

# The effect of soil organic matter on long-term availability of phosphorus in soil: evaluation in a biological P mining experiment

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## Abstract

The plant uptake of legacy phosphorus (P) from over-fertilised agricultural soils could offer a solution to decrease dependency on finite mineral P resources. This study evaluated the long-term availability of legacy P in soils with an accelerated biological mining assay, thereby testing to what extent this availability is affected by soil organic carbon (SOC). A 15-month-long pot trial was set-up, in which 25 soils with 1.2–24% SOC were mined for P by continuous cropping and harvesting of ryegrass (*Lolium perenne*) in a plant growth cabinet. The cumulative uptake of P was, on average, 19% of the P associated with poorly crystalline iron (Fe) and aluminium (Al) (oxy)hydroxides (oxalate-extractable P;  $P_{ox}$ ). On average, half of this P could be taken up at rates fast enough to maintain crop production at > 90% of its potential. This P taken up before a 10% reduction in yield occurred, termed the critical cumulative P uptake (CCP), strikingly matched with the isotopically exchangeable P or “E value” of a soil (median  $CCP/E_{24h} = 0.81$ ), whereas it was markedly underestimated by Olsen P (median  $CCP/P_{Olsen} = 1.51$ ). The fractions of plant-available  $P_{ox}$  increased at increasing ratios of either P or SOC to the sum of  $Fe_{ox}$  and  $Al_{ox}$ , suggesting that enhanced SOC contents reduce ageing of P by preventing its diffusion into micropores. That effect of SOC on P availability was more pronounced in soils with a low initial P saturation status. The comparison of the results from biological mining with available soil P pools determined in a (sterile) P desorption experiment could not confirm a significant contribution of organic P to plant P supply. Based on the set of soils in our study, our findings suggest that legacy P in well-fertilised agricultural soils could act as a sufficient P source for plants for several years to decades, and that this long-term availability is positively affected by SOC as long as the soil is not saturated with P.

## Abbreviations

DPS = degree of phosphorus saturation; CCP = critical cumulative P uptake

## Keywords

accelerated biological mining, ageing, availability, long-term, phosphorus, soil organic carbon

# 1. Introduction

Phosphorus (P) is a major essential nutrient for crop growth, meaning productivity of modern agricultural systems is highly dependent on its availability to plants in soil. To guarantee this availability, P fertilisers have been abundantly applied to soils in the past decades, resulting in a largely positive soil P budget at the global scale, despite large spatial variability in agronomic P imbalances (Sattari et al., 2012). In the first decade of this millennium, there was a total net accumulation of  $6.6 \times 10^9$  kg P year<sup>-1</sup> in soils worldwide, with the largest P build-up per hectare (ha) observed in Western Europe, while the low P soils of Africa remained largely deficient due to only a limited surplus (Sattari et al., 2012). An estimated 55% of cropland fertilisation came from mineral fertilisers derived from phosphate rock (Lun et al. 2018). As a result of such unsustainable use of this finite resource, reserves are dwindling fast, making it a global priority to reduce dependency on its availability to ensure food security in the future (Cordell and White 2015). In Europe, it is now a widely advocated strategy to promote the reduction or cessation of P fertilisation on heavily fertilised soils (Jordan-Meille et al. 2012) and thereby exploit the P reserves that have accumulated in agricultural soils, the so-called *legacy P* (Kleinman et al. 2011). This utilisation or “mining” of legacy P has been cited as a first step towards a more sustainable P use in agriculture (Withers et al. 2015). Moreover, the resulting decrease of soil P concentrations could moderate the negative consequences of excess P on the environment such as eutrophication (Schoumans et al. 2014), although it was recently projected that reaching environmental targets by this type of drawdown of P alone might take several decades (McDowell et al. 2020).

An accurate assessment of the consequences of mining soil legacy P on agricultural productivity is crucial for the success of the campaign to reduce dependency on mineral P reserves. This calls for an evaluation of the *long-term availability* of the legacy P in soils. It is well-known that the availability of this P remaining in soil, which is almost exclusively found bound to four oxygen atoms as phosphate (PO<sub>4</sub>), reduces over

time because P in soil is subject to *ageing*. After the fast, reversible sorption reaction at the surface of amorphous iron (Fe) and aluminium (Al) (oxy)hydroxides following P fertiliser applications, slower ageing processes take place that reduce the ability to immediately buffer P in the soil solution (Frossard et al., 1995; McGechan & Lewis, 2002). These ageing processes include diffusion of P into micropores of mineral oxides (Madrid and De Arambarri 1985; Warrinnier et al. 2018) or precipitation of P with cations such as  $\text{Fe}^{3+}$ ,  $\text{Al}^{3+}$  (Van Riemsdijk and Lyklema 1980; Ler and Stanforth 2003) or  $\text{Ca}^{2+}$  inside such pores or at the mineral surface (Tunesi et al. 1999). As a result, the size of the rapidly desorbable or “labile” P pool decreases over time. This has been substantiated by several isotope dilution studies showing a distinct difference in P availability of freshly added P compared with legacy P (Frossard et al. 1994; Oberson et al. 2010). Accordingly, ageing of P in soils is likely to cause a pronounced hysteresis in P availability to crops with respect to the period of P reserve build-up (Verloop et al. 2010; van der Salm et al. 2017). However, research has shown that aged P is not irreversibly fixed (Johnston and Poulton 2019). Slow P desorption can replenish the rapidly desorbable or labile P pool over the long-term and, thereby, contribute to maintaining a sufficiently high P flux towards the plant roots (Smolders et al. 2020). As a result, considerable fractions amounting to 23-70% of the P associated with amorphous Fe- and Al-oxides have been shown to become available after prolonged desorption times, both in chemical (zero P sink) or biological (plant) mining experiments (Koopmans et al., 2004; Lookman et al., 1995; Vermeiren et al. 2021). Observations from long-term field trials that showed continued adequate crop production for many years under negative P regimes corroborate these results (Ellmer et al. 2000; Gransee and Merbach 2000a; Johnston and Poulton 2019; overview in Nawara et al. 2017). Recently, the cumulative soil P that could be taken up by plants before a 10% reduction in yield occurred, i.e. the critical cumulative P uptake (CCP), was linked to several widely used indices of P availability in a biological mining experiment by Nawara et al. (2018). Ammonium-lactate extraction (P-AL) and the diffusive gradient in thin film (DGT) technique correlated well with this CCP, but P-AL extracted considerably more and DGTs less P, while the Olsen method (Olsen et al. 1954)

extracted similar amounts of soil P as the CCP, even though its correlation was only borderline significant. As the study of Nawara et al. (2018) was limited to a small number of soils (8), it did not explore the impact of soil properties such as soil organic matter (SOM) on the long-term availability of P. As a result, this impact remains, to this day, largely unknown.

From a soil chemistry point of view, it is likely that the hysteresis encountered in a P mining scenario will be less pronounced in soils with a higher organic matter content. As with  $\text{PO}_4$ , SOM is known to bind to amorphous Fe and Al (oxy)hydroxides, and can thereby act as a competitor for sorption sites. Indeed, studies have repeatedly shown a direct effect of competition and electrostatic interaction, both in batch sorption experiments using model Fe or Al compounds and humic or fulvic acids (Antelo et al. 2007; Weng et al. 2008) and in actual soil systems (Hiemstra et al. 2010; Regelink et al. 2015). The reduced  $\text{PO}_4$  loading resulting from this direct SOM effect can potentially alter the P concentration gradient towards the interior of oxide particles and, consequently, reduce its ageing. In addition, the large size of SOM molecules possibly enables them to occlude micropores in amorphous oxides (Kaiser and Guggenberger 2003) and thus prevent the ageing of P by diffusion into these pores. The latter was recently suggested in a P desorption study in which many soils were subjected to P mining using anion exchange membranes as a zero P sink (Vermeiren et al. 2021). However, the speculated positive effect of SOM on long-term soil P availability is not limited to its potential reduction of P ageing processes by competition with soil organic carbon (SOC) alone. Organic P ( $\text{P}_o$ ) present in SOM could additionally contribute to the long-term supply of P to crops through mineralisation processes. Such  $\text{P}_o$  constitutes, on average, 25% of total P in agricultural soils (Stutter et al. 2015) and is considered to be less prone to ageing due to its larger molecular size. Müller and Bünemann (2014) have shown that, under quasi steady-state conditions (i.e. no large changes in available P pools), net rates of  $\text{P}_o$  mineralisation are small. However, in a P mining scenario, mineralisation reactions of  $\text{P}_o$  could be enhanced due to increased secretion of plant or microbial phosphatase and/or other root exudates (Olander and Vitousek 2000; Spohn et al. 2013; Spohn and Kuzyakov 2013), thereby exceeding P

immobilisation rates. Moreover, with time, desorption rates will decrease in the absence of external P additions, potentially increasing the relative importance of  $P_o$  to sustain P uptake by crops (Koopmans et al. 2004; van der Salm et al. 2009). Both these soil chemical and soil biological mechanisms suggest a positive effect of SOM on the long-term availability of P in soil. From an agronomic perspective, this would imply that organic fertilisers with P such as farmyard manure, sludge and compost might be more efficient P sources than mineral fertilisers, as they keep soil P available for a prolonged time.

In summary, if utilisation of soil legacy P is to be promoted, there is a requirement for better information about the availability of this legacy P in soils. This study was set up to evaluate the potential long-term bioavailability of P in soils under P drawdown, and to identify the soil properties that affect it. Due attention is paid to the role played by SOM, as it is hypothesised that SOM can reduce ageing of P in soils and, consequently, keep P better available over time.

