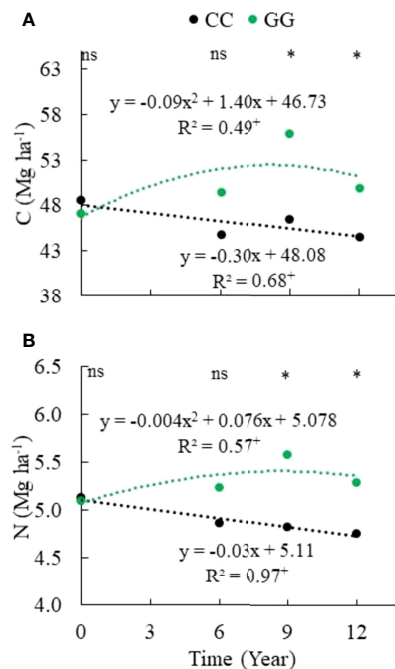


FIGURE 3 | Temporal change in (A) carbon and (B) nitrogen stocks in the 0-30 cm topsoil layer. CC-permanent conventional crop rotations (corn, winter wheat, and barley crops) and GG-permanent grassland. ^{ns}Not significant. ^{*}Significant in the Scott-Knott test ($p \leq 0.05$). ⁺ Significant at $p \leq 0.01$.

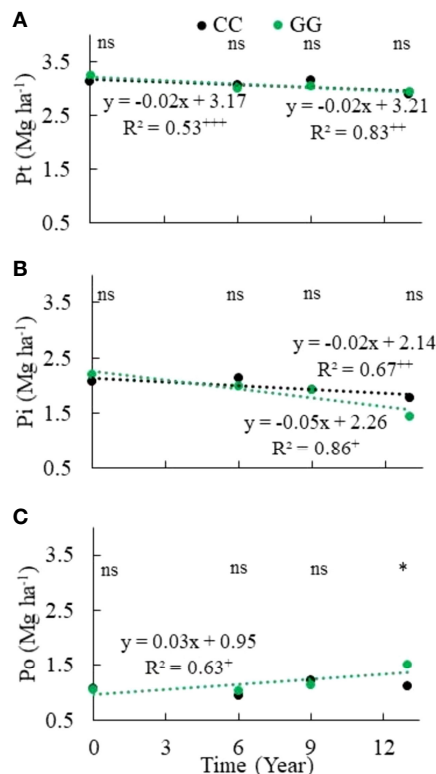


FIGURE 4 | Trends of (A) total phosphorus, (B) total inorganic phosphorus and (C) total organic phosphorus stocks in the 0-30 cm topsoil layer of soil subjected to different soil management systems. CC— permanent conventional crop rotations (corn, winter wheat, and barley crops) and GG—permanent grassland. ^{ns}Not significant. *Significant in the Scott-Knott test ($p \leq 0.05$). ⁺Significant at $p \leq 0.01$. ^{**}Significant at $p \leq 0.05$. ⁺⁺⁺Significant at $p \leq 0.10$.

treatments, representing a 47% decrease in the total P stocks. However, nonlabile P, assessed based on the 0.5 mol L⁻¹ NaOH extraction product, represented 22% of the total P stock in the 0-30 cm topsoil layer on average. Labile P depletion was 0.13 and 0.17 Mg ha⁻¹ in CC and GG, respectively; it represented a 23% and 32% reduction of its initial content and 53% and 56% of the total P stock depletion in CC and GG, respectively. Labile Pi and labile Po showed opposing trends over time. There was a labile Pi depletion of 0.16 and 0.21 Mg ha⁻¹ at rates of 0.012 and 0.016 Mg ha⁻¹ y⁻¹ in CC and GG, respectively (Figure 6), whereas labile Po stocks increased by 49% (from 27 to 69 kg ha⁻¹) under the GG soil management system, but they did not significantly change in CC (Figure 6).

