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# Dynamics of soil phosphorus measured by ammonium lactate extraction as a function of the soil phosphorus balance and soil properties

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## ABSTRACT

A sustainable supply of phosphorus (P) to agricultural soils should maintain soil fertility without jeopardizing the environment. This study analyzed the change in the soil P as a function of the net soil P mass balance, i.e. the input minus removal by the harvested crop. The P available for crops was estimated by the P extracted by ammonium lactate at pH 3.75 (P-AL). First, laboratory and pot-trial data showed that the net change in soil P-AL was only 68% of the change in total soil P, either when P had been added to soil or when P had been mined by plants for 1–2 years, indicating removal to or release from the non-extractable pool depending on the balance. Secondly, data were collated from 33 field trials in Europe with a wide range of cumulative soil P balances ($-1200$ to $+2500$ kg P $ha^{-1}$) in time spans ranging from 3 to 51 years. The average change in P-AL across the negative and positive balance was only 27% (95% confidence interval 25–30%) of the net balance. The change in P-AL was larger as the net balance increased and as the initial P-AL decreased. The slope of the change versus the balance was smaller as time increased and initial P-AL decreased. The other soil characteristics did not affect these changes, and the model developed was not different for negative or positive P balances. Our results suggest that a steady state P-AL concentration at optimal P supply for crops is obtained at a net P balance of about 1–10 kg P $ha^{-1}$. The P mass balance of a 51 year-old trial showed that the *aqua regia* soluble P of the 0–90 cm layer accounted for only 64% of the net P balances at the surface. This still incomplete P balance could be related to upward P transport by plant uptake from $>$ 90 cm soil layers, lateral P movement in the field, and changes in the soil P fraction that could not be extracted with *aqua regia*. This study quantified the long-term dynamics of P-AL, which is useful for agronomic and environmental purposes and policy, and pointed out the importance of non-extractable P and vertical P movement.

## 1. Introduction

Most phosphorus (P) fertilizer recommendation systems define a target range of values for the concentration of extractable P (Jordan-Meille et al., 2012). If the soil P concentration is lower than the target values, optimal yields cannot be obtained due to too low P availability for the crop. Phosphorus fertilizer prices can also be taken into account for recommendations in order to maximize the economic return (Valkama et al., 2011). Raising soil P availability above target values is not favorable (i) from an agronomical production point of view, due to the lack of further yield increases with increasing soil P, and (ii) from an environmental point of view given the larger P losses by leaching and runoff with increasing soil P (Pote et al., 1996; Ulén, 1999). Phosphorus fertilizer advice is needed to reach and maintain the target values for soil P concentrations. If the soil P concentration is smaller than the target value, it is advised to increase the P input above the amount of P

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exported by the harvested crop (Jordan-Meille et al., 2012). In some European regions, P input by fertilizer, manure or other organic materials is restricted, especially at high soil P (Amery and Schoumans, 2014). For soils in the P target range, the maximum allowed P application often equals the expected crop P offtake, i.e. targeting a net zero soil P balance. However, there are indications that soil P fertility can decrease at such balanced P application because of lateral P loss by runoff, vertical P loss by leaching and, especially, by movement of P to less accessible fractions by e.g. diffusive penetration into adsorbing particles (Torrent et al., 1992). On the other hand it is also possible that at zero P application the decrease in soil P is smaller than expected based on the mass balance (Johnston et al., 2014).

The analyses of so-called "available" P concentrations, further defined as soil P tests, are based on operationally defined methods. More than ten different methods are used in Europe, compromising the combination of results of field experiments from different countries. The soil P tests measure only a fraction of the total soil P. For example, P present in Ca-phosphates, sorbed by aluminum and iron in clays or (hydr)oxides or accumulated in organic P is incompletely recovered in most soil P tests (Sharpley and Rekolainen, 1997). It is well established that added P can progressively move from extractable to non-extractable pools and *vice versa* (Karpinets et al., 2004; Shuai et al., 2014).

There are few generic data and few models to calculate the P supply or soil P balance (inputs minus outputs) needed to reach the target values, or to maintain the target P at field scale. Several mechanistic models exist to predict the changes in soil P (Johnston et al., 2014; Karpinets et al., 2004; Smolders et al., 2020), but these models require measurements or estimations of several input variables. Since these data are often not available or have to be estimated by transfer functions which are not always accurate, models with limited input data requirement are preferred for predictions of general soil P changes (Uusitalo et al., 2016). More empirical models have been developed based on data of changes in the soil P test and associated soil P balance, but mostly using only one or a small number of field trials, or a relatively small range in soil P balances (Cao et al., 2012; Ekholm et al., 2005; Messiga et al., 2010; Tang et al., 2008; Uusitalo et al., 2016; van der Bom et al., 2017, 2019a). Several model approaches have been followed, such as simple linear models, multiple linear models and artificial neural networks (Alvarez and Steinbach, 2017). Especially for negative P balances, curvilinear relationships are more appropriate to describe changes in soil P (Dodd et al., 2012; Johnston et al., 2016; Schulte et al., 2010). Sometimes different approaches are chosen depending on the direction of the change in soil P, with linear increase and curvilinear decrease in soil P (Ma et al., 2009; Sucunza et al., 2018; Tang et al., 2008).