## **2. Materials and Methods**

Testing of our hypothesis requires sufficient soil P removal to induce P deficient conditions. Such P depletion can take years to decades, but was accelerated here by performing a pot experiment with ryegrass that was continuously grown and harvested at a high plant:soil ratio. Mining was performed on a large number of soils (25) with contrasting properties to compensate for the lack of existing field trials with long-term factorial applications of SOM and P. The long-term availability of P to plants was evaluated by determining both critical and total cumulative P uptake and correlated with selected soil properties. In addition, results from the biological mining were compared to P desorption data from a chemical mining experiment using anion exchange resins involving largely the same set of soils (Vermeiren et al. 2021). As the latter study focussed solely on the effect of SOM on P ageing and was, therefore, performed under abiotic conditions, comparison between the two experiments could provide valuable information of the contribution of organic P mineralisation to P availability in a mining scenario.

## 2.1 Soils

A total of 53 topsoils (0-10 cm) was collected from agricultural lands across the UK, preselected based on the likelihood of contrasting soil properties and a more than sufficient level of available P built up by heavy P fertilisation.. All soils were homogenised, air-dried and sieved to <4 mm for storage or <2 mm for characterisation. Soil pH was measured in 10 mM CaCl<sub>2</sub> at a soil:solution ratio of 1 g:5 mL. Sand, silt and clay contents of the soils were determined by laser diffraction analysis (NRM laboratories; Bracknell, UK), and their water holding capacity by gravitational draining of saturated soils for two days. Soil organic carbon (C) and nitrogen (N) content were determined by dry combustion of crushed soil samples that were acidified with 10% HCl to remove inorganic C, and subsequent measurement of released CO<sub>2</sub> and N<sub>2</sub> with an elemental analyser (EA1108, Thermo Scientific).

Isotopically exchangeable P and Olsen P were determined as indices of P availability. The Olsen P test (Olsen et al., 1954) is widely used as a standard soil P test to determine plant-available P, and its concentration was therefore included as a selection criterion for the soils used in this study (i.e. Olsen P index  $\geq 3$ ). The extraction was performed by shaking a 1:20 suspension of soil in 0.5 M NaHCO<sub>3</sub> (pH 8.5) on a horizontal shaker (130 rpm) for 30 minutes, after which the suspension was filtered over Whatman no. 2 filter paper. Extracted P was quantified colorimetrically with the molybdenum blue method of Murphy & Riley (1962). Absorbance of the coloured complex was measured at 890 nm, using a Perkin Elmer Lambda 25 spectrophotometer. The isotopically exchangeable P or the E<sub>24h</sub> value was determined according to Maertens et al. (2004). Soils pre-equilibrated in 5 mM CaCl<sub>2</sub> at a 1 g:10 mL soil:solution ratio were spiked with 30 kBq <sup>32</sup>P per g dry soil. After 24h of isotope dilution, the <sup>31</sup>P concentration (<sup>31</sup>P<sub>sol</sub> ; mg P L<sup>-1</sup>) and remaining <sup>32</sup>P activity concentration (<sup>32</sup>P<sub>sol</sub> ; Bq L<sup>-1</sup>) in solution were measured in 0.45 µm filtrates with malachite green (Ohno and Zibilske 1991) and liquid scintillation counting (Perkin Elmer, Tri-carb 2800 TR

Liquid Scintillation Analyser), respectively. These data were used to determine the specific activity of P in solution, i.e. the ratio  $^{32}\text{P}_{\text{sol}}/^{31}\text{P}_{\text{sol}}$  ( $\text{SA}_{\text{sol}}$ ;  $\text{Bq mg P}^{-1}$ ), and from this, the  $E_{24\text{h}}$  value was calculated as:

$$E_{24\text{h}}(\text{mg P kg}^{-1}) = \frac{1}{\text{SA}_{\text{sol}}(\text{Bq mg P}^{-1})} {}^{32}\text{P}_{\text{added}}(\text{Bq kg}^{-1}) \quad (\text{Equation 1})$$

Oxalate P ( $\text{P}_{\text{ox}}$ ), associated with amorphous Fe ( $\text{Fe}_{\text{ox}}$ ) and Al ( $\text{Al}_{\text{ox}}$ ) oxyhydroxides, was determined by the acid oxalate extraction method of Schwertmann (1964): air-dried soil was extracted for 2 h with a mixture of ammonium oxalate and oxalic acid at pH 3 in darkness. After passing through 0.45  $\mu\text{m}$  filters (Chromafil Xtra, PET -45/25), the extracts were analysed for P, Fe, Al and Mn with ICP-OES (Thermo Scientific iCap 7000). These oxalate-extractable elements were used to determine the degree of P saturation (DPS; (Van der Zee et al. 1990)) as:

$$\text{DPS}(\text{mol mol}^{-1}) = \frac{\text{P}_{\text{ox}}(\text{mol kg}^{-1})}{0.5 \cdot (\text{Fe}_{\text{ox}}(\text{mol kg}^{-1}) + \text{Al}_{\text{ox}}(\text{mol kg}^{-1}))} \quad (\text{Equation 2})$$

and the molar ratio of SOC versus oxalate-extractable oxyhydroxides ( $\text{SOC}/(\text{Fe} + \text{Al})$ ) as:

$$\text{SOC}/(\text{Fe} + \text{Al})(\text{mol mol}^{-1}) = \frac{\text{SOC}(\text{mol kg}^{-1})}{(\text{Fe}_{\text{ox}}(\text{mol kg}^{-1}) + \text{Al}_{\text{ox}}(\text{mol kg}^{-1}))} \quad (\text{Equation 3})$$

Additionally, dithionite extractions were performed on the soils to measure the sum of amorphous and crystalline oxyhydroxides (Fe, Al and Mn) as well as their associated P. In accordance with Mehra & Jackson (1960), air-dried soil was extracted with a dithionite-citrate-bicarbonate (DCB) mixture at a 1 g:50 mL soil:solution ratio by shaking the suspensions for 2h at 40°C and, subsequently, for 16h at 25°C. Samples were then centrifuged for 15 min at 1968 RCF, filtered over 0.45  $\mu\text{m}$  (Chromafil Xtra, PET -45/25) and analysed for P, Fe, Al and Mn with ICP-OES (Perkin Elmer optima 7500 DV). Finally, total soil P concentrations were determined by muffling of the soils at 550°C to convert  $\text{P}_\text{o}$  to inorganic P, and subsequent extraction of the ashes with 0.5M  $\text{H}_2\text{SO}_4$  (16 h) at a 1 g: 25 mL soil:solution ratio (Saunders and



Williams 1955). After filtration over 0.45  $\mu\text{m}$  syringe filters, extracted P was measured colorimetrically with malachite green.

Based on these characterisation results, 25 soils with contrasting soil properties and an Olsen P index of 3 or higher (20-58 mg P  $\text{kg}^{-1}$ ; AHDB (2022)) were eventually selected for the biological P mining experiment performed in this study (Table 1).

## 2.2 Biological mining experiment

The 25 selected agricultural soils were used in a 15-month-long pot experiment, in which they were subjected to mining of the soil P by continuously growing and periodically clipping ryegrass. To set up this experiment, soils were sieved to < 4 mm and mixed with quartz chips at a 1:1 ratio to prevent structural limitations caused by e.g. compaction due to daily watering during the prolonged pot experiment. Soils were wetted to 60% water holding capacity using deionised water and an all-inclusive (with the exception of P) nutrient solution, supplying 400 mg N  $\text{kg}^{-1}$  ( $\text{NH}_4\text{NO}_3$ ), 100 mg K  $\text{kg}^{-1}$  (KCl), and other macro- and micronutrients at rates shown in the Supplementary Information (SI; Table S1). The soil/quartz mixtures were divided over three replicate pots ( $d = 13.5$  cm,  $h = 8.5$  cm), with each pot containing the equivalent of 0.5 kg dry soil. The potted soils were sown with Italian ryegrass (*Lolium perenne*; Abermagic variety) seeds at a density of 1 g  $\text{kg}^{-1}$  dry soil to ensure an initial dense sward and rapid depletion of P from the soil. Plants were grown in a growth chamber, with 16h day ( $T=20^\circ\text{C}$ ; 550  $\text{mmol m}^{-2} \text{sec}^{-1}$ ) and 8h night ( $T=16^\circ\text{C}$ ) cycles. To keep the soil water content optimal for plant growth, gravimetric water losses were restored daily.

Ryegrass was grown for 448 days, or until plant growth failed, and harvested every 3-4 weeks. This resulted in a total of 18 harvests, i.e. on days 28, 47, 68, 89, 110, 131, 152, 182, 208, 238, 266, 287, 308, 337, 372, 394, 419 and 448 after sowing. At every harvest, shoots were cut at 2 cm above the soil surface and dried

at 60°C for analysis. After determination of the dry matter yield, dried plant samples were ground with a hammer mill and a subsample was digested in a heating block with a 85/15 v/v% mixture of HNO<sub>3</sub> and HClO<sub>4</sub>. Phosphorus and other macro- and micronutrients were measured in the digests with ICP-OES (Perkin Elmer Optima 7300 DV, Shelton, CT USA). To prevent the occurrence of nutrient deficiencies other than P, 240 mg N kg<sup>-1</sup> and 200 mg K kg<sup>-1</sup> were consistently applied after every harvest.