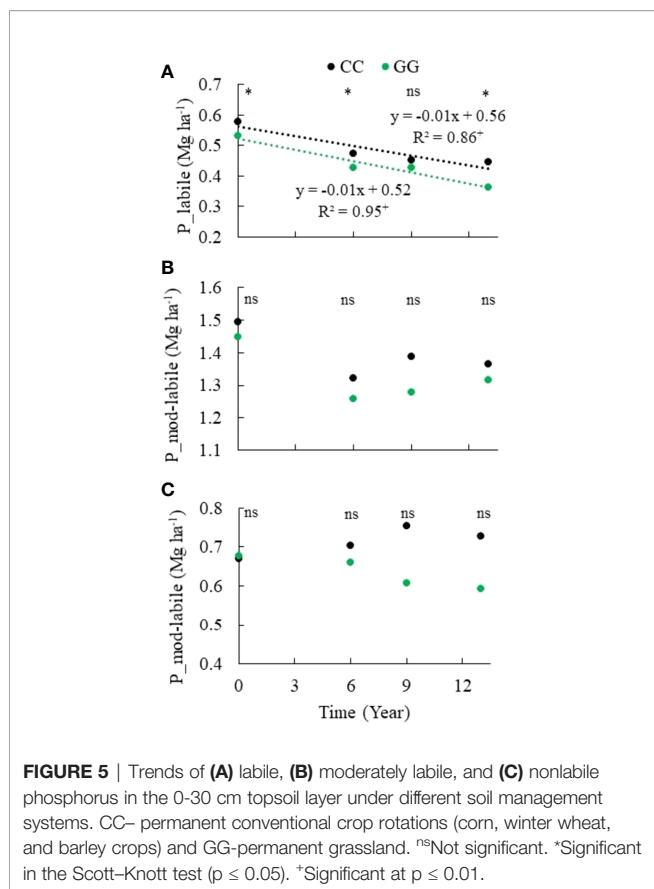
C:N:Po Stoichiometry

C, N and Po stoichiometry changed depending on the management systems due to changes in C and Po stocks (Figure 7). There was a significant reduction in the C:Po atomic ratio in the GG (from 116 to 86) and CC (from 117 to 104) systems over time. The N:Po atomic ratio decreased from 11 to 8 in GG and from 11 to 10 in CC. The C:N:Po atomic ratios were 117:11:1 and 116:10:1 in CC and GG, respectively, at the beginning of the experiment; they were 104:10:1 and 86:8:1 in CC and GG, respectively, 13 years later.

P Characterization by ³¹P-NMR

Soil Po and Pi forms at the beginning of the experiment (2005 - initial) and, after 13 years of cultivation (2018) under different soil management systems, were assessed after extraction with a solution containing 0.25 mol L⁻¹ NaOH + 0.05 mol L⁻¹ EDTA and a thorough ³¹P-NMR analysis. This solution extracted 57%, 54% and 51% of the total soil P at Pi/Po ratios of 2.7, 2.7 and 2.8 in the initial, CC and GG management systems, respectively (Table 1). The Pi/Po ratio in the extraction solution was higher than the rate in the soil (2.0, 1.6 and 1.0 in the initial, CC and GG management systems, respectively); this finding indicates greater Pi extraction than Po extraction.

Orthophosphate, orthophosphate monoesters, orthophosphate diester, pyrophosphate and adenosine diphosphate were identified through ³¹P-NMR (Figure 8). Although there was a slight indication in the spectra, peaks assigned to diester compounds (2 to -1 ppm) in CC and GG samples were below the detection limit available in the device. Orthophosphate (296, 250 and 250 mg kg⁻¹ at 72, 74 and 75%, respectively) prevailed in all spectra, followed by monoester (111, 92 and 88 mg kg⁻¹ at 23, 25 and 24%, respectively) in the initial, CC and GG management systems, respectively. Pyrophosphate and adenosine diphosphate accounted for lower contributions in the three samples (Table 1). *myo*-IHP, *scyllo*-IHP, α -glycerophosphate,

