



Legacy phosphorus lability shifts after Atlantic Forest conversion to sugarcane

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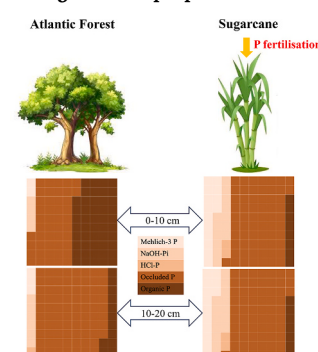
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HIGHLIGHTS

- Labile and moderately labile P fractions increased significantly under sugarcane.
- Organic P was depleted under sugarcane, especially in the surface soil layer.
- Occluded and total P remained largely unaffected by land use change.
- Limited legacy P accumulation and relatively high P use efficiency were observed.
- Occluded P stability suggests limited capacity for further fixation in these tropical soils.

GRAPHICAL ABSTRACT

Changes in the proportion of soil P fractions after forest-to-sugarcane conversion in Brazil.



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ABSTRACT

Phosphorus (P) fertilisation plays a central role in the high productivity of Brazilian sugarcane systems, yet concerns about inefficient P use and environmental risks from legacy P accumulation remain unresolved. Land use change from native vegetation to sugarcane cultivation in tropical regions can profoundly influence soil P dynamics, with implications for fertiliser efficiency and environmental sustainability. We investigated changes in soil P fractions at three long-term sugarcane fields in São Paulo State, Brazil, following conversion from Atlantic Forest. Using a rapid sequential P fractionation method, we evaluated labile, moderately labile, and non-labile P pools in surface (0–10 cm) and subsurface (10–20 cm) layers. Results showed a significant increase in labile and moderately labile P (Mehlich-3 and NaOH-Pi) under sugarcane, reflecting long-term fertiliser application, while organic P declined substantially, especially in surface soils. Despite two decades of cultivation at some sites, total P did not differ significantly between land uses in the surface layer, indicating limited accumulation of legacy P. Occluded P, representing the non-labile fraction, remained stable across land uses, suggesting limited capacity for further P fixation. Moreover, soils with higher clay content exhibited greater retention of organic P under native vegetation, highlighting the role of texture in modulating P dynamics. These findings challenge common

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assumptions about legacy P accumulation and suggest that sugarcane systems in Brazil may be more efficient in long-term P use than previously thought.

1. Introduction

Phosphorus (P) is an essential macronutrient that plays a central role in plant growth and productivity (Marschner, 2012). However, in many tropical ecosystems, including those of Brazil, P availability is limited due to the strong fixation of phosphate ions by highly weathered soils rich in iron (Fe) and aluminum (Al) oxides (Syers et al., 2008). This constraint presents a major challenge for sustainable agricultural production, particularly in intensive systems such as sugarcane cultivation, which is a dominant land use in Brazil (9 Mha) and a key contributor to the country's bioenergy and sugar economy. Brazil is the world's leading producer of sugarcane, accounting for approximately 40 % of global production (FAO, 2023). The South-Central region, particularly the state of São Paulo, contributes over 70 % of the national output (CONAB, 2024). In recent years, average sugarcane stalk yields in Brazil have ranged from 70 to 85 Mg ha⁻¹, with potential yields exceeding 100 Mg ha⁻¹ under optimal management conditions (Withers et al., 2018). In addition to its high biomass productivity, Brazilian sugarcane is a major source of both sugar and ethanol. On average, the crop produces 10 to 14 Mg ha⁻¹ of sugar and 6000 to 8000 l ha⁻¹ of ethanol, depending on cultivar and management practices (Goldemberg et al., 2018). This remarkable productivity underpins Brazil's strategic role in global bioenergy and food security, while also highlighting the importance of efficient nutrient use, especially P, to sustain high yields and minimise environmental impacts.

To compensate for low natural P availability, large quantities of P fertilisers are applied in Brazilian sugarcane systems. Although this can improve crop yields, it also raises concerns about the long-term efficiency of P use and the potential for P accumulation in non-available soil pools, contributing to environmental risks such as eutrophication (Withers et al., 2018). Furthermore, the high costs and limited global reserves of phosphate rock, an essential input for fertiliser production (Cordell et al., 2009), underscore the urgent need to optimise P management in tropical agroecosystems (Withers et al., 2018). Understanding how different P forms behave under changing land use is crucial for improving P use efficiency and maintaining soil fertility.

Land use change, particularly the conversion of native vegetation to cropland, can substantially alter soil P dynamics by modifying organic matter inputs, microbial activity, and soil mineralogy (Soltangheisi et al., 2019a). In Brazil, large areas of the Atlantic Forest biome have been converted to sugarcane cultivation over recent decades. While several studies have investigated total or available P in these systems (Chi et al., 2017; Medorio-García et al., 2020), few have comprehensively examined how different P fractions, ranging from readily available to highly recalcitrant, are affected by this land use transition (Cherubin et al., 2016; Wright, 2009). Such fractionation provides critical insights into the fate of fertiliser P, the stability of native P pools, and the long-term sustainability of nutrient management practices.

While P fertilisation has supported the high productivity of Brazilian sugarcane (dos Santos et al., 2018; Soltangheisi et al., 2019b), concerns have been raised about the long-term sustainability of this practice (Soltangheisi et al., 2019c). Due to low P-use efficiency and strong P-fixing capacity of weathered tropical soils, it is widely assumed that large amounts of fertiliser P accumulate over time, contributing to so-called "legacy P" pools (Pavinato et al., 2020). Many researchers have suggested that Brazilian agricultural systems, including sugarcane fields, hold substantial amounts of legacy P that could potentially be mobilised to meet crop demands. However, few studies have systematically quantified the distribution of P fractions in relation to land use change from native vegetation to sugarcane over decadal timescales. The key aims of this study were to evaluate whether long-term sugarcane

cultivation in Brazil has indeed led to significant legacy P accumulation, and to determine how this may affect P use efficiency and future fertiliser requirements.

2. Material and methods

2.1. Site description, experimental setup, and soil sampling

The study was conducted in South-Central Brazil (Fig. 1), representing the primary sugarcane-producing region of the world, encompassing three paired sites with contrasting soil textures (Table 1). The edaphoclimatic characteristics of each site are presented in Table 1. At all locations, the predominant land use change from Atlantic Forest to sugarcane cultivation was investigated. This conversion occurred approximately 20 years ago in Paraguaçu Paulista and São Pedro do Turvo, and 10 years ago in Tanabi. All three locations are situated in the state of São Paulo.

At the time of sugarcane establishment, soils at all three sites were prepared using conventional tillage practices, including plowing, disk-ing, harrowing, and furrowing. Lime and gypsum were applied prior to planting at rates tailored to each site's specific requirements (Table 1). This amendment strategy was repeated every five years during sugarcane re-establishment. Fertilisation management included both in-furrow and banded applications, beginning at planting and continuing 60 to 90 days afterward. Nutrient inputs consisted of nitrogen (90 kg N ha⁻¹) as ammonium nitrate, potassium (220 kg K₂O ha⁻¹) as potassium chloride, boron (2 kg B ha⁻¹) as ulexite, and zinc (10 kg Zn ha⁻¹) as zinc oxysulfate. For the first ratoon crop, fertilisers were band-applied over the sugarcane rows without mechanical incorporation, including 100 kg N ha⁻¹ and 150 kg K₂O ha⁻¹. Phosphorus fertilisation was applied as monoammonium phosphate (50 % P₂O₅), with 65 kg P ha⁻¹ applied in-furrow at planting, and 22 kg P ha⁻¹ in band-over for each ratoon cycle. This fertilisation regime reflects common commercial practices in Brazilian sugarcane systems.

At each site, a 100 × 100 m (1 ha) plot was established in both land uses, native vegetation (Atlantic Forest) and sugarcane cultivation. Within each plot, four sampling points were randomly selected. At each point, a 50 × 50 cm trench was excavated to a depth of 20 cm, and soil samples were collected from two distinct layers: 0–10 and 10–20 cm. All samples were collected in November 2024, during the rainy season, to ensure consistent soil moisture conditions across sites.