In Flanders, The Netherlands, Norway, Sweden, Hungary, Lithuania and Slovenia, the soil P test is based on soil extraction with ammonium lactate and acetic acid at pH 3.75 (P-AL) (Egnér et al., 1960). This method extracts more P than the well-known Olsen extraction with 0.5 M $NaHCO_3$ (Olsen et al., 1954) and performed similarly to other soil P tests in predicting crop response in long-term field experiments (Nawara et al., 2017) and in a soil P mining test (Nawara et al., 2018). The changes in soil P-AL have been estimated in relation to the soil P mass balances for several field trials but no attempt has yet been made to collate these data and to identify the factors that explain the dynamics of P-AL.

This study was set up to i) quantify the dynamics of P-AL in relation to the soil P mass balance at field scale across contrasting soils and time intervals and ii) to identify factors that statistically explain the trends. First, laboratory soil incubation data and greenhouse data were used in which the mass balance of P was accurately known along with net changes of P-AL. Secondly, the relationship between the soil P balance (P inputs minus P exports) and the related change in P-AL, and the effect of soil characteristics on this relationship, were investigated by using data of 33 European field trials. Examining such a large dataset allows the identification of general trends. Moreover, soil samples from two of these 33 field trials were available for making additional P measurements, necessary to explore the importance of non-P-AL fractions in the soil P dynamics.

## 2. Material and methods

### 2.1. Overview of experiments and P-AL extraction

Laboratory, greenhouse and field experiments were examined in order to relate changes in P-AL with soil P balances. The tests in the laboratory and greenhouse (Section 2.2) allowed the investigation of this relationship in closed, more controlled conditions. The setup of the greenhouse experiment with eight soils and results on various soil P tests were described in detail by Nawara et al. (2018). Data on the change of P-AL during this experiment, in two treatments with different soil P balances, are given and discussed here. In addition, the current study describes new laboratory experiments which were performed with the same eight soils (same sampling event) and one additional soil in order to enlarge the range in applied soil P balances (third treatment in soil P balance). For the field trials (Section 2.3), both already published and new results on changes in P-AL in 33 field trials were used.

In both the laboratory, greenhouse and field experiments the P-AL was measured in the initial and final soil samples by the method of Egnér et al. (1960). The extraction solution was 0.1 M ammonium lactate and 0.4 M acetic acid at pH 3.75. Extraction (shaking) was performed for 4 h in a soil:solution ratio of 1:20. Analytical details differ between the field trials (see references in Table S1). The change in P-AL (ΔP-AL) is

$$\Delta\text{P-AL} = \text{P-AL}_{\text{fin}} - \text{P-AL}_{\text{ini}} \quad (1)$$

with $\text{P-AL}_{\text{ini}}$ the initial and $\text{P-AL}_{\text{fin}}$ the final P-AL value, all values in mg $kg^{-1}$ units.

For some soil samples (see Section 2.4), a more complete P measurement was performed by extraction in *aqua regia* (P-AR, ISO 11466). For this, 9 ml HCl (37%) and 3 ml $HNO_3$ (65%) was added to 0.5 g of oven-dry soil (45 °C, sieved over 2 mm). The soil sample was digested for 30 min at 200 °C (at 1500 W) in a high performance microwave digestion system oven (Milestone ETHOS One). After filtration over a Macherey Nagel 640d filter, the P concentration of the filtrate was measured by ICP-OES (Agilent 5110 VDV, USA).

Changes in P-AL were related to the soil P balance. The cumulative soil P balance was calculated as the sum of all P inputs (fertilizers, manure, and others) minus all P exported by crop removal. For the field trials the P balance used here is partial, as it does not account for P export by leaching, runoff and erosion. It also neglects the P input by atmospheric deposition. However, the European median of this input is only 0.22 kg P $ha^{-1}$ $yr^{-1}$ (Tipping et al., 2014).

### 2.2. Changes in P-AL in laboratory incubation and greenhouse experiments

Eight contrasting soils of Flanders (Belgium) were subjected to three P removal or addition treatments in two replicates. The first treatment (negative soil P balance) consisted of soil P removal by perennial ryegrass that was grown for 535 to 667 days. The grass was periodically clipped and all nutrients except P were supplied to allow optimal growth (see Nawara et al. (2018) for more details). The second treatment (slightly positive soil P balance) was similar to the first but with regular additions of P ($KH_2PO_4$) in amounts slightly over-compensating the P removal by the grass. P-AL was measured at the beginning and the end (after 535 to 667 days) for both P treatments of the greenhouse experiment. More details from the greenhouse experiment with the two P treatments can be found in the study of Nawara et al. (2018). For the current study an additional third treatment (positive soil P balance) was performed in the laboratory without grass. Fertilizer P was added to the same eight soils at a rate of 147 mg P $kg^{-1}$ (by $KH_2PO_4$ solution).