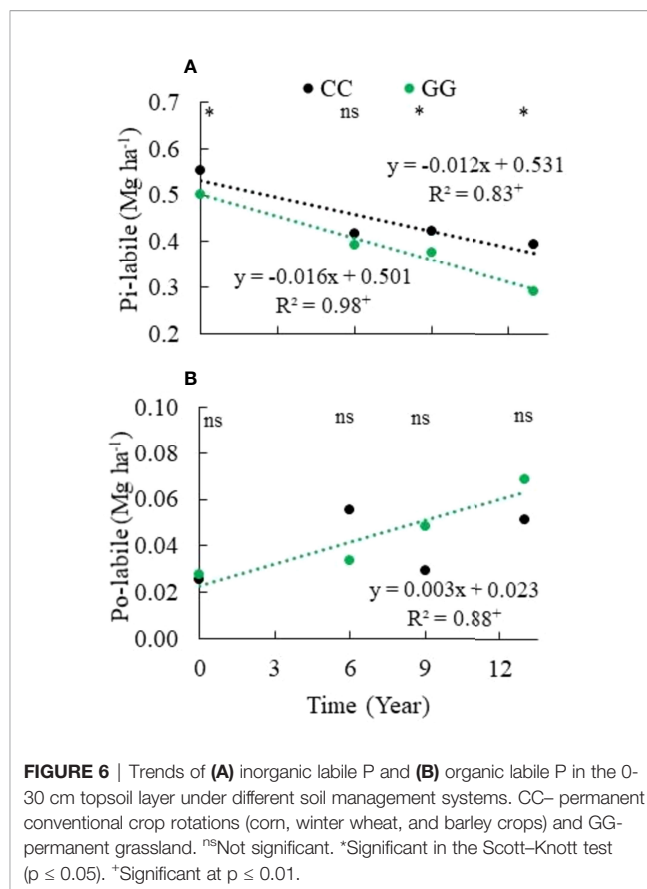


β -glycerophosphate and adenosine monophosphate were identified in the monoester region (Table 1 and Figure 8). The contribution of the last three represented 56%, 61% and 62% of the phosphate monoester, respectively.