## 2.3 Data analysis

For every soil of the biological mining experiment, two endpoints were selected to relate to soil properties and indices of available P in soil. The first endpoint is the cumulative P uptake during the experiment, which is the sum of the product of P concentration and dry matter yield of the above-ground biomass over all harvests. This cumulative uptake is further referred to as the total cumulative P uptake (TCP). The second endpoint is the critical cumulative P uptake (CCP; mg P kg<sup>-1</sup> dry soil), i.e. the cumulative P uptake up to the point where P becomes deficient. Several methods can be used to define that deficiency point, which are either based on plant growth, shoot P concentration or the product of both. Here, we defined the onset of P deficiency when above-ground (dry) matter yield was below 90% of that at Harvest 2, i.e. when the relative yield (RY) < 90% with RY defined as

$$RY(\%) = 100 * \frac{yield(t_i)/(t_i - t_{i-1})}{yield(t_2)/(t_2 - t_1)} \quad (\text{Equation 4})$$

with  $t_i$  the day of the harvest in question, and both yields expressed in g dry matter per kg dry soil. Harvest 2 (on Day 47) was chosen as a reference for relative yield calculations because it was assumed that the root system was, by then, well-established and, at the same time, no P deficiency had occurred. As the period between two consecutive harvests varied between 19 and 35 days, dry matter yields were corrected for number of growing days. Exceptionally, one harvest < 90% was allowed if the following >2 harvest were again above 90% to prevent premature cut-off. In the majority of cases, the point of deficiency coincided

more or less with the point where shoot P concentrations dropped below about 2 mg P g dry matter<sup>-1</sup>, but not always. Therefore, two additional definitions of the point of deficiency per soil were used: (1) the point at which shoot P concentrations decreased below 2 mg P g dry matter<sup>-1</sup> and (2) the point at which the shoot P uptake, corrected for growing period, was less than 90% of that at Harvest 2 for a hypothetical P concentration in the latter of only 2 mg P g dry matter<sup>-1</sup> (argument in SI; Equation S1). Figure S1 shows that these three definitions yielded comparable values for the CCP. More importantly, the conclusions about the most optimal relationships between CCP and both soil properties and different indices of soil P availability were unaffected (Figure S2).

The total and critical cumulative P uptake were subjected to correlation and regression analysis with the soil properties presented in Table 1. For these analyses, both endpoints were expressed as a fraction of  $P_{ox}$  in the soil. The acid oxalate extraction is one of the more aggressive soil extraction methods, generally capturing 40-90% of total P in soil (Neyroud and Lischer 2003; Stutter et al. 2015), and has been shown to perform well in capturing the plant P uptake mass balance in a P depletion experiment (Nawara et al. 2018). Here, the  $P_{ox}$  concentrations varied by more than a factor of ten among soils (Table 1). As it is logical that soils with a higher total P (and higher  $P_{ox}$ ) concentration contain more P that can be utilised, it was decided to normalise P uptake and P availability indices per unit of  $P_{ox}$  to better indicate the relative bioavailability among soils during mining.

Results from biological mining were compared to available soil P pools determined in a long-term P desorption experiment (“chemical mining”) that was performed in parallel on a selection (20 out of 25) of the same set of soils (Vermeiren et al. 2021). Briefly, these soils were subjected to 288 days of chemical P mining by using anion exchange membranes (AEMs; 551642S, VWR Chemicals) as a zero P sink. The cumulative P desorption ( $Q_{des}(t)$ ) versus time was described by a serial two-pool kinetic desorption model, with a fast desorbable P pool ( $Q_1(t)$ ) in direct contact with the soil solution and a slow desorbable P pool

( $Q_2(t)$ ) that replenishes the former. Fitting this model to the desorption data yielded estimates for the initial labile ( $Q_1$ ) and total desorbable ( $Q_1+Q_2$ ) P pool, with  $Q_1+Q_2 \leq P_{ox}$ , as well as their respective desorption rate constants. In addition, this two-pool model also allowed to extrapolate desorbed P concentrations to 448 days ( $Q_{des}(448d)$ ), i.e. the duration of the biological mining experiment, as an indication of the potential P desorption within the timeframe of the present study. This desorption experiment was performed in the presence of chloroform ( $CHCl_3$ ) to minimise organic P mineralisation during chemical P mining. As a result, the comparison between biologically and chemically mined P can be used as an indication for the impact of organic P mineralisation on long-term P availability.

All statistical analyses were performed using JMP Pro 16 (SAS, Cary, NC, USA). Several data distributions, such as oxalate- and dithionite-extractable elements in soil, were positively-skewed. For skewness coefficients exceeding the threshold of 1, the data were  $\log_{10}$  transformed prior to analysis (Webster 2001). For correlation and regression analyses, either among pools or between pool sizes and soil properties, Pearson correlation coefficients were calculated. Absolute comparison of pool sizes such as, for example, the  $Q_1$  pool and the CCP, was done by evaluating the means for statistical difference with t-tests. Levene's test was used to assess homogeneity of variances; in case this suggested inequality, the Welch's t-test was used instead of a student's t-test (Welch 1951). Under the null hypothesis that the means are equal, their correspondence is reported by means of p-values, with typical levels of  $p < 0.05$ ,  $< 0.01$  and  $< 0.001$  indicating a statistically significant difference.

## 3. Results

### 3.1 Soils

Properties of the 25 grassland and arable soils selected for the biological P mining experiment in this study are presented in Table 1. All soils had a  $P_{Olsen}$  content between 21-128 mg P  $kg^{-1}$  and could, therefore, be

categorised as P index  $\sim 3$  or more (AHDB 2022). All other soil properties varied considerably among soils. The pH ranged from 4.7 to 7.4 and the SOC content from 1.2% to a remarkably high 23.8%, yielding SOC/(Fe+Al) values of 7-50 mol mol<sup>-1</sup>. The  $P_{ox}$ , associated with poorly crystalline Fe and Al oxyhydroxides, ranged between 200 and 2600 mg P kg<sup>-1</sup>, constituting 38-78% of total soil P. This corresponded to DPS values of 0.18-0.56 mol mol<sup>-1</sup>, with the exception of calcareous Soil 7 with a DPS=1.00. Dithionite-extractable P correlated well with  $P_{ox}$  ( $R^2=0.86$ ;  $p<0.001$ ) and they corresponded remarkably well in size, despite the significantly higher extracted Fe content in the former ( $p < 0.001$ ), confirming the lower importance of crystalline oxides as adsorbents of P in soil.

### **3.2 Available P in biological mining and correlation to available P in soil P tests**

Ryegrass growth and P uptake over the 448 days of biological mining were substantially different between soils, despite the generally similar index of  $P_{Olsen}$  availability within the dataset. This is clearly illustrated in Figure 1, presenting significantly different total yield and cumulative P uptake data among four different soils with comparable initial  $P_{Olsen}$  and  $P_{ox}$  contents (Soil 1, 4, 15 and 16). Among all soils, maximum P uptake rates varied between 0.3 and 3.0 mg kg<sup>-1</sup> day<sup>-1</sup>, with plant P concentrations at the start of mining reaching up to 5 mg P g<sup>-1</sup> (dry matter). However, due to the high plant:soil ratio, P uptake rates soon decreased, with significantly reduced yields for all soils after only a couple of harvests. Ryegrass growth persisted for several more harvests under P deficient conditions, but eventually stopped well before the end of the experiment for the majority of the soils, except for Soils 1, 2, 15 and 22-25. The total dry yield of aboveground ryegrass biomass cumulated over the whole course of the experiment varied between 20 and 195 g per kg dry soil. This resulted in a TCP ranging between 32 and 446 mg P kg<sup>-1</sup> among soils, accounting for 4-50% of the  $P_{ox}$  (Figure 2). On average among all soils, half of this TCP (52%) occurred at rates high enough to maintain crop production at > 90% of its potential. This so-called CCP indicates the readily available soil P. It ranged between 16 and 385 mg P kg<sup>-1</sup>, constituting on average 11% of  $P_{ox}$ . In reality, both CCP and TCP would be

somewhat larger because P in the below-ground biomass was not accounted for in this study. However, a comparison between measured and predicted (i.e. taking only P uptake by the above-ground harvested biomass into account) soil DPS values at the end of the depletion trial showed that root P stock has only a marginal contribution (Figure S3).

Figure 3A shows that the fraction of  $P_{ox}$  that comprised the CCP corresponded fairly well to the isotopically exchangeable P fraction in soil ( $E_{24h}/P_{ox}$ ), both in terms of size (median  $CCP/E_{24h}$  ratio = 0.81; Table 2) and correlation ( $R^2=0.56$ ;  $p < 0.001$ ). Due to the close link between the CCP and TCP ( $R^2=0.80$ ;  $p < 0.001$ ; data not shown),  $E_{24h}/P_{ox}$  was also well correlated to the latter fraction of  $P_{ox}$ , but uptake exceeded the  $E_{24h}$  value (median  $TCP/E_{24h}$  ratio = 1.47;  $p = 0.05$ ). The widely used  $P_{Olsen}$  largely underestimated both the CCP ( $p < 0.05$ ) and TCP ( $p < 0.001$ ) for the majority of the soils (Table 2) and correlations were less strong compared to the  $E_{24h}$  value (Figure 3B).

The P uptake results from the biological mining experiment were also compared with P desorption data from the (sterile) chemical mining experiment in Vermeiren et al. (2021), which used AEMs as a zero P sink to mine P from largely the same set of soils (Figure 4; Table 5.2). The initial labile or “rapidly desorbable” P fraction ( $Q_1/P_{ox}$ ) corresponds markedly well to the CCP fraction of  $P_{ox}$ , both with respect to correlation ( $R^2 = 0.76$ ; Figure 4A) and size (median  $CCP/Q_1$  ratio = 0.72), as the means were not statistically different ( $p = 0.84$ ). Moreover, a strikingly identical mean (t-test  $p = 0.71$ ) of 11% was obtained for  $CCP/P_{ox}$  and  $Q_1/P_{ox}$  when considering not only the 20 soils in common but all the soils in the respective experiments (i.e.  $n=25$  for the biological and  $n=24$  for the chemical mining experiment). The total cumulative P uptake ( $TCP/P_{ox}$ ) was well correlated with desorbable P fractions determined by chemical mining. Nevertheless, biologically mineable P was, on average, significantly smaller than total desorbable P ( $Q_1+Q_2$ ; (median  $TCP/(Q_1+Q_2)$  ratio = 0.41;  $p < 0.001$ ) while it was well above the labile P ( $Q_1$ ), especially at higher fractions (Figure 4;  $p < 0.01$ ). The  $Q_1$  and  $Q_2$  pools are fitting parameters from the 288 days P desorption curves and, together,

signify an estimate for the total desorbable P. Hence, the time to reach such P desorption might be longer than the total plant growing time. Nevertheless, the chemically desorbed P extrapolated to the same duration as the biological mining experiment was, on average, still a factor 2.5 larger than biologically mined P at 448 days after seeding in the corresponding soil (Table 2).