Controls without P addition were included. The P-AL was measured on day 0, 29 and 119 after P application. To calculate ΔP-AL, the final P-AL value of the samples with P addition and the initial P-AL value of the control samples were used (Fig. 1). For this third treatment with positive soil P balance, additional soil samples were included from field experiment #20 (Gembloux, equilibrium K fertilizer addition, see below and Table S1). This was done for soils sampled in 2018 from the three P treatments with strongly different P-AL values. To these soil samples, 100 mg P $kg^{-1}$ was added as a $KH_2PO_4$ solution. The soils were incubated for 119 days at 21 °C, measurements and calculations were the same as the ones used for the eight soils described above.

### 2.3. Data collection of long term changes in P-AL in field trials

Data from European field trials were collected for assessment of changes in P-AL over time (Table S1). Field trials with the required information (see below) were selected by three methods: (i) literature search (key words: “P-AL”, “ammonium lactate” and “phosphorus”), (ii) measurements of P-AL on soils collected from field trials used in Nawara et al. (2017) and (iii) field trials executed by the Soil Service of Belgium, Flanders Research Institute for Agriculture, Fisheries and Food (ILVO) and Ghent University (Belgium). Each data entry consisted minimally of 1) P-AL concentrations in topsoil measured between a time interval t (year), i.e. the initial ($P\text{-}AL_{ini}$, mg $kg^{-1}$) and final ($P\text{-}AL_{fin}$, mg $kg^{-1}$) concentrations, 2) the cumulative soil P balance during that time interval (kg P $ha^{-1}$), 3) sampling depth (5 – 30 cm) and 4) soil texture (sand, sandy loam, loam, clay or peat). In some cases measured crop P export values were not available for all years. In that case, export values were estimated based on (in consecutive order) values measured in other treatments of the same field trial, values measured in previous years, or average values from the overview of crop P exports of Ehlert et al. (2009). The effects of these assumptions on the results were addressed by a sensitivity analysis (see Section 2.5). If available, additional soil characteristics were also collected: pH, organic matter (OM) content, clay content, iron (Fe), aluminum (Al) content and bulk density. Soil pH was entered as pH measured in 1 M KCl (ISO 10390). If soil pH was measured in water or 0.01 M $CaCl_2$, pH-KCl values were calculated from the formulas in Gavriloaiei (2012). If organic carbon content instead of organic matter data was available, OM was calculated as 1.72 times the organic carbon content (Van Bemmelen, 1890). The Fe and Al content was measured by ammonium oxalate extraction (Schwertmann, 1964). Each field trial could result in several data entries if the field trial consisted of treatments with different soil P balances (categorized by different treatment numbers), as common in P fertilizer trials. However, if several measurements of P-AL in time were available, only the first and last (or most recent) measurement, giving the longest time interval, were used. These intervals ranged 3 and 51 years. In total, 33 field trials from 5 countries with in total 265 data entries were selected (Table S1). For some field trials, information on the individual replicates was available (different replicate numbers within one treatment number).

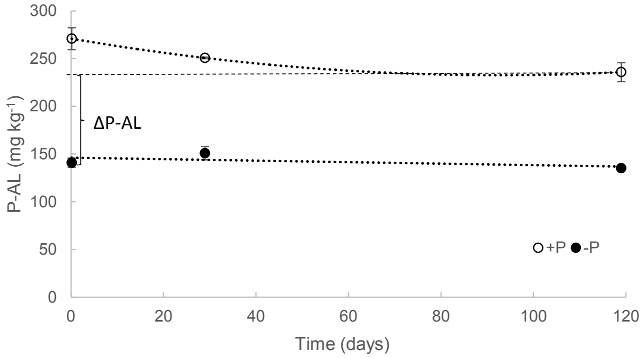

**Fig. 1.** Changes over time of P-AL of soil 8 after addition of 147 mg P $kg^{-1}$ (+P) or 0 mg P $kg^{-1}$ (−P) at day 0 of the laboratory experiment, with dotted trend lines. ΔP-AL is the difference between the final P-AL (day 119) of the +P treatment and the initial P-AL (day 0) of the −P treatment. Error bars represent the standard deviation (two replicates).

Soil P contents (P-AL and P-AR) were expressed as mg P $kg^{-1}$, but these can also be converted to the units kg P $ha^{-1}$ by taking the sampling depth (d, in m) and the soil bulk density (BD, in kg $m^{-3}$) into account:

$$P\left(kg\ P\ ha^{-1}\right) = P\left(mg\ P\ kg^{-1}\right) \times BD \times d \times 10000\frac{m^2}{ha} \tag{2}$$

If no measured soil bulk density was available, a soil density of 1450 kg $m^{-3}$ was assumed for loamy and sandy loamy soils and a soil density of 1250 kg $m^{-3}$ for sandy soils (BAM, 2019).