DISCUSSION

C, N and P Stock Trends

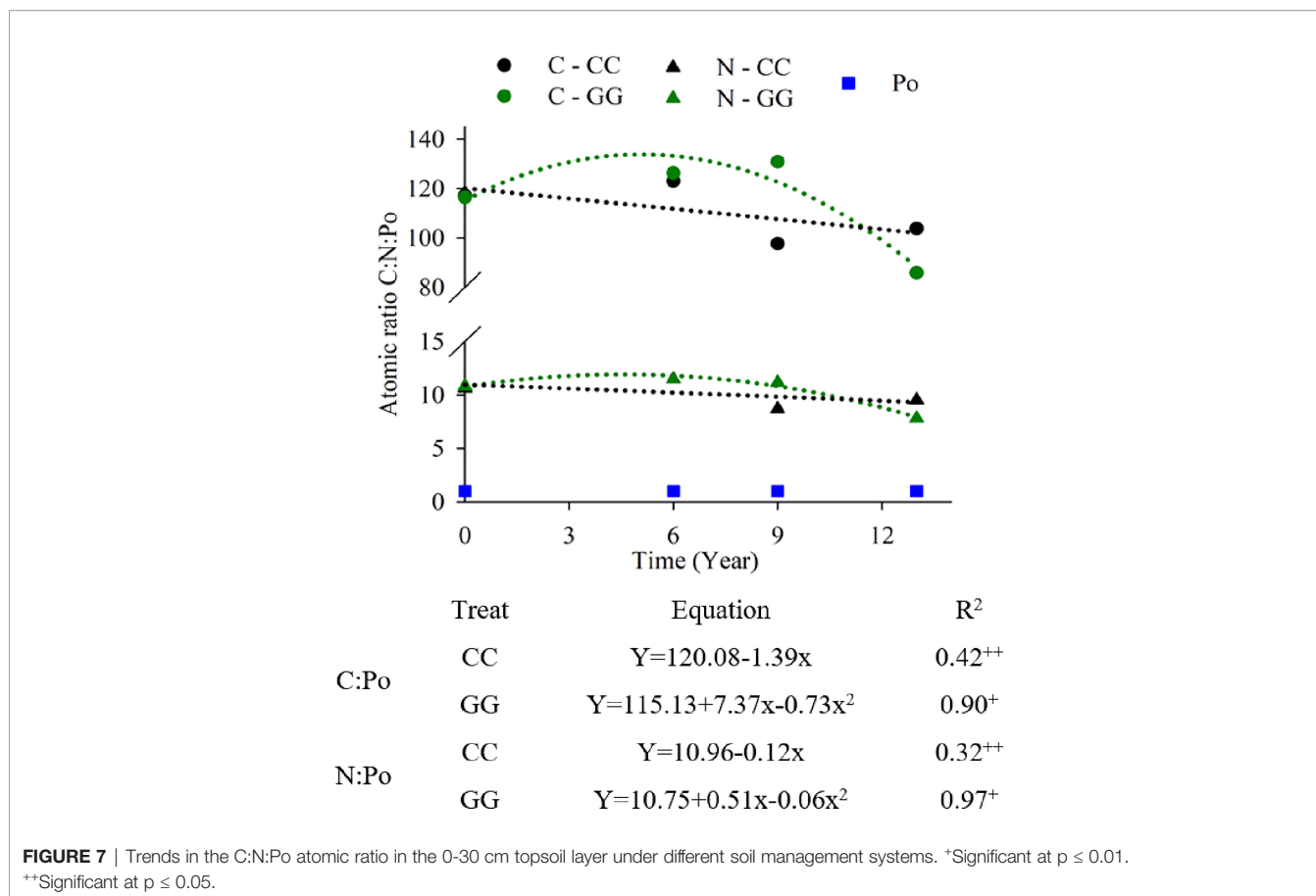
C, N and P stocks were analysed by focusing on changes in soil P lability observed over 13 years of cultivation (2005-2018) under contrasting soil management systems in soil presenting a high initial P stock (3.2 Mg ha^{-1} , on average), without supplementary P fertilization. The C and N stocks in the 0-30 cm topsoil layer were differentiated by management systems: the C and N stocks increased in GG and decreased in CC. The constant input of plant residues (leaves, stems and roots) and lack of soil disturbance were fundamental factors for the increase in C and N stocks under GG. Similar results were recorded for the 0-10 cm layer in the same experiment (25, 50). Soil ploughing in surface soil layers under tillage management favours SOM mineralization and C and N losses to the atmosphere by potentiating microbial activity through greater residue-soil contact, system aeration and SOM physical protection reduction (22, 26, 51, 52). In addition, grassland-managed soils have significant organic matter input, mainly through



underground rhizodeposition, which changes the soil C stock (53, 54).

The total P stock decreased in both treatments because of P export by plants (forage or grain) without P input due to fertilization (Figure 4). This long-term negative balance can lead to great P stock reduction, mainly in P available in the soil, which may constrain plant nutrition (32, 55, 56). Despite the total amount of exported dry matter was similar in the two management systems (112 and 110 Mg ha^{-1} for CC and GG, respectively), the amount of exported P differed between treatments due to the nature of the exported plant material. Grains were harvested (maize-wheat-barley) at a mean P concentration of 0.23% in CC, whereas leaves and stems of grasses (*D. glomerata*, *F. arundinacea* and *L. perenne*) were exported as forage at a mean P concentration of 0.28% in GG. This finding partly explains the difference of 27 kg ha^{-1} in the total exported P and the subsequent reduction by 0.3 Mg ha^{-1} in soil P in GG after 13 years.