The comparison of P release between biological (total cumulative P uptake, TCP) and abiotic chemical mining (total desorbable P,  $Q_1+Q_2$ ) showed that the  $TCP/(Q_1+Q_2)$  ratio was below 1, i.e. uptake by plants did not exceed abiotic desorption. Moreover, the ratio did not increase with SOC content of the soil and was even entirely unaffected by it (Figure 5; note that the Arthur Rickwood soil was excluded). The same was observed when comparing the TCP to the desorbed P after 448 days (the duration of the biological mining;  $Q_{des}(448d)$ ) rather than the total desorbable P, with the former being calculated by extrapolation of the chemical mining data using the two pool model (Table 2).

### **3.3 Soil properties affecting long-term P availability**

Total and critical cumulative P uptake, expressed as a fraction of  $P_{ox}$ , were linked to soil properties in a correlation analysis including all soils except the one from Plumpton (Soil 7). The latter was the only soil with a rather calcareous nature (8.1% inorganic C; data not shown) and was clearly an outlier in correlations involving mineral oxides. This is not illogical, since the acid oxalate extraction is known to perform less well in calcareous soils for quantification of the amorphous mineral oxides and their associated P (Guo and Yost 1999; argumentation in SI of Koopmans et al. 2020). Moreover, both the outlying behaviour of Soil 7 with respect to the DPS and correlations with oxides, and its low available P fractions, suggest the occurrence of interactions of P with  $CaCO_3$  in addition to those with Fe and Al (oxy)hydroxides (Frossard et al. 1995). Pearson correlation coefficients for the whole set of selected soil properties can be found in Table S3. The fraction of  $P_{ox}$  available for plant uptake was mainly dictated by the presence and occupation of mineral sorption surfaces in the soil. Both the DPS and  $\log_{10}(SOC/(Fe+Al))$  were significantly correlated with the CCP

(CCP/P<sub>ox</sub>; Figure 6), and  $\log_{10}(\text{SOC}/(\text{Fe}+\text{Al}))$  also most significantly explained the total cumulative uptake of P (TCP/P<sub>ox</sub>) ( $R^2=0.35$ ,  $p < 0.01$ ). Clustering of the soils revealed a distinctly different contribution of the DPS and SOC/(Fe+Al) ratio in explaining plant P uptake depending on the initial P saturation of the soil. For soils with a relatively low P saturation (DPS < 0.30), both the CCP and TCP (as % of P<sub>ox</sub>) most significantly increased with increasing SOC/(Fe+Al) ratio of the soil (Figure 6B;  $p < 0.01$ ), while they were unaffected by its DPS (Figure 6A; n.s.). In contrast, for more P saturated soils (DPS > 0.30), an increase in P uptake (as % of P<sub>ox</sub>) was mainly explained by an increase in DPS of the soil (Figure 6A;  $p < 0.05$ ).

In addition to oxalate-extractable elements, results from dithionite extraction were included in the correlation analysis. Involving more crystalline oxides in this way did not yield better correlations, leading to the conclusion that these sorption surfaces do not significantly contribute to explaining the variability in plant availability of P in soil. This also validated the use of P<sub>ox</sub> as the relevant P pool to relate to (i.e. CCP/P<sub>ox</sub> etc) throughout this study.

## 4. Discussion

### 4.1 Long-term availability of P in soil

Recent years have seen an increased incentive to mine and reutilise the legacy P built-up in heavily fertilised arable soils. This incentive is typically put into practice by imposing a negative P balance on those soils that have a high to excessive value for a certain soil P test (e.g. Olsen P, P-AL; Jordan-Meille et al. 2012). The Olsen P index of  $\geq 3$  measured in the soils presented here marks an elevated available P concentration compared to the target value for arable lands and grasslands in the UK, i.e. 16-25 mg P L<sup>-1</sup> soil or 12-19 mg P kg<sup>-1</sup> if considering a soil bulk density of 1.3 kg L<sup>-1</sup> (AHDB 2022). Accordingly, all soils in the ensemble are considered to have a “more than sufficient” soil P availability and fall into the P drawdown category, i.e. a negative P balance on those soils is recommended according to the UK Nutrient Management Guide (AHDB



2022). This substantiates the relevance of the selected set for assessment of the long-term availability of P in a P mining scenario. Due to the high plant:soil ratio, optimal climatic conditions and unlimited availability of other nutrients, plant P demand was maintained at a high level throughout the experiment and P deficiency could be induced in all soils within the timeframe of the study. The CCP in these settings varied substantially among soils, with a factor of >20 difference between the extremes despite the rather limited variation in P availability index as determined by the widely used Olsen extraction. To put the numbers into perspective, the ranges observed in our study would be equivalent to an uptake of 60-1500 kg P ha<sup>-1</sup> when extrapolating to the field, considering the top 30 cm of soil and assuming a typical bulk density of 1300 kg m<sup>-3</sup>. At a P uptake rate around 26 kg P ha<sup>-1</sup> year<sup>-1</sup>, roughly based on numbers from the Dutch (CBS, 2019; (Ehlert et al. 2009)) and Belgian (Lenders et al. 2012) government as well as the UK Nutrient Management Guide (AHDB 2022), this could sustain current crop production levels for 2 to 60 years, with a median of 8 years, at zero P input. In reality, it will likely take even longer before crop yield losses occur under field conditions, since the plant growth rate, and consequently the plant P uptake rate expressed as mg P kg<sup>-1</sup> per unit of time, is smaller in the field. This plant growth rate critically affects the point (i.e. the quantity of P removed from the soil) at which P deficiency is induced, as was experimentally confirmed by different N fertilisation regimes in a P mining experiment to temper the growth rate, and further substantiated by modelling (Nawara et al. 2018; Smolders et al. 2020). In addition, the readily available pool will be continuously replenished by slowly desorbable P under P depleting conditions. In the timeframe of the experiment, that latter process might have been too slow to sustain adequate P uptake. However, typical values of crop P export (kg P ha<sup>-1</sup>; AHDB (2022); Manure Action Plan 5 (2015)) and duration of growing seasons (days) suggest that, in the field, uptake rates of 0.1 mg P kg<sup>-1</sup> day<sup>-1</sup> should be ample for crop production. Therefore, the true CCP in the field might be closer to the TCP rather than the CCP determined in this accelerated mining experiment. This would imply that the available legacy P built-up in well-fertilised agricultural soils could be approximated by the TCP. A quick calculation using the same assumptions as

before (bulk density of  $1.3 \text{ kg L}^{-1}$ ; annual P uptake of  $26 \text{ kg P ha}^{-1}$ ), this would be, for the soils here, equivalent to a P source for plants for 5-70 years, with a median of 15 years. With a median TCP of  $97 \text{ mg P kg}^{-1}$  or  $378 \text{ kg P ha}^{-1}$ , this study suggests that half of the estimated  $765 \text{ kg P ha}^{-1}$  net accumulation of legacy P that has built-up in Western-European soils through over-fertilisation (Sattari et al. 2012) could be re-used. These results are in line with field observations from several long-term trials comparing different P fertilisation regimes (Ellmer et al., 2000; Gransee & Merbach 2000; Rubæk & Sibbesen 2000). At zero P input, significant yield losses in trials on Danish (Rubæk and Sibbesen 2000) and German (Gransee and Merbach 2000b) agricultural soils were only observed after 16 to 25 years, even at higher P uptake rates. Even more notable are the long-term field trials of Gembloux (BE; Nawara, 2018) and the Static Nutrient Deficiency Experiment in Thyrow (GE; Ellmer et al., 2000): crop yields for barley had only decreased to ~90% relative yield after 45 and 60 years of suspended P fertilisation respectively. Similarly, annual P uptake by crops in the Exhaustion Land field trial at Rothamsted remained at a nearly constant level for over a century after P fertilisation ceased (Johnston and Poulton 2019).

Nevertheless, a significant fraction of oxalate-extractable P (81% on average) could not be utilised in the mining experiment as it could not become available at rates relevant for crop growth; this fraction was, therefore, considered to be less bioavailable. It likely comprises P that has migrated into micropores of amorphous Fe and Al (oxy)hydroxides and/or has precipitated with dissolved  $\text{Fe}^{3+}$ ,  $\text{Al}^{3+}$  or  $\text{Ca}^{2+}$  (Warrinnier et al., 2018). Slow diffusion out of the micropores might decrease the relevance of such aged P in supply to crops (Ryden and Syers 1977; Koopmans et al. 2004), especially under accelerated mining conditions. It can, however, not be ruled out that this P might become more relevant at slower depletion rates that occur in the field under e.g. only mildly negative P balances. Moreover, even when limited yield losses do occur, they should always be evaluated in an economic context (Otinga et al. 2013). They might be acceptable as

long as the reduced income by lower crop yields does not considerably exceed the costs that were saved by reducing fertilisation efforts and environmental risks.