### 2.4. Additional P measurements on field trial soil samples

From the field trial #19 in Ath (Vanderdeelen et al., 1985) and #20 in Gembloux (Nawara et al., 2017), samples were available from the topsoil (0 – 25 cm) and deeper soil layers (Gembloux only, 25 – 50 cm and 50 – 90 cm) for additional P analyses (P-AL and P-AR). For the field trial of Gembloux a bulk density of 1350 kg $m^{-3}$ for the 0 – 25 cm layer and 1500 kg $m^{-3}$ for the 25 – 50 cm and 50 – 90 cm layer were assumed, based on data from adjacent fields (Goidts, 2009). Unfortunately only the final, and not the initial samples were available. However, it can be assumed that the initial soil P values in the field trial treatments receiving different P application rates were the same or showed only minor differences compared to the soil P differences created after 20 (Ath) or 51 years (Gembloux) of varying P fertilizer doses. Therefore, the final soil P contents of the treatments were compared with the reference treatment, i.e. the treatment with the soil P balance closest to zero (P1 treatment, Tables S2 and S3), by calculating the residual P-AL or P-AR as (van der Bom et al., 2019b):

$$residual\ P_{treatmentX} = P_{treatmentX} - P_{reference} \tag{3}$$

with all terms in units of kg P $ha^{-1}$. For Gembloux, soil samples were taken from the six replicates of the three P dose treatments receiving balanced K application: P0 (zero P application), P1 (approximately balanced P application) and P2 (surplus P application). For the field trial of Ath, P-AR was measured on soil samples that were a mixture of the six replicates for every P treatment (from 0 to 100 kg P $ha^{-1}$ $yr^{-1}$ application) sampled in 1985 (no replicates available).

### 2.5. Data analysis

Data analysis was carried out with the statistical program JMP Pro 14.1.0 (SAS, Cary, NC, USA). First, correlations among variables were checked by Multivariate analysis. Then, mixed multiple linear regression was performed on the field trial data (265 data entries) with ΔP-AL (Eq. (1), mg P $kg^{-1}$) as dependent variable. The independent variables were $P\text{-}AL_{ini}$, time difference (t, years) between the P-AL measurements, the cumulative soil P balance, soil texture (categorical variable: sand, sandy loam, loam, clay or peat) and the interaction terms among these variables. The categorical field trial number (from 1 to 33), the categorical treatment number nested within the field trial number and the categorical replicate number nested within the field trial and treatment number were entered as random effects. The homogeneity of variance was checked with diagnostic plots. Due to the large sample size (>200) distribution of the means across the samples is normal based on the central limit theorem. Non-significant terms ($p > 0.05$) were removed stepwise from the model until a basic model was generated with only parameters with statistically significant effect. We tested whether the model was significantly different for positive versus negative soil P balances. This was done by introducing in the basic model an additional categorical variable, i.e. “positive balance” or “negative balance” depending on the sign of the soil P balance, both as single term and as interaction term with the other variables of the basic model. Soil characteristics for which not all data entries had measured values (pH ($n = 235$), clay ($n = 175$), OM ($n = 199$), iron ($n = 188$) and aluminium content ($n = 188$)) were added separately to the basic model (single and interaction terms with the variables from the basic model) to check if the additional effect of these soil characteristics on ΔP-AL was significant. A sensitivity analysis was performed to assess the effect of deviation of the assumed crop P export data values on the modelling. For this, mixed multiple linear modelling was done on 225 of the 265 data entries for which there were data on P input and (sometimes partly assumed) crop P export. For the remaining 40 cases, we had only data on the net cumulative P balance. The mixed multiple linear modelling was performed four times on the 225 data entries with 1) measured and assumed crop P export values, as described above; 2) assumed P export values decreased by 30% (measured values not changed); 3) assumed P export values increased by 30% (measured values not changed); and 4) cumulative P input instead of cumulative P balance as explanatory variable.

In a second analysis of the field trial data, the change in P-AL and the soil P balance were expressed per time unit (annual values) and linear regression was performed. We checked whether the fit was improved by adding the average P-AL (P-AL$_{av}$), i.e. the average of P-AL$_{ini}$ and P-AL$_{fin}$ of the field experiment. In this analysis P-AL$_{av}$ was preferred over P-AL$_{ini}$ because we attempted to explain annual changes in P-AL during the whole time interval.

Thirdly, mass balance calculations were done. Changes in P-AL and P-AR in kg ha$^{-1}$ were compared with cumulative soil P balances in the same unit by linear regression. The effect of deviation of the assumed bulk densities from the real values was addressed by performing a second analysis with 1) 20% lower and 2) 20% higher values than the assumed bulk densities (measured values not changed).