Although total P stock depletion was more evident in the system managed with perennial pasture (GG), it was slow in both systems because annual dry matter export was low; it was 19.1 and $21.0 \text{ kg ha}^{-1} \text{ y}^{-1}$ in CC and GG, respectively, despite the 3-4 mowings per year and plant shoot material removal from the perennial pasture. However, according to the literature, nutrient export by plants in grazed systems (e.g., not studied here), tended to be lower, as some of the nutrients exported by plants were



returned to the soil through animal urine and dung (57, 58), which is not the case in mowed grasslands. Although P exportation was low overall, it had a stronger impact on soils presenting low P stock, mainly due to low P stock availability.

The Pi stock decreased in both treatments simultaneously with the total P stock reduction. This process resulted from plant uptake of inorganic P and from further P export (2) or return to soil organic P forms. The Pi stock decrease in GG stood out (Figure 4) because, in addition to Pi exportation, there was Pi transformation and accumulation into Po. The increase of 42% in Po indicates the high potential of grassland to transform Pi into Po. This transformation occurs when P is absorbed by plants; incorporated into essential plant metabolism; returned to the soil through leaves, stems and roots; and remains in the soil as organic matter (59).

Po accumulation in grassland is the consequence of an increase in SOM due to organic matter input to the soil and to nonploughing during the experimental period (25). Overall, pasture soils have increased organic P forms and C and N due to SOM accumulation (27, 29). SOM represents the coupling of P, C and N cycles in the soil organic phase (Rumpel et al., 2015). Moreover, Pi incorporation into organic molecules in rangelands or croplands can be larger if mining plants are used (e.g., *Lolium* sp. and *Lupinus* sp.), as well as a mix of plants or rotation, with more nutritionally demanding plants (i.e., leguminous crops) (60; Crème et al., 2016; 55).

The C:N:Po atomic ratio was also changed by the management systems because of changes in C and Po stocks (Figure 7). The stoichiometry values (104:10:1 and 86:8:1 for CC and GG, respectively) were below the means of 166:14:1 (61) and 169:11:1 (62) recorded for pasture and above the mean of 64:5:1 (62) observed for cropland values reported in the literature for bulk soil at a global scale. The lower values observed in the present study were mainly caused by high soil Po contents, which resulted from the history of P input before the experiment and from the soil Po stock increase caused by permanent grassland cultivation. The temporal reduction in the C:N:P stoichiometry reveals that there is no decrease in OM input and thus in the amount of P even after 13 years without P fertilization, suggesting that there is internal P turnover (see below). Alternatively, a limitation of N in the SOM can be addressed when there is a reduction in the atomic N:P ratio. However, this likely did not occur in our treatments, as there were increases in N stocks over time.

P Depletion and Dynamics

A decrease in total P stocks was first observed through changes in labile and moderately labile P stocks, since turnover in these fractions can last from minutes to months (13). Moreover, labile P is the most available P fraction for plant uptake. Based on the current results, decreases of 0.13 and 0.17 Mg ha⁻¹ in labile P recorded for CC and GG, respectively, represented most of the

TABLE 1 | Total organic and inorganic P extracted by NaOH + EDTA and P forms analysed through ^{31}P -NMR in Cambisol managed under permanent cropland (CC) and permanent grassland (GG) for 13 years under a temperate climate.