2.2. Soil P fractionation procedure

For chemical analysis, soil samples were air-dried, and then passed through 2 mm sieve. Soil P forms were evaluated using the sequential chemical fractionation method described by Gatiboni and Condron (2021). A 0.5 g of soil sample was placed in 15 ml Falcon tubes and sequentially extracted with 10 ml of specific chemical solutions. Each extraction step involved shaking the tubes in an end-over-end shaker at 30 rpm for 60 min, except for the Mehlich-3 extraction, which was performed for 5 min. Following each extraction, samples were centrifuged at 3500 rpm for 20 min, following the protocol developed by Gatiboni and Condron (2021), which ensures effective separation of soil particles from the extract without resuspension, thus improving the reliability of P fraction measurements. The extractants were applied in the following sequence: Mehlich-3 (Mehlich, 1984) (to extract available P), 0.5 mol l⁻¹ NaOH (to extract NaOH-P), and 1.0 mol l⁻¹ HCl (to extract HCl-P). An aliquot of each extract was used to determine P concentration via the colorimetric method of Murphy and Riley (1962).

In the NaOH extract, inorganic P was determined directly, while total

P concentration was measured after acid digestion in an autoclave. For the digestion, 0.5 ml of concentrated sulfuric acid (H_2SO_4) and 5 ml of 7.5 % (m/v) ammonium persulfate ($(\text{NH}_4)_2\text{S}_2\text{O}_8$) were added to the extract. The tube was covered with a sheet of aluminum foil and autoclaved for 120 min. After cooling, distilled water was added to adjust the final volume to 8 ml. Phosphorus concentrations were determined using the method of [Murphy and Riley \(1962\)](#). Organic P was calculated by subtracting inorganic P from the total P concentration.

Total P content was estimated from a separate aliquot of the soil sample using the method proposed by [Olsen and Sommers \(1982\)](#). For this analysis, 0.1 g of soil was placed in a 50 ml digestion tube, followed by the addition of 2 ml of concentrated H_2SO_4 , 2 ml of 37 % H_2O_2 , and saturated MgCl_2 . The tubes were then placed in the digestion block at 350 °C for 120 min. After digestion, distilled water was added to bring the final volume to 50 ml. An aliquot of the resulting solution was used to determine P concentration using the colorimetric method of [Murphy and Riley \(1962\)](#).

Occluded P was calculated based on the data obtained from the chemical fractionation of soil P. Occluded P was defined as the difference between total P and the sum of inorganic and organic P fractions extracted by Mehlich-3, 0.5 mol l⁻¹ NaOH, and 1.0 mol l⁻¹ HCl as shown in Eq. (1).

$$\text{Occluded P (mg kg}^{-1}\text{)} = \text{Total P} - (\text{Mehlich} - 3 \text{ P} + \text{NaOH} - \text{Pi} + \text{NaOH} - \text{Po} + \text{HCl} - \text{P}) \quad (1)$$

2.3. Statistical analysis

To assess the effects of land use change from native vegetation to sugarcane cultivation on soil P fractions, we applied linear mixed-effects models using the lme4 package in R (version 4.3.1). In these models, land use (sugarcane vs. native vegetation) was treated as a fixed effect, reflecting our primary interest in evaluating the direct impact of land use conversion. Site was included as a random effect to account for unmeasured environmental and spatial variability among the three study locations, which were considered representative of a broader regional context. Each P fraction was analysed separately as a response variable.

Model significance for fixed effects was assessed using Satterthwaite's approximation for degrees of freedom, as implemented in the lmerTest package. Outliers were assessed using boxplot diagnostics with the ggplot2 package in R. One clear outlier was detected for organic P at the 10–20 cm soil depth. This point was removed from both the statistical analysis and visual representation due to its undue influence on distributional assumptions. No other outliers were identified or excluded. Assumptions of normality and homoscedasticity were checked by inspecting residual plots. When necessary, data were log-transformed



Fig. 1. Geographic location of the three study sites, Paraguaçu Paulista, São Pedro do Turvo, and Tanabi, in the state of São Paulo, South-Central Brazil. All sites are located within the region of Atlantic Forest biome conversion to sugarcane cultivation. Inset map shows the location of São Paulo state within Brazil.

to meet model assumptions. At the 0–10 cm depth, Mehlich-3 P, inorganic NaOH extractable P, organic P, and HCl extractable P were not normally distributed and were therefore log-transformed. At the 10–20 cm depth, Mehlich-3 P, HCl extractable P, and occluded P also showed non-normal distributions and were log-transformed accordingly.

All statistical analyses were performed in R (R Core Team, 2025), and results were considered significant at $p < 0.05$.

3. Results

3.1. Effect of land use change on soil P fractions

Mehlich-3 P concentrations differed markedly between native vegetation and sugarcane cultivation across both sampled soil depths (0–10 and 10–20 cm) (Fig. 2). At the 0–10 cm depth, soils under sugarcane exhibited substantially higher Mehlich-3 P levels compared to those under native vegetation, with median values exceeding 60 mg kg⁻¹ under sugarcane and less than 10 mg kg⁻¹ under native vegetation (Fig. 2A). This difference was statistically significant ($p < 0.0001$), indicating a strong enrichment of plant-available P following conversion to sugarcane cultivation. A similar trend was observed at the 10–20 cm depth (Fig. 2B), where Mehlich-3 P concentrations under sugarcane remained significantly elevated relative to native vegetation ($p < 0.0001$). However, the magnitude of difference was slightly reduced compared to the topsoil layer, suggesting a potential gradient of P accumulation with depth.

Inorganic NaOH-extractable P (NaOH-Pi), representing moderately labile P associated with Fe and Al oxides, showed significant enrichment following land use change from native vegetation to sugarcane cultivation across both sampled soil depths (Fig. 3). At the 0–10 cm depth, NaOH-Pi concentrations were significantly higher under sugarcane cultivation compared to native vegetation ($p < 0.0001$) (Fig. 3A). Median values in sugarcane soils exceeded 55 mg kg⁻¹, whereas native vegetation soils showed median concentrations below 20 mg kg⁻¹. This indicates a substantial accumulation of moderately labile P in the surface layer under sugarcane, likely due to long-term fertilisation practices and changes in soil redox and binding capacity. At the 10–20 cm depth, the trend remained consistent, with sugarcane fields exhibiting significantly greater NaOH-Pi concentrations ($p < 0.0001$) than native vegetation (Fig. 3B). Although concentrations at this deeper layer were slightly lower than in the surface soil, the difference between land uses remained pronounced, suggesting that P accumulation under sugarcane extends beyond the surface horizon.

HCl extractable P (HCl-P), representing P associated with Ca compounds and extracted using HCl, showed significantly higher concentrations under sugarcane cultivation compared to native vegetation across both soil depths (Fig. 4). At the 0–10 cm depth, the median HCl-P concentration under sugarcane was nearly three to four times greater than that under native vegetation (Fig. 4A). This difference was highly significant ($p < 0.0001$) and reflects the accumulation of Ca-associated P due to liming and P fertilisation commonly applied in sugarcane

systems. At the 10–20 cm depth, a similar pattern was observed (Fig. 4B). HCl-P remained significantly elevated under sugarcane ($p < 0.0001$), although the overall concentrations were lower than in the surface layer, suggesting vertical movement of HCl-P is limited but still evident.