The results from this biological P mining experiment showed similar trends to those obtained by chemical mining of the same soils in a P desorption experiment (Vermeiren et al. 2021). Both the total desorbable P and the P extrapolated to be desorbed after 448 days correlated well with the TCP, but both consistently overestimated the P available to plants. This is logical, as P desorption in the chemical mining is measured in a well-shaken system where diffusion of the desorbed  $\text{PO}_4$  was not limiting. In the biological mining, diffusion can control P uptake by plants, resulting in a higher solution concentration in the soil outside the rhizosphere than in the chemical mining and, hence, slower desorption (Smolders et al. 2020). Similarly, this decrease in diffusive fluxes with time most likely explains why the CCP was well correlated with the fast desorbable “labile”  $Q_1$  but was slightly overestimated by the latter. However, care should be taken when comparing these two fractions. The  $Q_1$  pool determined by chemical mining is a “snapshot”, a momentary pool size characterised by an average desorption rate constant (Nawara et al. 2018), while the plant-available P is a dynamic factor. It partially depends on the plant P demand rate, which affects the time allowed for other forms of P in the soil to buffer the readily available P pool. This explains the observation that in some soils, with the best example being Barnfield 86 (Soil 25), the CCP exceeded the labile P pool: for some time, desorption from the slow P pool likely replenished the labile pool at a sufficient rate to sustain an adequate P uptake at crop demand rates imposed in the mining experiment.

For the same reason, the isotopically exchangeable P pool matched with, but slightly overestimated the CCP. This confirms suggestions made by earlier studies that this E value, either determined at 1 minute, several days or even weeks, can act as a reliable index of soil P bioavailability (Frossard et al. 1994; Warrinnier et al. 2018; Braun et al. 2019; Lemming et al. 2019). However, the duration of the isotopic exchange likely dictates the nature of the relationship, i.e. if there is an actual correspondence in size (as

was observed here in the 24h test), or merely a good correlation, to be expected when using e.g.  $E_{1min}$ . The same applies to the widely used Olsen extraction method: despite it being significantly correlated with the CCP, it consistently underestimated its size. While this test thus fails to tell us the remaining availability of P in a soil, it should be noted that this inability to capture the absolute value of plant-available P does not contradict the good performance of the Olsen extraction method in predicting crop response in many, but not all datasets (Johnston et al. 2013; Nawara et al. 2017; Nawara et al. 2018; Tandy et al. 2021; Steinfurth et al. 2022). Rather, the Olsen P provides an index of available P: as long as extractable P values remain above a certain critical P concentration, i.e. the lower limit of the target category (Index 2), it can be expected that P will not be the limiting factor for crop production (Nawara et al. 2017). It should be noted, however, that even for Olsen P values above this lower limit, some P dosing is recommended to maintain an adequate soil fertility; only when the soil is classified as “excessively” fertilised will the extreme scenario of zero P input be recommended (Jordan-Meille et al. 2012).

#### **4.2 Effect of SOM on long-term P availability**

Substantial differences in P uptake, even for soils with similar  $P_{Olsen}$  and  $P_{ox}$  contents, confirmed the importance of soil properties in determining the long-term availability of P in soils. The DPS and  $SOC/(Fe+Al)$  ratio most significantly explained the variability in both critical and total cumulative P uptake fractions among soils, but the relative importance of these properties depended on the soil’s initial DPS (i.e.  $<$  or  $>$  0.30). The effect of DPS on bioavailable P is rather obvious: a higher DPS means a larger fraction of P sorption sites that are occupied and larger  $PO_4$  concentration in the soil solution, contributing to higher diffusive fluxes (Braun et al. 2019; Smolders et al. 2020). In contrast, the observation of an increased long-term P availability, here assessed by plant P uptake, with increasing  $SOC/(Fe+Al)$  ratio is rather new, but confirms the speculated mitigation effect of SOC content on the ageing of P in soils. First indications of such a positive effect were observed by Regelink et al. (2015), who showed that information on the SOM loading of metal oxides was required in addition to the DPS to describe the distribution of  $PO_4$  over the solid and

liquid phase. More recently, Vermeiren et al. (2021) found that increased SOC/(Fe+Al) ratios were related to larger fractions of  $P_{ox}$  that were (readily) desorbable, i.e. soil P remained more available over time when SOC/(Fe+Al) was high, although only for soils that were not saturated with P. This was explained by the hypothesis that SOC can prevent migration of P into micropores because the relatively large molecules can spread out over the sorption surface, thereby occluding the pores, and improving P availability in the long-term. This effect is not related to the absolute content of SOC, but rather to its concentration relative to that of Fe and Al (oxy)hydroxides. An alternative explanation could be that site competition and electrostatic effects keep  $PO_4$  from sorbing to oxides in the first place (Hiemstra et al. 2010), which reduces its concentration gradient towards the interior of the particles and, therefore, also the ageing of P. However, the latter does not explain the distinction between soils with low and high DPS and is, therefore, likely only partially responsible for the positive effect of SOC/(Fe+Al) on P uptake fractions.

Interestingly, the impact of DPS and SOC/(Fe+Al) ratio on long-term availability of P to plants shifted depending on the initial P saturation status of the soil. The CCP and TCP fractions of  $P_{ox}$  in highly P saturated soils (i.e.  $DPS > 0.30$ ) correlated best with the DPS, while those in soils with a low P saturation ( $DPS < 0.30$ ) were best explained by the SOC/(Fe+Al) ratio of the soil. Similar observations were made in the chemical mining experiment of Vermeiren et al. (2021) and comparable mechanisms may be at work: at low DPS, sufficient sorption sites are available for the SOM molecules to spread out over the surface and hinder diffusion into micropores. In contrast, at high levels of P saturation, effective competition between  $PO_4$  anions and SOM molecules can force the latter into a more extended conformation, hampering their efficiency in occluding micropores. Ample evidence exists showing that SOM tends to decrease its number of groups complexed to the sorption surface due to steric and electrostatic hindrance at elevated surface loadings (Kaiser and Guggenberger 2003; Kaiser and Guggenberger 2007; Moens 2020). Admittedly, these

studies focused on the competition among SOM molecules, but this effect can plausibly be translated to competition between SOM and  $\text{PO}_4$  ions.

In addition to the effect of SOM on P ageing, the contribution of organic P mineralisation to P availability in a P mining scenario was evaluated by comparing the results from the biological mining with the sterile chemical mining experiment. It was hypothesised that a significant net organic P mineralisation would manifest itself as an increased ratio of total plant-available (TCP) over total desorbable ( $Q_1+Q_2$ ) P at elevated concentrations of SOC. However, such an increase in  $\text{TCP}/(Q_1+Q_2)$  was not observed at all; in fact, the ratio was unaffected by the SOC content of the soil. Therefore, it could not be confirmed that organic P mineralisation had a significant role in P supply to plants in this accelerated P mining scenario. This is in line with recent estimates from two novel isotope dilution studies showing that net organic P mineralisation rates in soils are often small (Müller and Bünemann 2014; Wanek et al. 2019). In these studies, net mineralisation rates in four out of six temperate grassland or tropical forest soils (Wanek et al. 2019; deduced from their Figures 7 and 8) and in two grassland soils (Müller and Bünemann 2014), were estimated to be below  $0.1 \text{ mg P kg}^{-1} \text{ day}^{-1}$ . This is considerably lower than estimates made with earlier, less sensitive techniques, which were as high as  $0.5\text{-}0.9 \text{ mg P kg}^{-1} \text{ day}^{-1}$  (Bünemann et al., 2007). Such high mineralisation rates would be, in any case, highly unlikely since the average P uptake rate over all soils in the biological mining experiment was already below  $0.5 \text{ mg P kg}^{-1} \text{ day}^{-1}$  after only a couple of harvests. It should be noted, however, that the highly organic Arthur Rickwood soil was not included in the comparison between biological and chemical mining as it was not included in the latter experiment. In that soil, both the CCP and TCP comprised remarkably high fractions of  $\text{P}_{\text{ox}}$ , despite the low DPS, suggesting that in this extreme case, organic P mineralisation might have played a significant role in P supply to plants.

The positive effect of the  $\text{SOC}/(\text{Fe}+\text{Al})$  ratio on the long-term availability of P to plants is an argument for improving OC management in agricultural soils. Increasing carbon sequestration and, thereby, elevating

SOC contents in such soils can be achieved by adapting soil management strategies (Lal 2008). Adaptations can aim at reducing OC losses from the soil, e.g. by reducing soil tillage (McConkey et al. 2003), or by increasing OC input, typically done by promoting incorporation of crop residues into the soil. Along the same lines, the addition of OC accompanying the application of organic fertilisers such as farmyard manure results in a slow but steady build-up of OC in soil (Annaheim et al. 2015; De Clercq 2017; Johnston and Poulton 2018). The results in our study thus suggest that organic fertilisers with P could enhance the efficiency of P use in agriculture since, at equal P input, they keep the added P more available in the long-term, but only at the lower range of P saturation. It furthermore suggests that the sustainable use of mineral P in soils with deficient levels of P, e.g. highly weathered soils, should make sure there is sufficient SOC to limit irreversible loss of P and low  $\text{PO}_4$  use efficiency, whether this is obtained by improved soil management for carbon sequestration or through the use of organic fertilisers with P. Positive effects of farmyard manure on long-term availability of P have already been observed in multi-year field studies on highly P deficient soils (Otinga et al. 2013; Andriamananjara et al. 2018; Andriamananjara et al. 2019), but also in long-term P depletion trials by Rothamsted Research: crops grown on soils amended with farmyard manure showed an elevated P uptake compared to mineral P fertilised soils at nearly equal P input in the > 100 years following cessation of P fertilisation (Johnston and Poulton 2019). However, such positive impact has not been replicated for more degraded organic materials such as compost; the chemical mining experiment from Vermeiren et al. (2021) even suggested a negative impact on the long-term availability of P, potentially caused by complexation of  $\text{PO}_4$  with stable OC compounds through metal cation bridging and/or the formation of poorly soluble calcium phosphates (Leytem et al., 2005; Vanden Nest et al., 2016). Therefore, care should be taken with the choice of fertiliser.