Parameter	Initial	CC	GG
P in soil			
Total soil P (mg kg ⁻¹)	719	637	657
Total Po (mg kg ⁻¹)	239	245	335
Total Pi (mg kg ⁻¹)	480	392	322
Pi : Po ratio	2.0	1.6	1.0
% of total Po	33	38	51
% of total Pi	67	62	49
P extracted by 0.25 mol L⁻¹ NaOH + 0.05 mol L⁻¹ EDTA			
Total P (mg kg ⁻¹)	407	342	338
Organic P (mg kg ⁻¹)	111	92	88
Inorganic P (mg kg ⁻¹)	296	250	250
Pi : Po ratio	2.7	2.7	2.8
% of total soil P	57	54	51
% Po	27	27	26
% Pi	73	73	74
P forms analysed through ^{31}P-NMR			
Orthophosphate (%)	72	74	75
Monoester (%)	23	25	24
<i>myo</i> -IHP (%)	< 0.01	8	15
α - Glycerophosphate (%)	27	23	19
β - Glycerophosphate (%)	15	18	15
Adenosine monophosphate (%)	14	19	29
<i>Scyllo</i> -IHP (%)	14	14	11
Other monoesters (%)	29	18	11
Diester (%)	0.2	< 0.01	< 0.01
Pyrophosphate (%)	1.8	0.7	0.9
Adenosine diphosphate (%)	2.8	0.5	0.5
Pi : Po ratio	2.7	3.0	3.2

reduction in total P stocks (mean of 55%); P was higher in GG due to greater P export under this soil management system. With respect to the total Pi and Po stocks, labile Pi and Po presented opposite patterns. Labile Pi decreased in both treatments due to the absorption of inorganic P forms (i.e., HPO_4^{2-} and H_2PO_4^-) (2). On the other hand, labile Po significantly increased under the GG treatment due to SOM accumulation in the soil. These results are consistent with the total C, N and Po soil stock increases (Figures 3, 4) and indicate an increase in C, N and Po coupling in the P pool, which showed greater availability. Labile Po corresponding to P from organic compounds was changed as a result of soil management systems (and thus *via* soil microbial biomass, soluble organic molecules, and organic orthophosphates less adsorbed by soil colloids). Therefore, it was possible to identify more significant changes in the labile P pool over the analysed period (13 years).

The mean decrease observed in the moderately labile P (0.13 Mg ha⁻¹) represented the remaining fraction of the total P stock reduction (47%, on average). This behaviour was similar in both soil management systems and showed the buffering effect of moderately labile P on labile P (12). These authors highlighted that Po presenting moderate lability (extracted by 0.1 mol L⁻¹ NaOH) in cropping systems with tillage accounts for a higher contribution (94%) to labile Pi buffering as ploughing enhances mineralization. However, this effect was not observed in the current research, since moderately labile P reduction was similar in both treatments and moderately labile P reduction was not significant in the CC treatment (data not shown).

The nonlabile P stock was not changed by the soil management systems throughout the evaluated 13-year period. Nonlabile P extracted with 0.5 mol L⁻¹ NaOH represented a more recalcitrant organic (inside soil aggregates) and inorganic P pool (inner sphere absorbed through iron oxihydroxides and clay minerals) (63). Therefore, there was low sensitivity and minor P pool turnover, regardless of the adopted soil and management system (13). Furthermore, the high labile and moderately labile P stocks that had a history of fertilization were able to fulfil crop demand without the need to access the nonlabile P pool.

Chemical Nature of Soil Organic Phosphorus

The extraction solution NaOH + EDTA extracted more than 50% of the total P from the soil. This observation is common in soils from temperate regions, given soil mineralogy and moderate pedogenic evolution. The soil in the present study was classified as Cambisol with loamy texture in the upper soil horizon with high P content, mainly Pi (67%, 62% and 49% of total P in Pi in the initial, CC, and GG management systems, respectively), due to the history of phosphate fertilization used prior to the beginning of the experiment. Doolette et al. (64) extracted 21% to 89% of the total soil P by using the same NaOH + EDTA solution (with prevalence > 60%) in a study carried out with soils presenting different uses and textural characteristics in New South Wales, South Australia and Tasmania. Abdi et al. (65) extracted 51% of the total soil P, on average, from soils collected in Canada (from temperate regions), presenting clayey texture