Occluded P, which represents the fraction of total P not extracted by conventional chemical methods and is often considered highly stable or occluded, showed inconsistent patterns with depth in response to land use change (Fig. 5). At the 0–10 cm depth, Occluded P concentrations were significantly higher under sugarcane cultivation compared to native vegetation ($p = 0.0110$) (Fig. 5A). The median value under sugarcane was approximately 230 mg kg⁻¹, compared to 190 mg kg⁻¹ under native vegetation. This suggests that land use change may lead to a modest accumulation of occluded or mineral-associated P in the surface soil, possibly due to increased P inputs and subsequent transformation into less labile forms. However, at the 10–20 cm depth, no significant difference in occluded P was observed between sugarcane and native vegetation soils ($p = 0.1731$) (Fig. 5B). While variability remained high in both land use types, the median values were similar, indicating that the impact of land use on this stable P pool is depth-dependent and less pronounced below the surface layer.

Organic P, which represents P associated with soil organic matter, was significantly affected by land use change from native vegetation to sugarcane cultivation (Fig. 6). At the 0–10 cm depth, organic P concentrations were markedly higher under native vegetation compared to sugarcane cultivation ($p < 0.0001$) (Fig. 6A). Soils under native vegetation had median values exceeding 200 mg kg⁻¹, while those under sugarcane showed values below 50 mg kg⁻¹. This substantial reduction in organic P under sugarcane is likely due to lower organic matter inputs and greater microbial mineralisation associated with intensive agricultural management. At the 10–20 cm depth, organic P remained significantly higher under native vegetation ($p = 0.0131$) (Fig. 6B), although the difference between land uses was less pronounced compared to the surface layer. Nonetheless, the trend indicates a consistent reduction in organic P stocks following land use conversion, even at subsurface depths.

Total P, which encompasses all organic and inorganic P forms in the soil, exhibited variable responses to land use change from native vegetation to sugarcane cultivation (Fig. 7). At the 0–10 cm depth, no statistically significant difference in total P concentrations was observed between land uses ($p = 0.3738$) (Fig. 7A). Despite slightly higher median values under sugarcane, the large variability within each land use class likely contributed to the lack of significance. This suggests that although specific P fractions (e.g., labile or organic forms) respond strongly to land use change, total P stocks in surface soils may remain relatively stable over the time frame and management intensity represented in this study. In contrast, at the 10–20 cm depth, total P was significantly greater under sugarcane compared to native vegetation ($p = 0.0023$) (Fig. 7B). This indicates a possible downward redistribution or accumulation of P with depth under sugarcane cultivation, potentially as a result of long-term fertilisation, tillage, or changes in soil chemistry

Table 1
Detailed site information for the study locations.

Site	Geographic coordinates	Altitude (m)	Soil classification ^a	Clay content (g kg ⁻¹)	Soil texture	Climate ^b	Sugarcane variety	Amendments (Mg ha ⁻¹)		
								Lime	Gypsum	Filter cake
Paraguaçu Paulista	22°27'42" S 50°31'8" W	475	Typic Quartzipsamment	125	Sand	Cfa	RB975201	3.5	1.0	8.0
São Pedro do Turvo	22°48'11" S 49°47'31" W	520	Rhodic Hapludox	200	Clay	Cfa	CTC 4	6.8	2.0	20.0
Tanabi	20°24'02" S 49°34'29" W	501	Rhodic Hapludox	564	Loamy sand	Aw	RB966928	1.4	0.5	–

^a According to USDA-Soil Taxonomy (Soil Survey Staff, 2014).

^b According to Köppen's climate classification: Aw: tropical climate with dry winter; Cfa: humid subtropical climate with hot summer without dry season (Alvares et al., 2013).

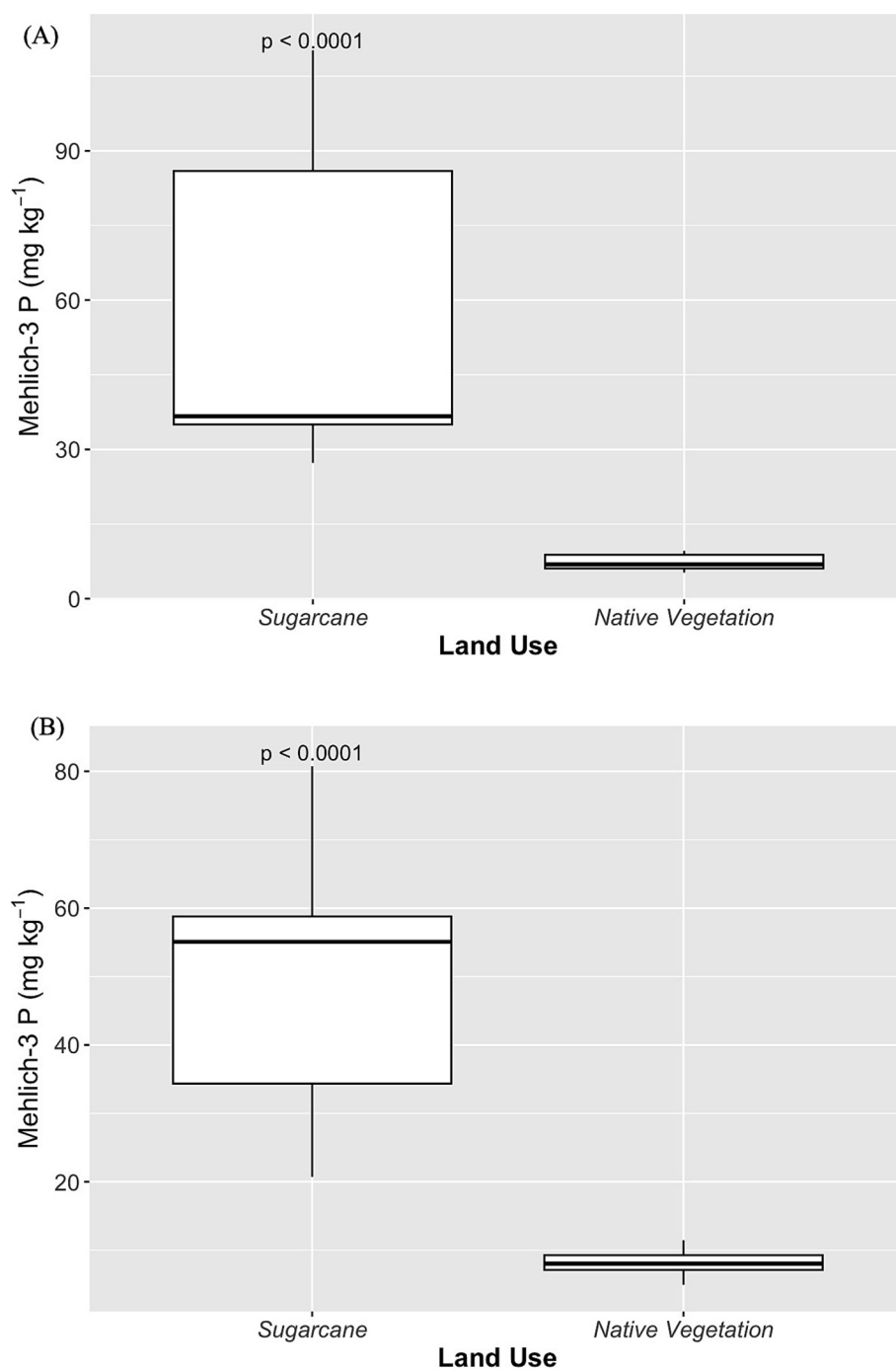


Fig. 2. Boxplots of Mehlich-3 P concentrations under sugarcane cultivation and native vegetation at two soil depths: 0–10 cm (A) and 10–20 cm (B). Data represent combined results from all three study sites. A threshold of $p < 0.05$ was considered statistically significant.

influencing P mobility. This pattern may also reflect the common practice of placing fertiliser at the bottom of the planting furrow, typically 15 to 25 cm deep.