## 5. Conclusion

This study showed a total uptake of legacy P from well-fertilised agricultural soils ranging between 30-450 mg P kg<sup>-1</sup>, suggesting that the soil P reserves could act as a sufficient P source for plants for several years to decades at zero P input. The fraction of this legacy P that can be taken up by crops is larger if the SOC content relative to the amorphous Fe and Al oxide concentrations is larger, as long as the soil is not highly saturated with P. These results imply that SOC can effectively reduce ageing of P in soils. As a mechanism for this observation, it was postulated that SOM molecules cover micropores in these oxides, and thereby reduce the ageing of P by diffusion into such pores. This suggests that the use of organic fertilisers with P could be considerably more sustainable than mineral P at equal P input. However, further work is still required to confirm this mechanism and the processes responsible for these results. Moreover, the observation that not one main soil property alone, but rather a combination of the SOC/(Fe+Al) ratio and the DPS, could explain the long-term availability of P across all soils once again emphasises the need for tailoring soil P management to specific soil types.

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## **Conflict of Interest**

None of the authors declare a conflict of interest.

## **Data Availability Statement**

Most data generated for this study are presented in the manuscript or the Supporting Information. Data that are not shown can be made available upon request to the corresponding author.



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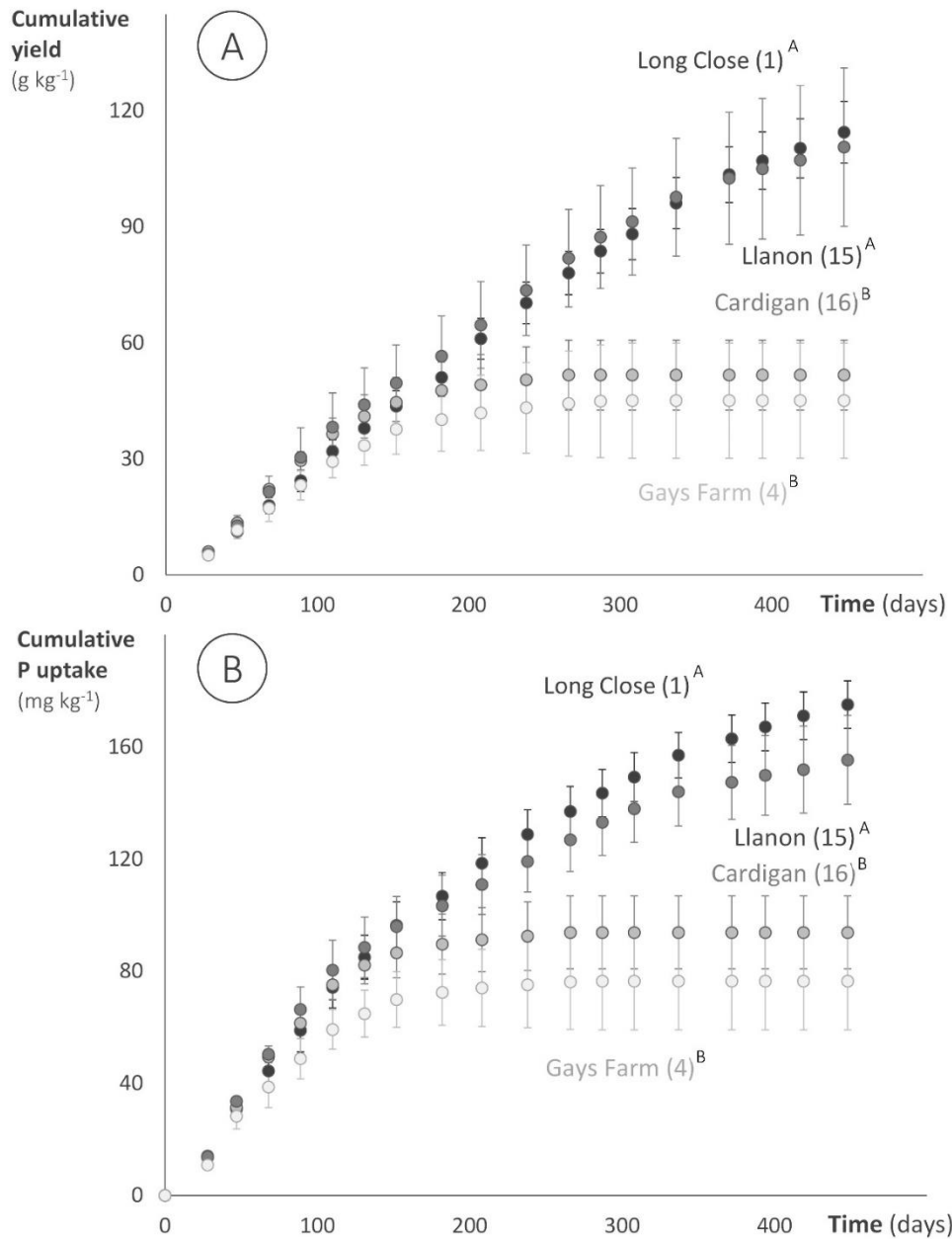
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**Table 1: Soil properties.** Selected properties of 25 UK soils used in the biological P depletion experiment; all soils without \* were in parallel subjected chemical mining of P (Vermeiren et al. 2021). A = arable, G = grassland, C = clay, L = loam, Si = silt, Sa = sand. All concentrations are expressed in mg per kg dry soil.

		General soil properties					Oxalate extraction					Dithionite extraction		
		Land use	Texture	pH	SOC (%)	Total P (mg kg <sup>-1</sup> )	P <sub>ox</sub> (mg kg <sup>-1</sup> )	Fe <sub>ox</sub> (mg kg <sup>-1</sup> )	Al <sub>ox</sub> (mg kg <sup>-1</sup> )	DPS (mol mol <sup>-1</sup> )	SOC/(Fe+Al) (mol mol <sup>-1</sup> )	P <sub>di</sub> (mg kg <sup>-1</sup> )	Fe <sub>di</sub> (mg kg <sup>-1</sup> )	Al <sub>di</sub> (mg kg <sup>-1</sup> )
1	Long Close	G	CL	4.7	4.0	1420	880	9130	1900	0.24	14	950	21180	2730
2	Duchy	A	SiL	6.0	3.3	1730	1180	8710	5840	0.20	7	1110	25520	5270
3	School Field	G	CL	5.5	3.9	1270	770	6760	1470	0.28	19	760	14480	1650
4	Gays Farm	G	L	5.2	3.5	1280	810	6200	1710	0.30	17	930	18970	1900
5	Horsey Park	G	CL	5.3	5.0	1330	790	9890	1830	0.21	17	750	15820	2000
6	Shuttleworth*	A	SaCL	7.2	1.1	530	210	1880	530	0.26	18	320	19810	1120
7	Plumpton	G	SiCL	7.4	2.3	1470	680	1120	640	1.00	43	260	3150	340
8	Boxworth	G	C	7.4	1.7	820	350	1570	1010	0.34	22	270	23700	1630
9	Vale Farm	G	SaL	5.9	1.3	630	450	3850	530	0.33	12	370	7570	500
10	Morton Morrel	A	C	5.8	3.8	840	450	4640	1250	0.22	24	250	8270	830
11	Kingston Maurward	A	SiL	5.8	1.6	930	590	3490	1140	0.36	12	580	9060	1170
12	Conygre	A	SaL	5.5	1.9	520	310	1340	310	0.56	44	270	2290	300
13	IBERS*	A	SiCL	5.8	3.4	1360	890	7420	2060	0.27	14	1010	14120	2450
14	TSB	G	L	5.4	3.3	1420	890	8490	4070	0.19	9	870	20450	4190
15	Llanon	A	L	5.6	3.1	1310	820	6360	2010	0.28	14	820	12620	2150
16	Cardigan	A	L	5.5	2.5	1160	780	7350	2610	0.22	9	790	15630	2720
17	Harper Adams	A	SaCL	5.6	2.0	620	420	3540	770	0.29	18	300	6440	600
18	Broadmead	A	C	7.3	2.6	930	410	4480	1110	0.22	18	360	21070	1380
19	Weston	A	CL	7.3	1.5	520	210	2200	710	0.20	19	200	14020	1130
20	Inschfield	A	SaL	6.0	4.7	2950	1790	13110	9420	0.20	7	1340	28850	6920
21	Great Field*	A	SiL	6.0	1.6	840	320	3500	810	0.22	15	590	17220	1670
22	Arthur Rickwood*	A	SiC	5.4	23.8	1610	1130	13430	4210	0.18	50	980	18360	2990
23	Mill Dam Close*	A	CL	5.9	4.4	3300	2570	20130	800	0.43	9	2770	45680	1350
24	Barnfield 85	G	SiCL	5.4	2.6	930	500	4190	1070	0.28	19	600	19040	2220
25	Barnfield 86	G	SiCL	6.0	2.8	1390	660	2360	1030	0.53	29	680	18190	2080
<b>Min</b>		-		4.7	1.2	520	210	1120	310	0.18	8	200	2290	300
<b>Median</b>		-		5.8	2.8	1270	680	4640	1140	0.27	17	680	17220	1670
<b>Max</b>		-		7.4	23.8	3300	2570	20130	9420	1.00	50	2770	45680	6920