## 3. Results

### 3.1. *The dynamics of P-AL in laboratory incubation and in greenhouse experiments*

The laboratory and greenhouse studies allow the identification of changes in P-AL with adequate control on the mass balance (no lateral and downward transport), unlike the field trials. The changes in P-AL with varying P balance (8 soils with three balance treatments and the additional Gembloux samples) were strongly related to the soil P balance ($R^2 = 0.78$, Fig. 2). The slope of the linear fit was 0.68 (95% confidence interval: 0.60 – 0.76). The slope <1 indicates that P is lost from the AL extractable fraction at excess P balance and that P is released from soil to the extractable P pool under negative P balance. In addition, the negative intercept indicates that a slightly positive soil P balance is needed to keep P-AL constant. The P balance required to give zero change in P-AL was + 29 mg P kg$^{-1}$ (95% confidence interval: +19 to +39 mg P kg$^{-1}$). There was no significant difference in ΔP-AL among the Gembloux soil samples with a strong difference in initial P-AL but equal soil P balance.

### 3.2. *The dynamics of P-AL in the field*

The cumulative soil P balance varied from largely negative (−1220 kg P ha$^{-1}$) to highly positive (+2500 kg P ha$^{-1}$) in the field trials. The change in P-AL in the field samples from the 33 trials ranged from –145 to +258 mg P kg$^{-1}$ and was largely determined by the cumulative soil P balance (Fig. 3A, slope of 0.081, $R^2 = 0.54$). The negative intercept indicates a loss of P-AL in the topsoil at equilibrium P fertilizer application, i.e. at zero net P addition. No significant correlations between the cumulative soil P balance (Bal$_P$, kg P ha$^{-1}$) and the measured soil characteristics were found. Mixed multiple linear regression after removing non-significant terms yielded the following relationship ($R^2 = 0.93$):

$$\Delta\text{P-AL} = 20.3 - 0.17 \times \text{P-AL}_{\text{ini}} - 0.52 \times t + (0.12 + 0.00020 \times \text{P-AL}_{\text{ini}} - 0.0022 \times t) \times Bal_P \quad (4)$$

in which ΔP-AL and P-AL$_{ini}$ in mg P kg$^{-1}$, t in years and Bal$_P$ in kg P ha$^{-1}$. The interaction term of time and the cumulative P balance was statistically significant. The initial P-AL was significant both as single term as in combination with the cumulative P balance. The main effect of time was not significant ($p = 0.15$). The intercept was statistically different from zero ($p = 0.02$). The change in P-AL was not statistically determined by the soil texture.

The categorical variable defining whether the soil P balance was positive or negative was not significant, both as a single term and in interaction with the other variables. Therefore, one single model for both positive and negative soil P balances can be used. None of the other soil characteristics, i.e. percentage OM, soil pH, percentage of clay, Fe and Al soil contents were statistically significant when added alone and in interaction with the variables of the basic model.

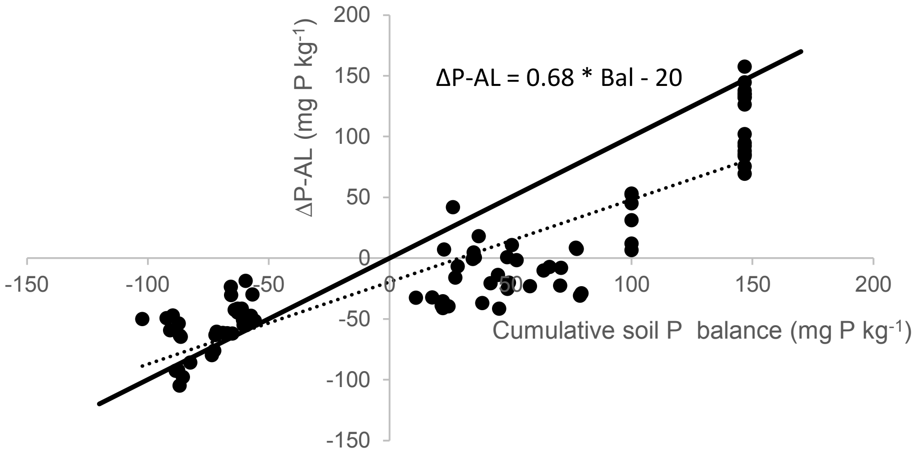

**Fig. 2.** The changes in ammonium lactate extractable P (ΔP-AL, mg P kg$^{-1}$) versus the cumulative soil P balance (Bal, mg P kg$^{-1}$) for eight contrasting soil samples used in a long-term greenhouse study with a P mining scenario (negative soil P balance), a P fertilized scenario (slightly positive balance) and in a laboratory incubation experiment with P fertilized soils (positive balance). For the latter, also 3 soil samples with different P-AL from the Gembloux field trial #20 were included. The dashed line is the linear fit ($R^2 = 0.78$, equation in figure) of the individual data points (2 replicates), the solid line represents the 1:1-line.