3.2. Effect of land use change on proportional distribution of soil P fractions

The relative distribution of soil P fractions revealed marked differences between land uses and depths (Fig. 8). In the 0–10 cm layer, the proportion of Mehlich-3 P, a proxy for labile plant-available P, was substantially higher under sugarcane (14.2 %) compared to native vegetation (1.9 %) (Fig. 8A). Similar trends were observed for NaOH-Pi

(12.6 % under sugarcane vs. 4.2 % under native vegetation) and HCl-P (1.9 % vs. 0.4 %), indicating significant enrichment of both labile and moderately labile P forms under sugarcane cultivation. Conversely, organic P accounted for nearly half of the total P pool under native vegetation (48.0 %) in the 0–10 cm layer, whereas it represented only 8.0 % under sugarcane (Fig. 8A). The largest proportion of total P in all treatments was in the occluded P fraction, although its share varied by land use and depth. At 0–10 cm, occluded P made up 63.3 % under sugarcane and 45.5 % under native vegetation (Fig. 8A). In the 10–20 cm layer, occluded P increased in both land uses, accounting for 64.3 % and 78.5 % under sugarcane and native vegetation, respectively, highlighting that non-labile P forms dominate at depth, particularly in

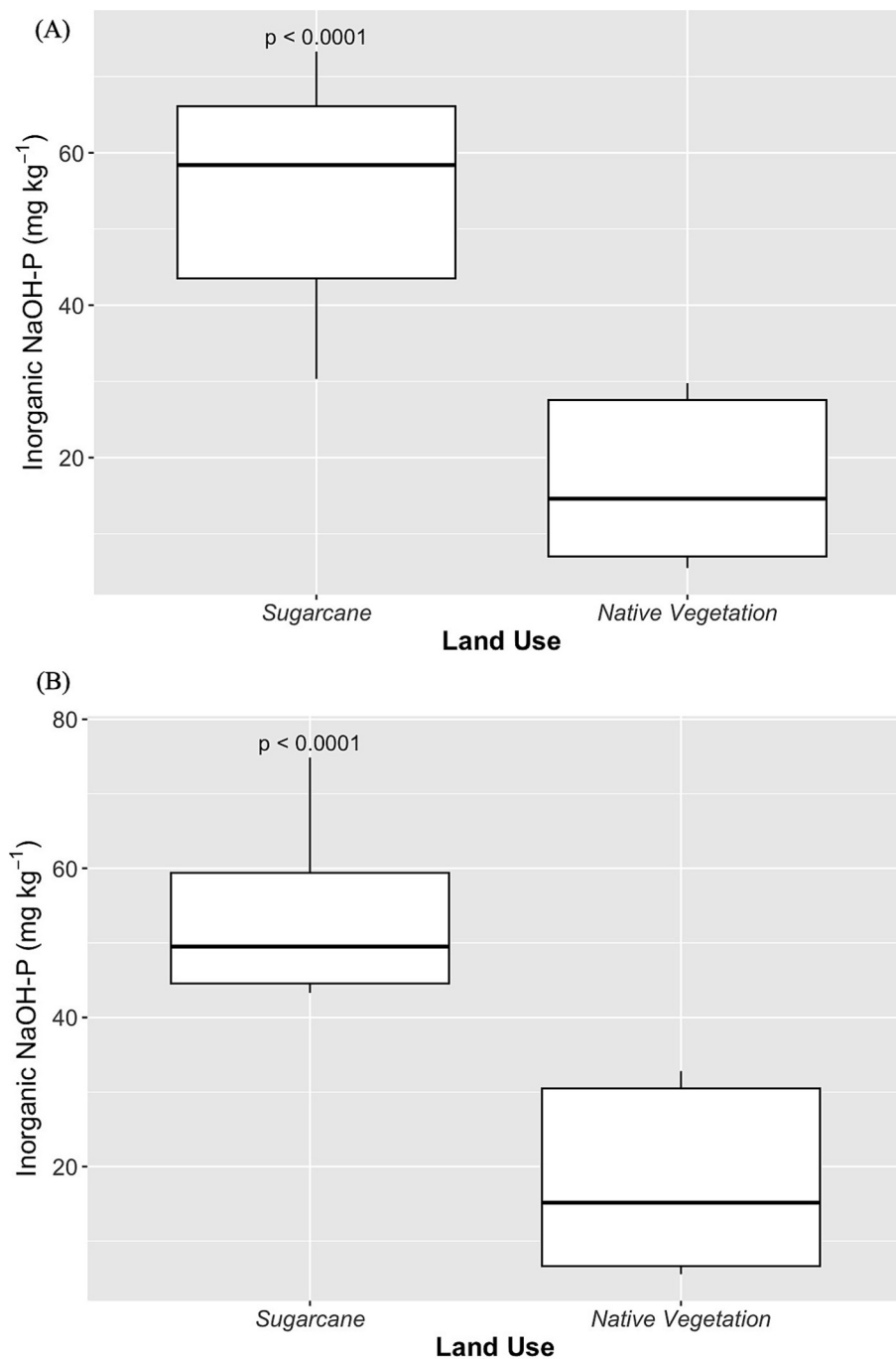


Fig. 3. Boxplots of inorganic NaOH extractable P (NaOH-Pi) concentrations under sugarcane cultivation and native vegetation at two soil depths: 0–10 cm (A) and 10–20 cm (B). Data represent combined results from all three study sites. A threshold of $p < 0.05$ was considered statistically significant.

undisturbed soils (Fig. 8B).

4. Discussion

Our results indicate that land use change from native vegetation to sugarcane cultivation leads to a pronounced and statistically significant increase in labile P in the upper soil layers. The elevated Mehlich-3 P under sugarcane likely reflects long-term fertilisation practices associated with intensive cropping, which may have implications for P mobility and environmental risk in these systems. Mehlich-3 is capable of extracting Fe and Al that are weakly bound to soil exchange sites, as well as those associated with low-crystallinity oxide minerals (Mehlich, 1984). The anion exchange resin method is the most widely used soil P

test for fertiliser recommendations in Brazilian sugarcane fields (Raij et al., 1986). This method relies on the transfer of orthophosphate ions from the soil to the resin (Silva and Raij, 1999). Valadares et al. (2017) reported a strong linear correlation between P extracted using the anion exchange resin and Mehlich-3 P in Brazilian Oxisols. The correlation coefficients were exceptionally high, 0.975 in the surface layer of a medium-textured Oxisol, 0.959 in the surface layer of a fine-textured Oxisol, and 0.909 in the subsurface layer of a fine-textured Oxisol. Given these strong correlations, the rapid P fractionation method developed by Gatiboni and Condron (Gatiboni and Condron, 2021) can be confidently applied in place of the commonly used Hedley P fractionation in Brazilian sugarcane fields. This rapid method is both faster and simpler, requiring only 16 h to complete all extraction steps and