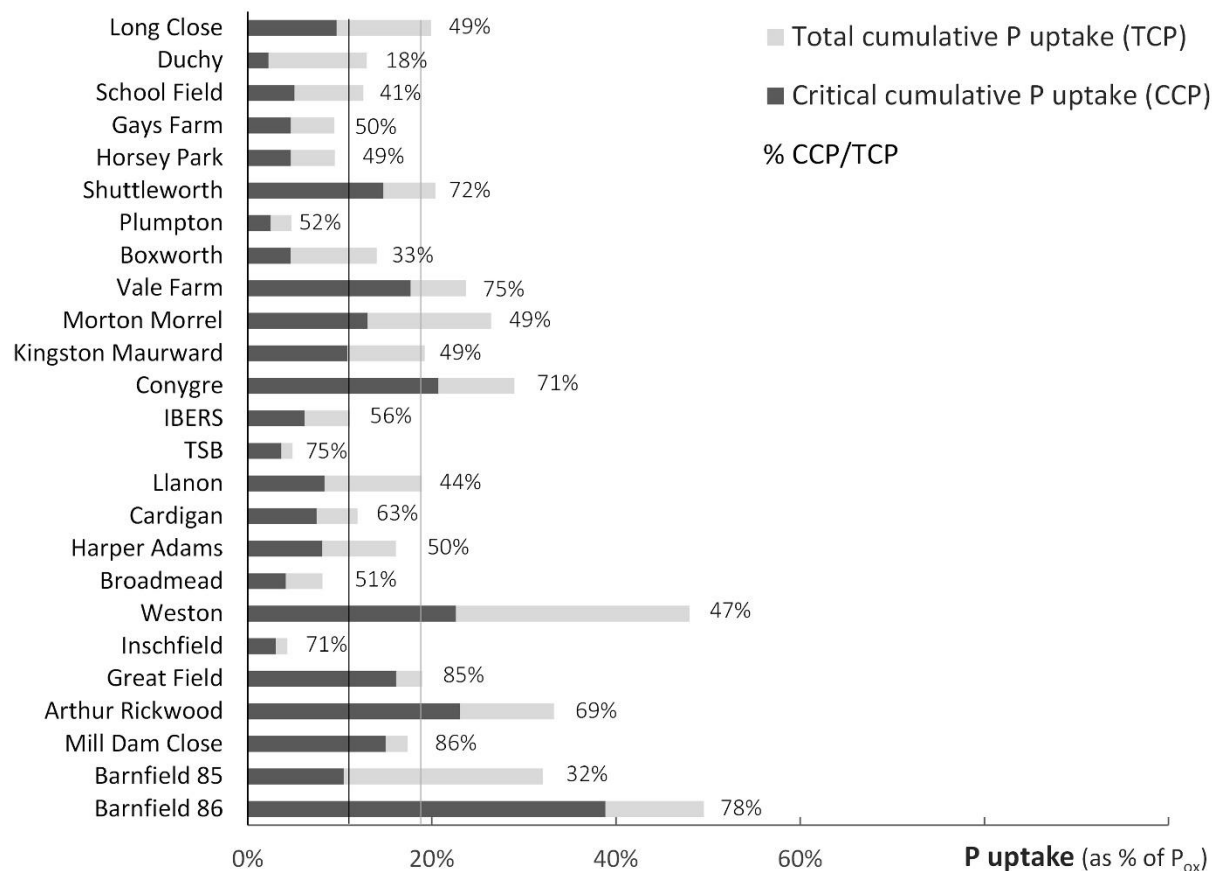
**Table 2: P uptake and availability indices.** Summary of the results from the biological P mining experiment and how they relate to different indices of P availability. Total cumulative P uptake (TCP) and critical cumulative P uptake (CCP) are expressed per unit dry soil and were calculated as explained in Section 2.3. The labile ( $Q_1$ ) and total desorbable ( $Q_1+Q_2$ ) P pool as well as the desorbed P after 448 days ( $Q_{des}(448d)$ ) were determined in a P desorption experiment and only include 20 out of the 25 soils used for biological mining (see Table 1). For the sake of comparison, min-med-max values including only those 20 soils are added between brackets. The results per soil can be found in the Supplementary Information (Table S2).

	Ryegrass data			P availability indices									
	Total yield (g kg <sup>-1</sup> )	TCP (mg P kg <sup>-1</sup> )	CCP (mg P kg <sup>-1</sup> )	P <sub>Olsen</sub> (mg P kg <sup>-1</sup> )	CCP/P <sub>Olsen</sub>	E <sub>24h</sub> (mg P kg <sup>-1</sup> )	CCP/P <sub>E24h</sub>	Labile Q <sub>1</sub> (mg P kg <sup>-1</sup> )	CCP/Q <sub>1</sub>	Total desorbable Q <sub>1</sub> +Q <sub>2</sub> (mg P kg <sup>-1</sup> )	TCP/(Q <sub>1</sub> +Q <sub>2</sub> )	Desorption at 448 d (mg P kg <sup>-1</sup> )	TCP/Q <sub>des</sub> (448d)
<b>Min</b>	20 (20)	32 (32)	16 (16)	21	0.41	15	0.22	29	0.31	138	0.16	104	0.20
<b>Med</b>	<b>57</b> (57)	<b>97</b> (95)	<b>52</b> (49)	<b>35</b>	<b>1.51</b>	<b>67</b>	<b>0.81</b>	<b>62</b>	<b>0.72</b>	<b>244</b>	<b>0.41</b>	<b>221</b>	<b>0.43</b>
<b>Max</b>	195 (141)	446 (328)	385 (257)	128	6.97	387	2.13	142	1.81	434	0.94	346	0.95



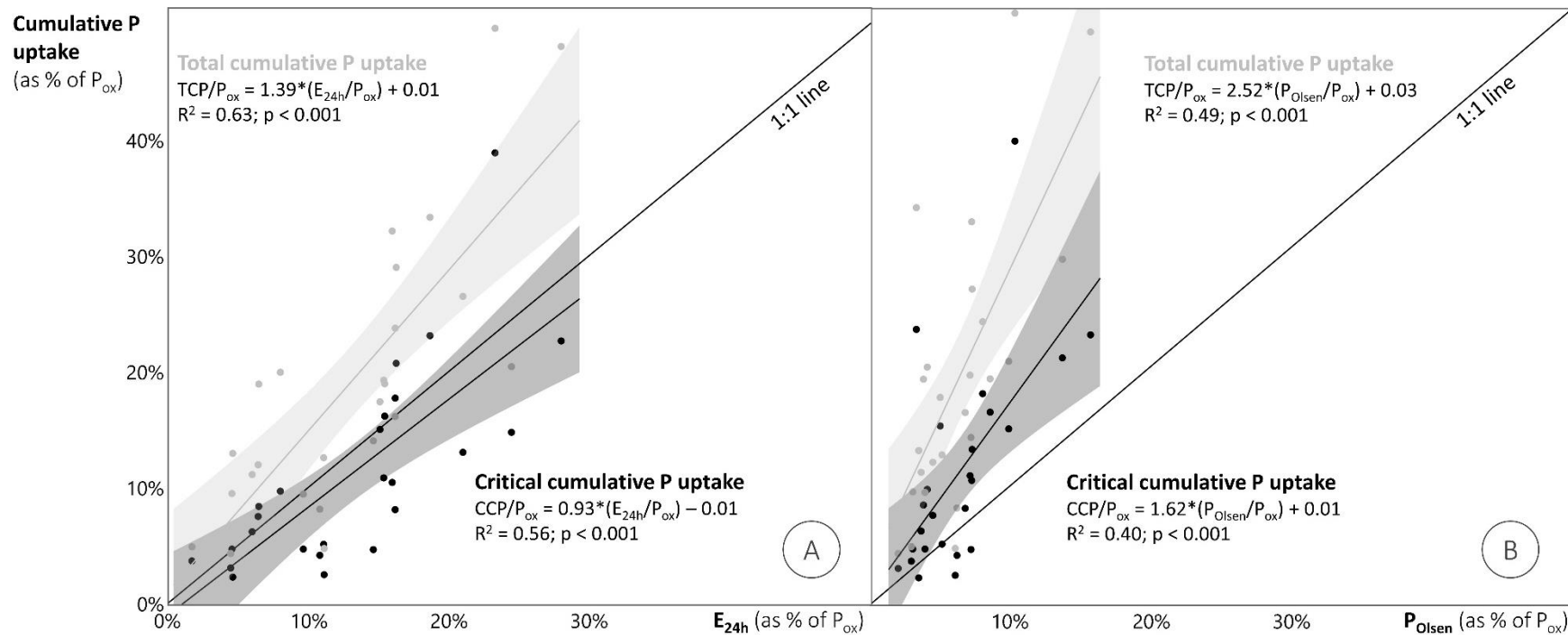
**Figure 1: Cumulative ryegrass yield and P uptake.**

Cumulative dry matter yield (A) and P uptake (B) during the biological P mining experiment for Soils 1, 4, 15 and 16. Despite having similar contents of  $P_{ox}$  and  $P_{Olsen}$ , the soils performed significantly differently in a P depletion scenario, hinting at the impact of other soil properties on the long-term availability of P. Different letters next to the soil names indicate statistically different final yields or differences (Tukey test;  $p < 0.01$  in all cases).