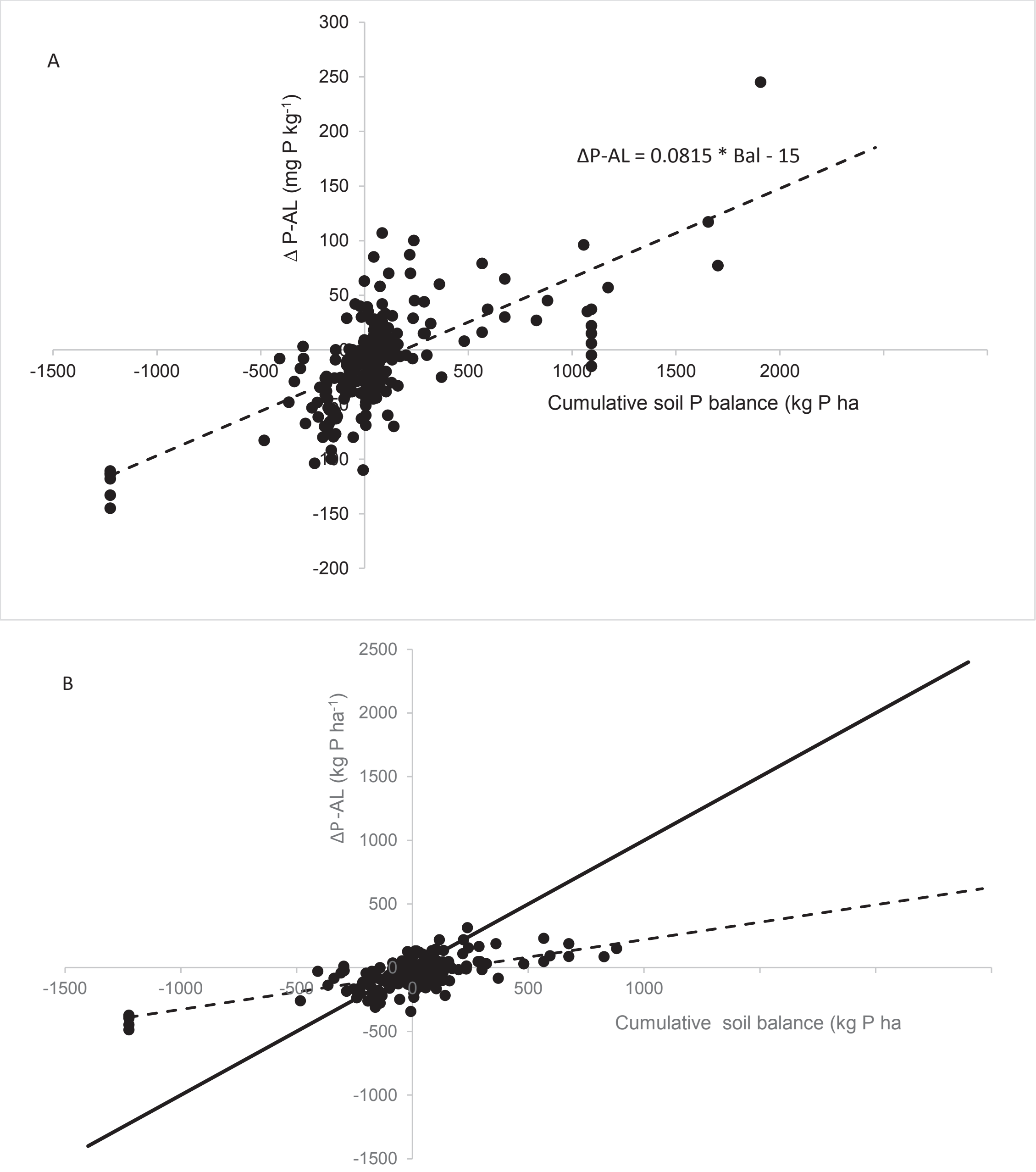

**Fig. 3.** The change in ammonium lactate extractable P (ΔP-AL) in 33 long-term field trials (265 data entries) is related to the cumulative soil P balance (kg ha$^{-1}$). The top figure (A) gives the soil weight based P concentration in mg P kg$^{-1}$; the bottom figure (B) is the area based stock of P in kg P ha$^{-1}$. The dashed lines are the linear fits through the data points, the solid line is the 1:1-line.

Mixed multiple linear modelling of the 225 data entries with separate information on P input and crop P export, yielded a very similar model ($R^2 = 0.91$) compared to the model derived from the whole dataset (Eq. (4)). If the assumed crop P exports were replaced by −30% or +30% of the assumed values, again a very similar model was obtained. This was also the case for the model that resulted from the mixed multiple linear modelling with P input instead of cumulative P balance as variable ($R^2 = 0.92$).