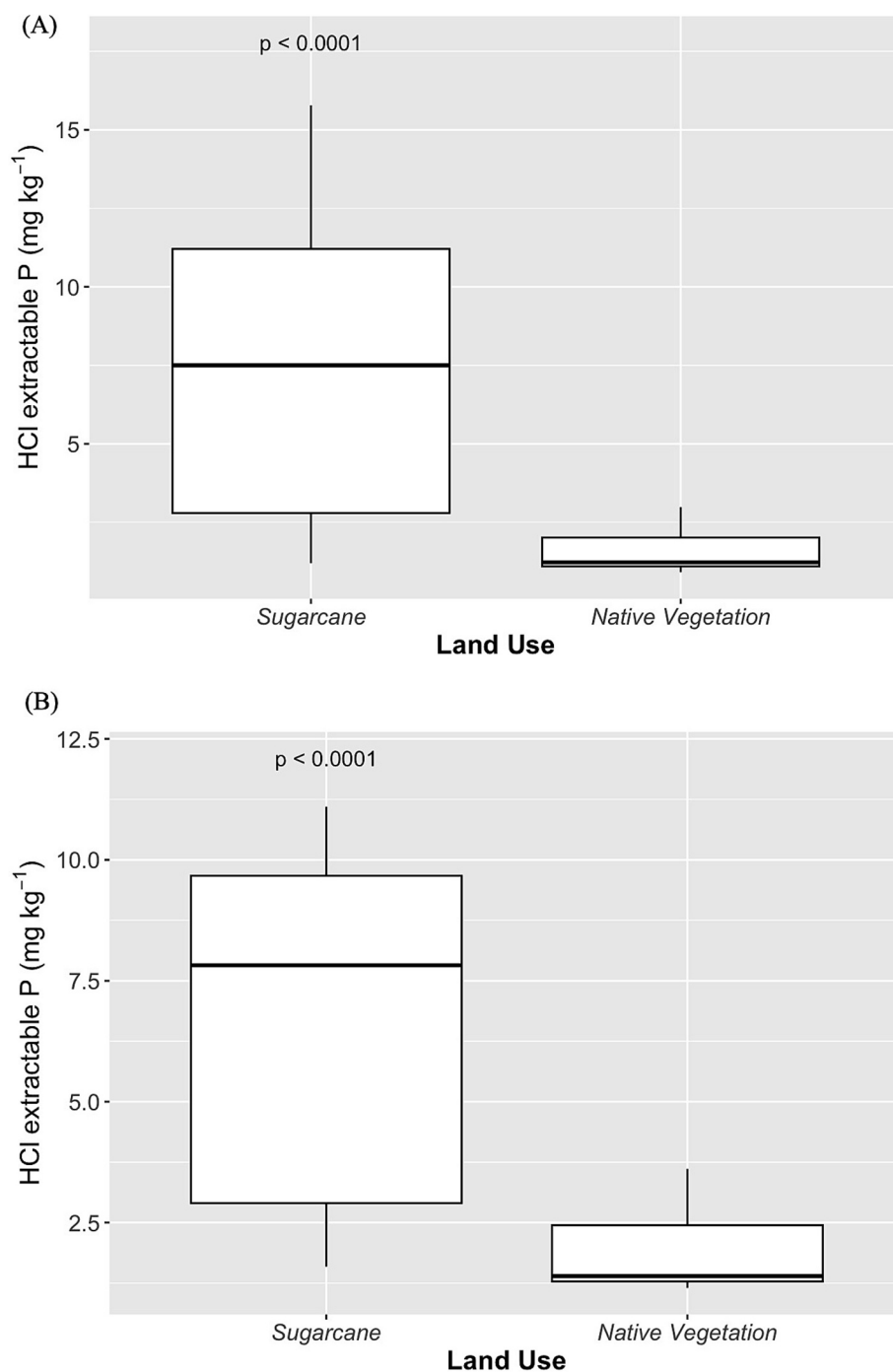


Fig. 4. Boxplots of HCl extractable P (HCl-P) concentrations under sugarcane cultivation and native vegetation at two soil depths: 0–10 cm (A) and 10–20 cm (B). Data represent combined results from all three study sites. A threshold of $p < 0.05$ was considered statistically significant.

obtain P fractions, compared to the seven days typically needed for the full Hedley procedure. Since calibration data for Mehlich-3 P are currently unavailable for Brazilian sugarcane systems, a critical level of 40 mg kg^{-1} can be adopted, as recommended by the International Plant Nutrition Institute (IPNI, 2015). Mehlich-3 P concentrations in both soil depths at Paraguaçu Paulista and Tanabi exceeded the critical level, whereas values at São Pedro do Turvo were below the critical threshold (Supplementary Table 1). This indicates that soils in Paraguaçu Paulista and Tanabi likely contain sufficient plant-available P to support optimal sugarcane growth, reducing the immediate need for additional P fertiliser. In contrast, the lower Mehlich-3 P levels in São Pedro do Turvo suggest a higher risk of P deficiency, meaning that sugarcane in this

region would benefit from P fertiliser applications to achieve optimal productivity. This spatial variability highlights the importance of site-specific soil testing and fertiliser management in sugarcane production systems. Mehlich-3 P concentrations were much higher under sugarcane cultivation than under native vegetation in both soil layers, indicating that fertiliser application enhances plant-available P even in deeper portions of the soil profile. This highlights the critical role of subsurface layers in the P nutrition of sugarcane. In Brazilian sugarcane systems, P fertilisers are typically applied at depths of 10–15 cm during crop establishment in planting furrows and subsequently broadcasted for ratoon crops. In this study, we assessed Mehlich-3 P down to a depth of 20 cm; however, it is recommended that deeper soil layers also be

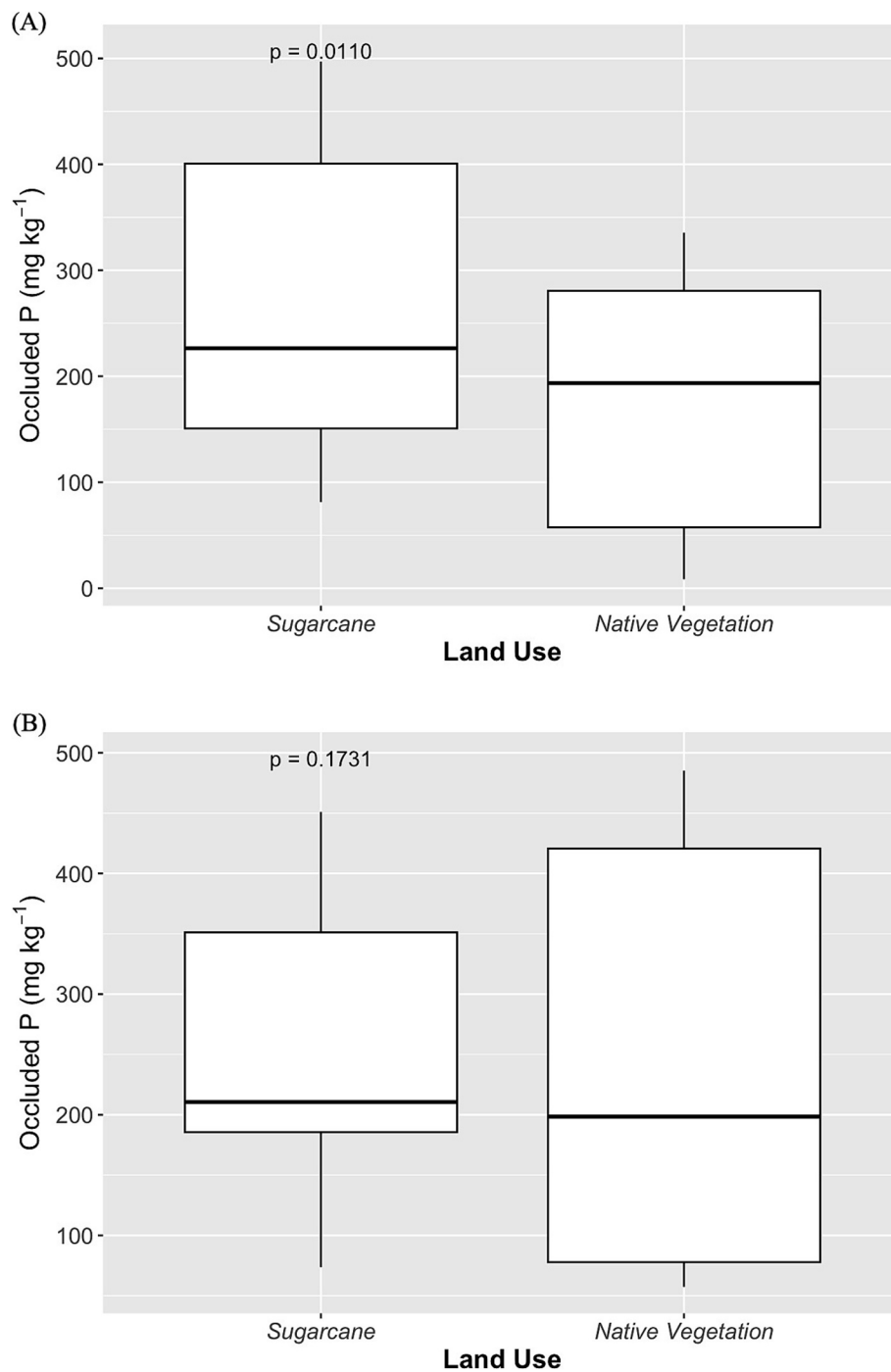


Fig. 5. Boxplots of occluded P concentrations under sugarcane cultivation and native vegetation at two soil depths: 0–10 cm (A) and 10–20 cm (B). Data represent combined results from all three study sites. A threshold of $p < 0.05$ was considered statistically significant.

evaluated and considered when managing P nutrition in sugarcane cultivation. Mehlich-3 P concentrations were consistently very low ($<10 \text{ mg kg}^{-1}$) across all sites and soil depths under native vegetation (Supplementary Table 1). Despite these low values, the native Atlantic Forest vegetation was healthy and thriving, indicating that conventional soil test methods such as Mehlich-3 may not be suitable for assessing P nutritional status in undisturbed ecosystems. These tests are primarily calibrated for agricultural systems and do not account for the adaptations of native plant species to low-P environments or their ability to access P from less labile pools. Therefore, caution should be exercised when interpreting soil test P values in natural vegetation using agromonic extraction methods.