**Figure 2: Phosphorus uptake as a fraction of the oxalate-extractable P ( $P_{ox}$ ).**

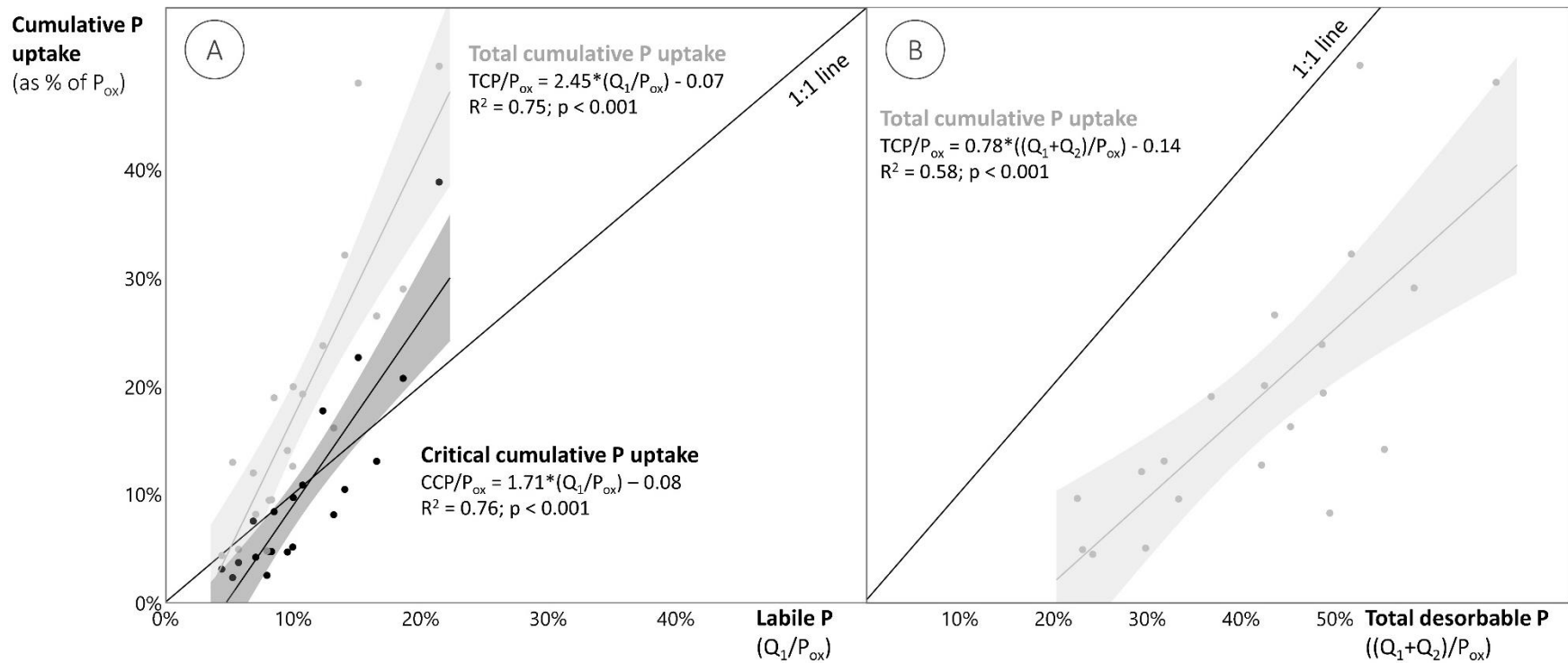
Total uptake varied between 4 and 50% of  $P_{ox}$ , with an average of 19% (grey vertical line). Of this total P uptake, 52% (median) occurred at rates fast enough to support crop growth without P deficiency (% next to the bars), corresponding to a critical cumulative P uptake (CCP) of 2-39% of the  $P_{ox}$  or 11% on average (black vertical line). This CCP is defined as the cumulative P uptake up to the point where the dry matter yield, corrected for the number of growing days, decreases below 90% of the yield at Harvest 2.



**Figure 3: Cumulative P uptake vs. typical P availability indices.**

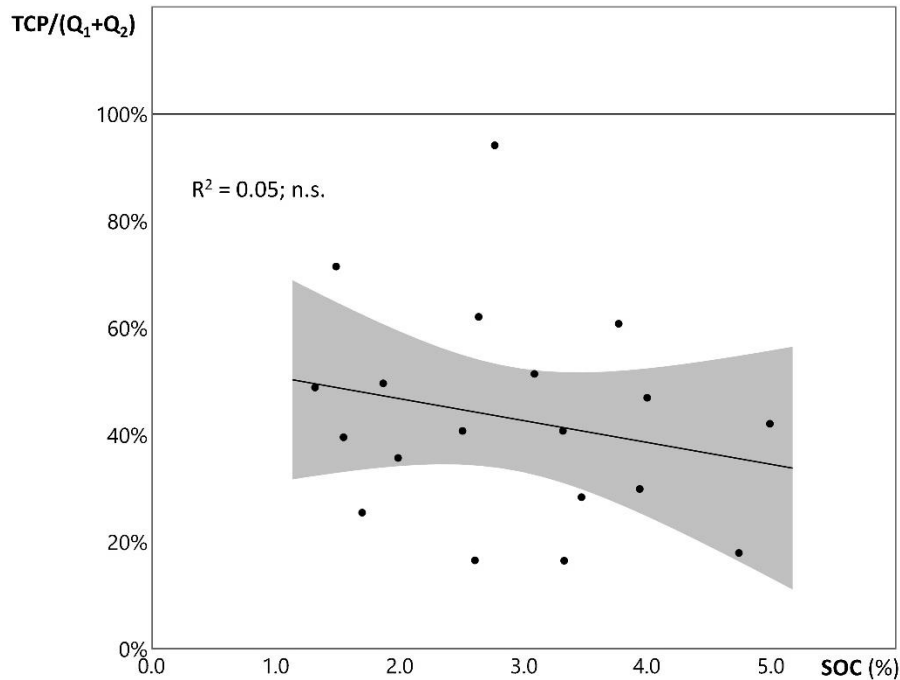
The isotopically exchangeable soil P concentration ( $E_{24h}$  value) strongly matched with the critical cumulative P uptake by ryegrass in the biological P mining experiment, but underestimated the total cumulative P uptake (A). Conversely, these P uptake values were generally underestimated by the widely used  $P_{Olsen}$  extraction (B). Note that both uptake and extraction data are presented as a fraction of the  $P_{ox}$  to normalise the data. Grey areas indicate the 95% confidence region for the fitted line.





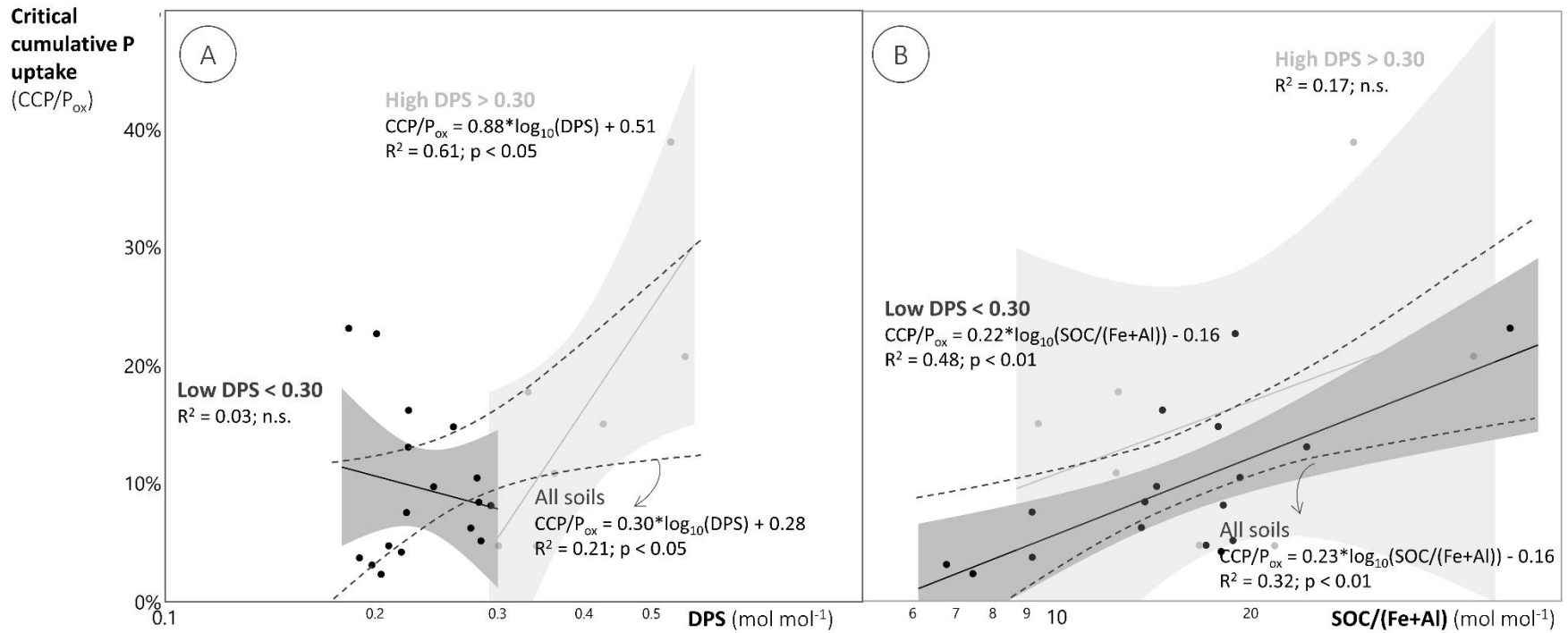
**Figure 4: Available P fractions in biological vs. chemical mining.**

Comparison between available P pools (as % of  $P_{ox}$ ) as determined by plant uptake in the biological mining experiment (y-axes) and by desorption in the chemical mining experiment from Vermeiren et al. (unpublished) (x-axes). These results only include 20 out of 25 soils (Soil 6, 13 and 21-23 were not subjected to chemical mining). Grey areas indicate the 95% confidence interval for the fitted lines.



**Figure 5: Effect of SOC on biological vs. chemical mining.**

The effect of SOC content on the ratio of total available P as determined in the biological mining (TCP) and sterile chemical mining ( $Q_1+Q_2$ ) experiment. The effect of organic P mineralisation on P uptake would suggest a positive trend of that ratio with SOC content, however, that increase was not observed. These results only include 20 out of 25 soils (Soil 6, 13 and 21-23 were not used in the chemical P desorption experiment).



**Figure 6: Effect of DPS and SOC/(Fe+Al) ratio on bioavailable P fractions.**

The CCP fraction of P<sub>ox</sub> was mainly explained by the ratios of P (A) and OC (B) to sorption surfaces in the soil. Depending on the soil's initial state of P saturation, i.e. the DPS at the start of the P depletion trial, the importance of DPS and the SOC/(Fe+Al) ratio in explaining the variability among soils shifts. Similar results are obtained when plotting the total P uptake as a fraction of P<sub>ox</sub> (not shown). Grey areas indicate the 95% confidence region for the fitted lines of the DPS clusters, dashed lines for the whole set of soils. Note that both DPS and the SOC/(Fe+Al) ratio are presented on a log<sub>10</sub> scale.