The annual change in P-AL was explained by the average annual soil P balance ($R^2 = 0.40$, Fig. S1). The fit was improved by adding the average P-AL ($R^2 = 0.46$):

$$\frac{\Delta\text{P-AL}}{\Delta t} = 0.7 + 0.15 \times \frac{Bal_P}{\Delta t} - 0.018 \times \text{P-AL}_{av} \tag{5}$$

in which $\frac{\Delta P\text{-}AL}{\Delta t}$ the annual change in P-AL in mg P kg$^{-1}$ y$^{-1}$, $\frac{Bal_P}{\Delta t}$ the average annual soil P balance in kg P ha$^{-1}$ y$^{-1}$ and P-AL$_{av}$ the average P-AL in mg P kg$^{-1}$. Eq. (5) allows the required annual soil P balance to be calculated in order to attain steady state for P-AL, i.e. no change in P-AL value. Such a steady-state is advised for agro-environmental target values of P-AL. Inverse prediction of $\frac{Bal_P}{\Delta t}$ from Eq. (5) with $\frac{\Delta P\text{-}AL}{\Delta t} = 0$ for several target values of P-AL$_{av}$ from the literature gave slightly positive values (Table 1), i.e. a limited surplus in the balance is needed in order to keep P-AL at the target value. Larger surpluses would be needed for a steady-state at larger P-AL values.

The slope of the linear fit of ΔP-AL expressed in kg P ha$^{-1}$ versus the cumulative soil P balance ($R^2 = 0.60$) was only 0.27 (95% confidence interval: 0.25 – 0.30), i.e., much smaller than 1 (Fig. 3B). If the analysis was done with 20% smaller or larger assumed bulk densities, the slope was still significantly lower than 1 (0.23 or 0.32, respectively). A slope of 1 would be expected if all P applied and all P removed by plants can or

**Table 1**
The required annual soil P balance to maintain the ammonium lactate extractable P (P-AL) constant as predicted by Eq. (5). The annual balances to maintain target P-AL depend on the critical levels of P-AL that differ among different field trials or legislations. Values in parentheses are the limits of the 95% confidence interval.

| Category P-AL value | P-AL (mg P kg$^{-1}$) | Required annual soil P balance for steady state (kg P ha$^{-1}$ y$^{-1}$) |
|---|---|---|
| Target P-AL range in Flanders (Belgium) (Jordan-Meille et al., 2012) | 120 – 180 | 10 (6 – 14) – 17 (13 – 21) |
| Average target P-AL range in European regions other than Flanders (Belgium) (Jordan-Meille et al., 2012) | 45 – 70 | 1 (−6 – 7) – 4 (−2 – 9) |
| Critical P-AL for crops Europe (Nawara et al., 2017) | 73 | 4 (−1 – 9) |
| Critical P-AL for crops Flanders (Martens et al., 2020) | 109 | 8 (4 – 13) |
| Average P-AL Flanders 2015–2019 (Soil Service of Belgium) | 300 | 31 (23 – 39) |

could be measured by AL-extraction of a sample of the topsoil (5 – 30 cm depth). Again, this slope was smaller than 1 and it was also much smaller than that in the laboratory and greenhouse trials (0.68, see above).

The analysis of P-AR in soils rather than P-AL allows a more complete balance estimation. The ratio of P-AR to P-AL varied from 3.4 to 16 in the soil samples collected from the field trials of Gembloux and Ath (Tables S2 and S3). The ratio was larger in deeper soil layers and in the treatments with zero P fertilizer addition (P0). The P-AL differentiates more sensitively between the P fertilizer treatments than the P-AR: the ratio of P measured in the treatment with the highest cumulative soil P balance over the P measured in the zero fertilizer treatment was 3.4–3.5 for P-AL and only 1.5 for P-AR. Even after more than 50 years of zero P application, the soil P-AR stock was larger than the residual P-AR (Eq. (3)) measured by applying 1.5 to 2 times the P amount exported by the crop for more than 50 years (Table S3). The residual P-AR was similar (Ath, Table S2) or less than two times larger (Gembloux, Table S3) than the residual P-AL measured in the 0 – 25 cm layer, and much lower than the cumulative soil P balance. Therefore, the slope of the residual P in the 0 – 25 cm layer versus the cumulative soil P balance was still far below 1 for P-AR (Fig. S2): 0.40 (95% confidence interval 0.28 – 0.52) in Ath and 0.35 (0.27– 0.42) in Gembloux.

The analyses of P in deeper soil layers allowed us to assess the importance of these soil layers in the soil-fertilizer-plant dynamics. Both P-AL and P-AR in the 25 – 50 cm layer of Gembloux were larger in the P2 compared to the P0 treatment (Table S3). The differences in soil P between treatments were not statistically significant in the 50 – 90 cm layer. The residual P-AL was only 33% (P0) to 69% (P2) larger in the whole 0 – 90 cm profile compared to the residual P-AL in the top 0 – 25 cm layer. Therefore, the slope of the residual P-AL taking into account the deeper soil layers (0 – 90 cm) versus the cumulative soil P balance was still far below 1 (0.29, 95% confidence interval 0.21 – 0.36, Fig. S2). However, the residual P-AR increased to 58% (P2) – 103% (P0) by including the deeper soil layers. This results in an increased slope of 0.64 of the residual P-AR (0 – 90 cm) versus the cumulative soil P balance, however the 95% confidence interval of the slope is very wide (0.23 – 1.05).