Higher NaOH-Pi under sugarcane cultivation in both depths highlight that the conversion of native vegetation to sugarcane promotes the accumulation of moderately labile P throughout the upper soil profile, which could have implications for both crop nutrient availability and long-term P mobility in these systems. This accumulation likely reflects the long-term application of P fertilisers, which contribute to the enrichment of this fraction. Cross and Schlesinger (1995) noted that NaOH extractants primarily recover inorganic P bound to Fe and Al oxyhydroxides, suggesting that the NaOH-Pi fraction is associated with these mineral components. This fraction is considered to have intermediate lability, meaning it is not readily available under normal conditions but can be accessed by plants with high P uptake efficiency or

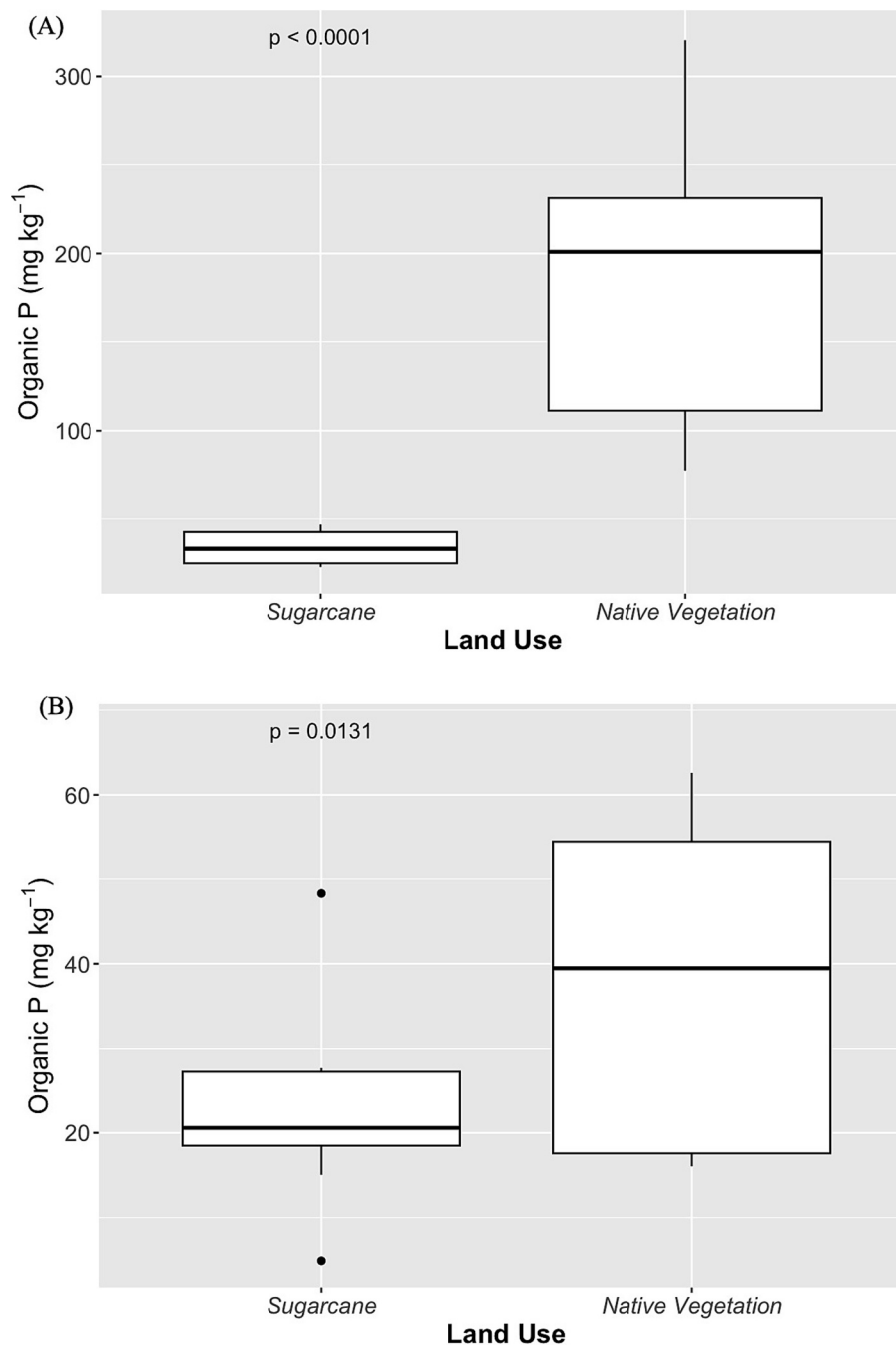


Fig. 6. Boxplots of organic P concentrations under sugarcane cultivation and native vegetation at two soil depths: 0–10 cm (A) and 10–20 cm (B). Data represent combined results from all three study sites. A threshold of $p < 0.05$ was considered statistically significant.

under conditions of severe P deficiency (Guo and Yost, 1998; Gatiboni et al., 2007; Gatiboni and Condon, 2021). The much lower NaOH-Pi concentrations observed under native vegetation suggest that this fraction can be depleted by plants in the absence of fertiliser inputs. This provides strong evidence that NaOH-Pi, while not immediately available, serves as a reservoir of plant-available P over the long term, particularly in nutrient-limited natural ecosystems. Therefore, the accumulation of this fraction in sugarcane soils and its depletion under native vegetation underscore its role as a moderately labile pool that can contribute to P nutrition under both managed and natural conditions.

The most common natural sources of P in soils are calcium-P minerals, particularly those in the apatite group (Walker and Syers, 1976). These forms are typically found in soils with high pH and tend to be

unstable under acidic conditions. As a result, P extracted using 1 mol l⁻¹ HCl in P fractionation techniques is generally interpreted as being associated with apatite or other Ca-bound forms (Cross and Schlesinger, 1995). In our study, HCl-P concentrations were consistently low (<13 mg kg⁻¹) across both land uses, reflecting the inherently low content of Ca-bound P in these soils. This is consistent with the highly weathered nature of Brazilian soils, where primary P minerals such as apatite have largely been depleted through pedogenic processes (dos Santos et al., 2018; Soltangheisi et al., 2019a). Given its consistently low concentrations and classification as a non-labile P pool, the HCl-P fraction is unlikely to play a significant role in sugarcane nutrition, particularly in areas where rock phosphate is not applied. Its limited availability further reinforces the minimal contribution of Ca-bound P to plant