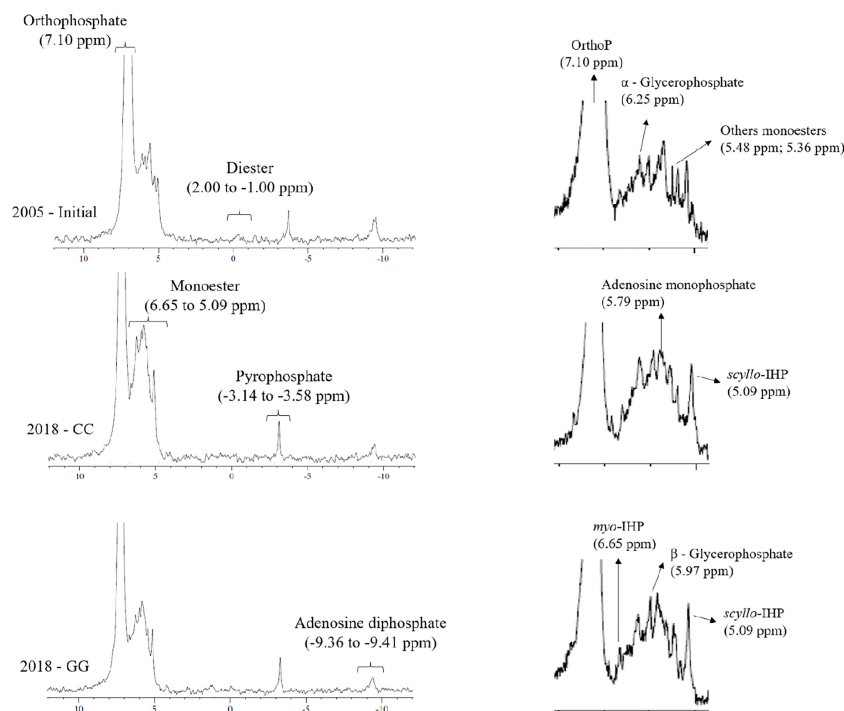


FIGURE 8 | ^{31}P -NMR spectrum of a representative composite of soil samples at the beginning (2005) and after 13 years of CC and GG soil management practices. CC-permanent cropland and GG-permanent grassland.

and cultivated with grassland. On the other hand, Rheinheimer et al. (66) found much lower P recovery (15.1%, on average) in subtropical soil. These authors attributed the P recovery rate recorded for subtropical to its clayey texture and a mineralogy dominated by soil kaolinite and iron oxides.

Similar to other current studies, and in compliance with all previously reported cases, in this study there was a prevalence of orthophosphate and monoesters and a small contribution of diesters and pyrophosphate in the analysed extract. More complex organic compounds, such as *myo*-IHP, *scyllo*-IHP, α -glycerophosphate, β -glycerophosphate and adenosine monophosphate, were identified in the spectrum shift attributed to the monoester. These compounds have also been identified in soils cultivated with annual crops and grassland in temperate regions (65, 67, 68). The present study stands out for its evaluation of the temporal effect of the conventional crop rotation system and permanently mowed grassland on the chemical nature of soil organic P. This approach contemplated the need to analyse the temporal change in organic P forms, as evidenced by Cade-Menun (68).

The permanent cultivation of cropland and grassland for 13 years did not significantly change the orthophosphate concentration. The total Pi stock was identified, despite the decrease in the total P stock (Figure 4). Likewise, no relevant temporal increase in organic P forms in GG was observed through ^{31}P -NMR (extracted by NaOH + EDTA), most likely because of the high initial total Po stock and C and N contents.