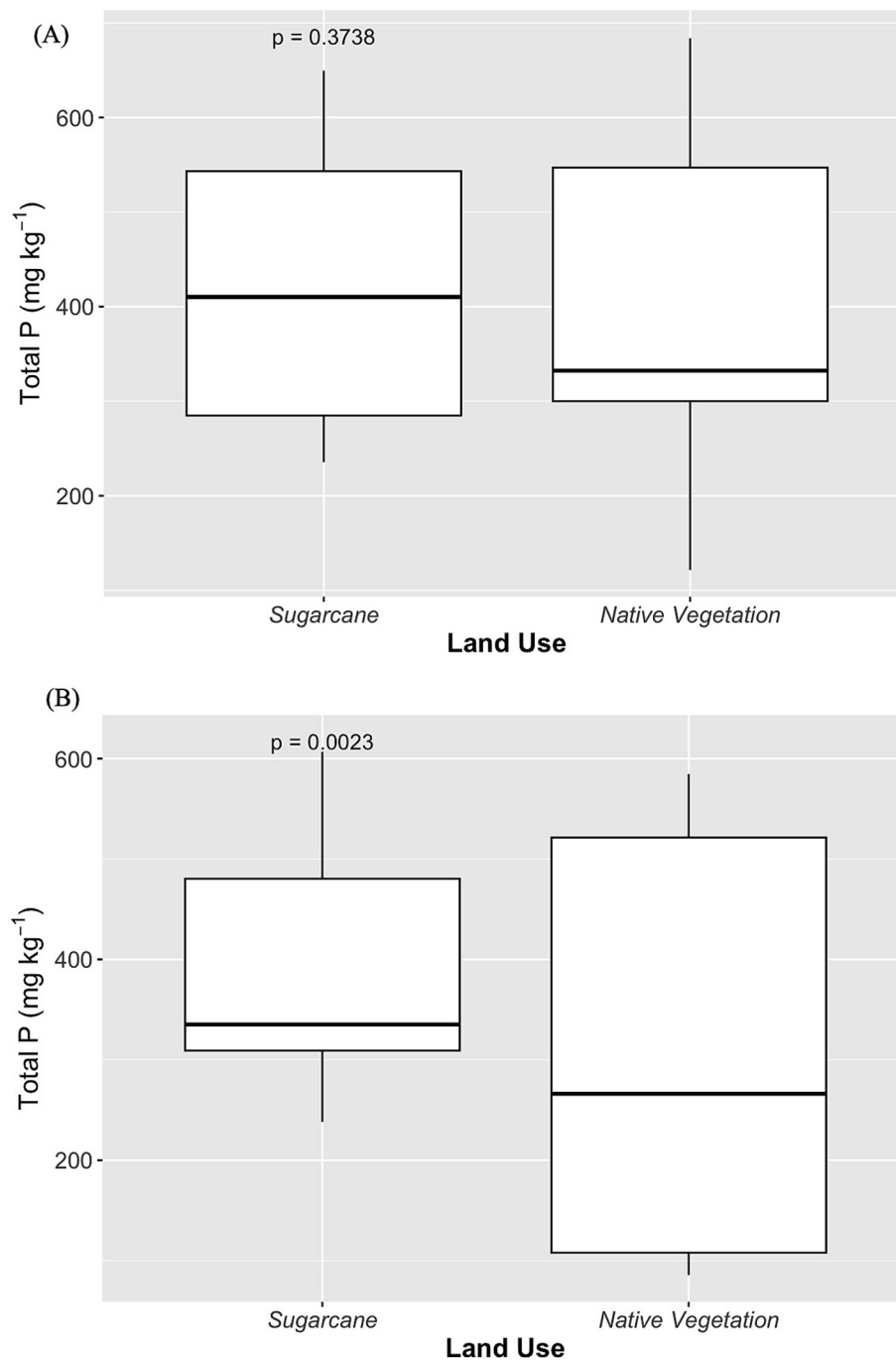


Fig. 7. Boxplots of total P concentrations under sugarcane cultivation and native vegetation at two soil depths: 0–10 cm (A) and 10–20 cm (B). Data represent combined results from all three study sites. A threshold of $p < 0.05$ was considered statistically significant.

uptake under the prevailing soil conditions in these highly weathered Brazilian soils. Higher concentration of HCl-P under sugarcane compared to native vegetation is likely due to the application of lime and gypsum during the establishment of sugarcane, which can enhance the formation of Ca-associated P compounds. Gatiboni and Condron (2021) argued that in highly weathered subtropical soils of Brazil, the presence of Ca-bound P minerals is unlikely due to extensive pedogenesis, and that any P extracted using HCl may result from artefactual formation of Ca–P during the fractionation process or from the dissolution of other P compounds, as also suggested by Gu et al. (2020). However, our results do not support this interpretation. In our study, HCl-P concentrations were consistently near zero under native vegetation but significantly higher under sugarcane cultivation. If the presence

of this P fraction were merely a methodological artefact, such as in-procedure Ca–P formation or dissolution of non-Ca-bound compounds, similar levels should have been observed across both land uses. The fact that this P fraction was almost undetectable under native vegetation while markedly enriched in sugarcane soils strongly suggests that Ca–P compounds have indeed formed in situ, possibly as a consequence of repeated lime and gypsum applications. These findings challenge the assumption that Ca-bound P is absent in these systems and highlight the capacity of agricultural amendments to alter the chemical nature of soil P pools over time.

Occluded P consists of highly recalcitrant inorganic and organic P forms that are not extracted by any of the preceding extractants (Turner et al., 2005). Due to their extremely low solubility (in the case of

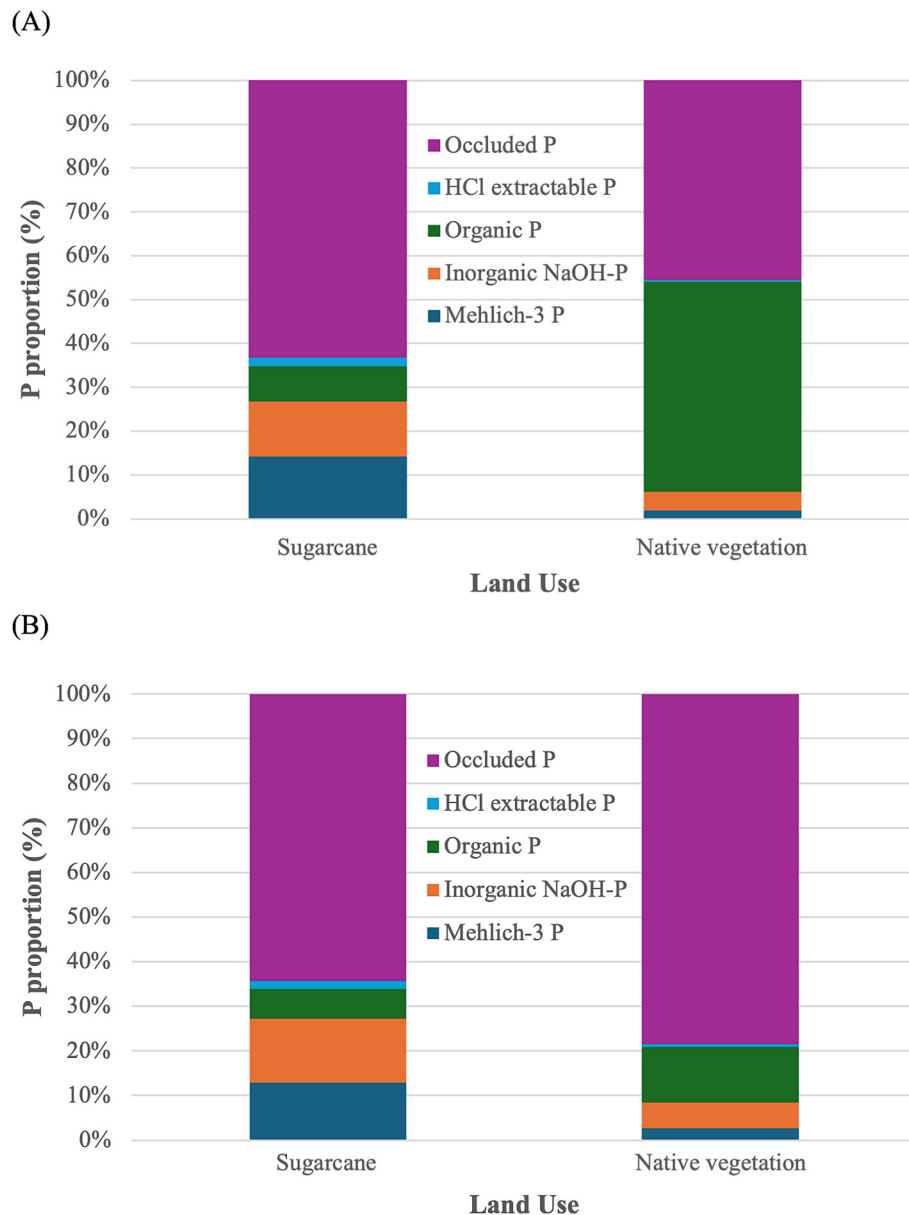


Fig. 8. Relative distribution (%) of soil P fractions under sugarcane cultivation and native vegetation at two soil depths: 0–10 cm (A) and 10–20 cm (B). Data represent the average proportional contribution of each P fraction across all three study sites.

inorganic P) and slow mineralisation rates (for organic P), these forms are largely inaccessible to plants and are therefore classified as non-labile P (Gatiboni and Condron, 2021). Unlike the labile and moderately labile P fractions, occluded P is only marginally affected by land use conversion in the upper soil layer and remains largely unaffected at greater depths. This is encouraging, as it suggests that fertiliser P applied in sugarcane fields has not accumulated in this non-labile pool. This indicates a reduced risk of P fixation into non-labile forms, contributing to improved P use efficiency in sugarcane systems over time. It seems that in these highly weathered tropical soils, the capacity to further incorporate P into occluded forms appears limited, implying that additional P inputs are more likely to enrich labile and moderately labile fractions rather than being fixed into recalcitrant pools.

The significant reduction in organic P under sugarcane likely reflects a combination of factors. While microbial mineralisation is a key driver, due to enhanced microbial activity and soil disturbance under intensive management, other contributing mechanisms should also be considered. Reducing organic matter inputs from litter and root biomass, changes in

plant community composition, and disruption of soil structure due to agricultural practices may further influence the decline of organic P in sugarcane systems. These factors collectively weaken the soil's natural capacity to stabilise and cycle organic P. Despite the relatively stable nature of organic P, these results highlight its vulnerability to management-induced changes. Moreover, the depletion of this pool under sugarcane cultivation underscores a shift away from slower-release P forms toward more inorganic, fertiliser-derived P pools. According to Cross and Schlesinger (1995), NaOH-extractable organic P represents stable organic matter-bound P, which may mineralise slowly over time. While this pool is typically considered moderately labile, its decline in sugarcane systems suggests long-term reductions in the soil's natural P buffering capacity, potentially reducing resilience to future fertiliser withdrawal. Organic P plays a crucial role in the natural P cycling of native vegetation and its loss under sugarcane cultivation may have long-term implications for soil fertility and sustainability. These findings reinforce the need for management practices that maintain or restore organic matter inputs to safeguard the organic P reservoir in

tropical agroecosystems. The results also highlight the critical importance of the surface soil layer (0–10 cm) for P nutrition in Atlantic Forest ecosystems. In native vegetation, organic P concentrations dropped sharply in the 10–20 cm layer, both in absolute and proportional terms, indicating that the majority of organic P, and thus biologically mediated P cycling, occurs near the surface. Notably, in the 10–20 cm layer, organic P levels under native vegetation were no longer significantly different from those under sugarcane, suggesting a convergence in subsurface P dynamics across land uses. This emphasizes the functional importance of the topsoil in supporting P availability in undisturbed forest systems and underscores the vulnerability of this layer to degradation following land use change. Maintaining the integrity of the surface soil is therefore essential for preserving the organic P reservoir and sustaining long-term nutrient cycling in Atlantic Forest remnants. Across the three native vegetation sites, organic P concentrations were notably higher in São Pedro do Turvo in both soil layers, which is characterized by a clayey soil texture, compared to the sandier soils of Paraguaçu Paulista and Tanabi. This difference is likely due to the greater physicochemical protection of organic matter in clay-rich soils, which reduces microbial access and decomposition rates. Clay minerals can stabilise organic compounds through adsorption and aggregation processes, thereby promoting the accumulation of organic P (Six et al., 2002).

In contrast to the labile and moderately labile P fractions, total P concentrations did not differ significantly between sugarcane cultivation and native vegetation in the 0–10 cm layer, and only a modest increase was observed under sugarcane at 10–20 cm depth. This stability in total P suggests that long-term P fertiliser use in sugarcane systems has not resulted in substantial accumulation of legacy P. The lack of significant legacy P build-up implies that much of the applied P is being taken up by crops or retained in more accessible P pools, rather than being fixed into highly stable forms such as occluded P. These results point to a higher long-term P use efficiency in Brazilian sugarcane systems than is often assumed (Pavinato et al., 2020). However, the fact that total P remains relatively stable also indicates an opportunity to further improve P management strategies, optimising fertiliser use to enhance crop uptake while minimizing losses and environmental impacts. This is especially relevant in the context of finite global P resources and the need for more sustainable agricultural practices.

Patterns of changes in proportional distribution of soil P fractions indicate that land use change from native vegetation to sugarcane cultivation shifts the soil P pool from stable and organic forms toward more labile and inorganic forms, especially in the surface horizon. This transformation likely reflects long-term fertilisation, organic matter loss, and changes in P cycling associated with intensive agriculture.

Soil texture and classification played a notable role in shaping P dynamics across sites. The clayey soil of São Pedro do Turvo exhibited the highest concentrations of organic P under native vegetation, suggesting enhanced physicochemical protection of organic matter through mineral associations and aggregate formation. This aligns with previous studies showing that clay-rich soils can stabilise organic P and reduce decomposition rates (Kaiser and Guggenberger, 2003; Six et al., 2000; Oades, 1988). In contrast, the sandy soils of Paraguaçu Paulista and Tanabi had lower organic P, likely due to reduced mineral protection. Additionally, the lower Mehlich-3 P levels observed in São Pedro do Turvo under sugarcane may be attributed to higher P fixation capacity in clayey soils rich in Fe and Al oxides, which are typical of highly weathered tropical Oxisols. Although we did not include soil texture as a formal variable in our statistical model due to the limited number of sites, these patterns underscore the relevance of edaphic characteristics in influencing P fractionation under contrasting land uses.

5. Conclusion

This study demonstrates that the long-term conversion of Atlantic Forest to sugarcane cultivation in Brazil has significantly altered the distribution of soil P fractions, especially in the upper soil layers. The

accumulation of labile and moderately labile P forms under sugarcane reflects the effect of fertiliser inputs and amendment practices, while the substantial decline in organic P highlights the degradation of natural P reservoirs linked to soil organic matter. Importantly, the lack of a corresponding increase in total and occluded P suggests that, despite long-term fertilisation, there has been limited accumulation of legacy P in non-labile forms. This finding challenges prevailing assumptions regarding widespread legacy P accumulation in Brazilian cropping systems and reveals opportunities to enhance P use efficiency by improving fertiliser strategies and managing existing soil P stocks. These insights are critical for developing more sustainable nutrient management practices in tropical agroecosystems and support calls for region-specific recommendations that account for both soil type and historical land use. Future studies should explore deeper soil layers (>20 cm) to fully quantify the vertical distribution and potential legacy P pools in sugarcane systems. Additionally, linking soil P fractions to crop P uptake data and yield response trials would strengthen our understanding of fraction-specific contributions to sugarcane nutrition.

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CRediT authorship contribution statement

Amin Soltangheisi: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Gabriel Pinheiro Silva:** Writing – review & editing, Methodology, Data curation, Conceptualization. **Wellington Soares:** Formal analysis. **Rafael Otto:** Methodology, Conceptualization. **Paulo Sergio Pavinato:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Amin Soltangheisi reports financial support was provided by British Council. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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