Moreover, no relevant changes in Po forms between GG and CC were identified, mainly because the Po rate in the NaOH + EDTA extract was similar between treatments (GG and CC - 27%, on average). Pi extraction selectivity was observed for the initial, CC and GG samples. Lower Pi rates (67%, 62%, 49% of Pi in the initial, CC and GG samples, respectively) were observed in the soil than in the analysed NaOH+EDTA extract (73%, 73%, 74% of Pi in the initial, CC and GG samples, respectively). Selectivity for Po compounds was more often observed with the use of the NaOH + EDTA extraction solution; this was also reported by Rheinheimer et al. (66) for soils from subtropical regions.

Dry matter was removed annually on the order of $7.9 \text{ Mg ha}^{-1} \text{ y}^{-1}$ in the GG treatment; this reached 110 Mg ha^{-1} within 13 years. Dry matter export from pasture tends to reduce Po content in the soil in the long term, mainly the monoester forms detectable through ^{31}P -NMR. Boitt et al. (32) found decreased rates of monoester compounds in the NaOH+EDTA extract when they simulated pasture dry matter grazing and exporting for 20 years in temperate pastures in New Zealand. Similarly, Deiss et al. (67) observed decreased monoester compounds in NaOH+EDTA extract due to animal grazing in experiments that lasted 7 to 12 years in winter forage under a subtropical climate in Brazil.

The main changes resulting from soil cultivation with different management systems were decreased α -glycerophosphate, diesters, pyrophosphate and adenosine diphosphate forms and increased *myo*-IHP and adenosine monophosphate forms in both treatments (Table 1). Significant differentiation between treatments was

observed only in monoester compounds (Table 1). Therefore, cultivation with grassland had the strongest effect on α -glycerophosphate reduction and on *myo*-IHP and adenosine monophosphate increases. Higher values of α -glycerophosphate and lower values of *myo*-IHP and adenosine monophosphate in permanent crop rotation are linked to greater availability of substrate in the soil for microbiological development (69). In this case, soil rotation favours the contribution of organic material and its incorporation into the soil; consequently, the microbial activity is high. In this case, there is marked production of α -glycerophosphate, with temporal accumulation of *myo*-IHP and adenosine monophosphate.

CONCLUSION

The aims of the current study were to analyse soil organic matter and P stock depletion trends and the chemical nature of soil organic P and C:N:Po stoichiometry in long-term field experiments based on contrasting soil management systems, namely, permanent crop rotation and permanent grassland, over 13 years. Based on the present results, there was greater P export and, consequently, greater total P stock depletion in the treatment with permanent grasslands. P depletion mainly affected the P pool, presenting the highest lability (P-labile). However, the depletion observed 13 years after experimental installation was not high enough to cause an apparent reduction in plant yield due to the buffering capacity of less labile P pools (moderately labile P).

There was a change in inorganic P into organic P in the most labile reservoir in the permanent grasslands and an increase in soil C and N stocks. This process led to a reduction in the C:N:P atomic ratio value and, consequently, to C, N and P cycle coupling in the soil. This means that due to the conversion of Pi to Po, there is tighter coupling as all elements are in SOM, compared to a system with organic C and N but mainly inorganic P. Based on the ^{31}P -NMR technique, permanent mowed grasslands significantly reduced α -glycerophosphate and increased *myo*-IHP and adenosine monophosphate contents in comparison to the permanent cropland.

Although productivity was not significantly affected, depletion of available P due to a lack of P fertilization in the soil should be monitored over time, especially in mowed permanent grasslands, to avoid possible nutrient limitation.

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Moreover, in soil with less available and total P stock, it is expected that crop P supply constraints will be felt in a shorter time frame and should require more attention from farmers. Avoiding nutrient depletion is essential for maintaining productivity and increasing C and N stocks in agricultural soils, in both arable and grassland systems.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

AS: Conceptualization, Methodology, Writing - original draft. LC: Writing - review and editing. IC: Methodology, review and editing. DR: - review and editing. AC: conceptualization of the experimental design, Methodology, Writing - review and editing. All authors contributed to the article and approved the submitted version.

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