## 4. Discussion

### 4.1. Phosphorus mass balance

The mass balance of the field trials showed that the soil P-AL changes by 0.081 mg P kg$^{-1}$ per unit of kg P ha$^{-1}$ of the net balance, i.e. fertilizer addition minus removal by the harvested crop. For P-Olsen, slopes of 0.014 to 0.057 mg P kg$^{-1}$ have been reported (Cao et al., 2012; Messiga et al., 2010; Tang et al., 2008; van der Bom et al., 2017, 2019a). This corresponds to the P-AL value of this study divided by the conversion factor of 3.2 between P-AL and P-Olsen obtained for 218 soil samples across Europe (Nawara et al., 2017). When P-AL is expressed in kg P ha$^{-1}$ the slope becomes 0.27 (Fig. 3B), i.e. 27% of the net P balance, either net addition or withdrawal, is measured in the P-AL fraction of the sampled topsoil, leaving a gap in the mass balance of 73%. Possible explanations of this large gap are (i) lateral and vertical P export from or input to the topsoil of each plot that were not accounted for in these open field experiments, (ii) participation of P that is not extracted by the AL extraction in the P field dynamics.

Field experiments are open systems, in contrast to lab and greenhouse experiments. For the soils tested in the greenhouse depletion trial and lab experiments, the recovery of the added P in the P-AL fraction was, on average, 68% (Fig. 2). This larger recovery compared to the field trials (Fig. 3B) can be attributed to the closed versus open system. In the greenhouse and lab experiments, no P leaching to or crop uptake from deeper soil layers, nor lateral soil movement by e.g. tillage, is possible. In addition, the larger recovery could also partly be explained by the smaller time frame of the greenhouse (2 years) and lab experiments (119 days) compared to the field trials (3 – 51 years). The extractability of added P can decrease in time (Karpinets et al., 2004). The recovery of the added P in the lab fertilizer treatment (positive soil P balance) was slightly larger when measured on the day of the fertilizer addition (on average 80%) instead of 119 days later (on average 64%), but still less than 100% (details not shown). This was not an analytical error since spiking the ammonium lactate extract with $KH_2PO_4$ showed 100% recovery (details not shown). Similarly, after 90 days only 2 to 61% of added P was recovered in the P-AL fraction of 28 Portuguese soils (Indiati et al., 1999). Part of P added to soil gradually moves to a fraction that is not recovered by ammonium lactate extraction, explaining the gap in the mass balance in this closed system (Fardeau, 1993). In addition, part of P removed by the grass in the closed greenhouse system originates from a fraction that is not measured by ammonium lactate extraction of the soil.

We attempted to close the mass balance gap for the field trials by using the P-AR extraction, i.e. by including P that is not extracted by ammonium lactate. The P-AR was 3.4 to 16 times larger than P-AL (Tables S2 and S3). However, the *aqua regia* soluble P in the topsoil still leaves a mass balance gap of 60% – 65% for the Ath and Gembloux field trials (Fig. S2). Other field trial studies also observed large P mass balance gaps for P-AR (Requejo and Eichler-Lobermann, 2014) or P extracted by other strong acids (Garz et al., 2000; Lemming et al., 2019; Oehl et al., 2002; Richards et al., 1995; van der Bom et al., 2019b). Median and Q90 values of 2108 soils from the GEMAS database (Reimann et al., 2014) show that P-AR measures 83–84% of the total P measured by X-ray fluorescence. Part of the total soil P, especially within Fe-oxides and –hydroxides, is possibly not completely accessible by *aqua regia* (Acksel et al., 2019). This is not problematic for the mass balance calculations as long as this non-AR-extractable fraction does not change. This P fraction is likely to be of only minor significance for plant P uptake or recently added P. Moreover, this P fraction is small in comparison to the observed mass balance gap in the Ath and Gembloux field trials.

A large part of the applied or withdrawn P does not end up in or originate from the topsoil. For the Gembloux field trial, the mass balance for P-AR improved from 35% to 64% by including not only the topsoil but all 0 – 90 cm P-AR. Smaller mass balance gaps by including subsoil P were also observed in other field studies (Garz et al., 2000; Oehl et al., 2002). A first possible explanation is the P uptake by plants from soil layers deeper than the topsoil. Phosphorus uptake by plants from the subsoil can range from 3 to 85% of the total P uptake (Kautz et al., 2013). This P taken up by the plants can be partly returned to the topsoil by plant debris, resulting in a net upward P transport. Secondly, deep tillage can mix soil from the topsoil with deeper soil layers. Thirdly, fertilizer or soil P can be leached to deeper soil layers. Significant phosphorus enrichments have been found up to 70 – 90 cm depth (De Bolle et al., 2013; Rubaek et al., 2013; Warrinnier et al., 2020).

A mass balance of 64% using 0 – 90 cm soil still leaves a considerable gap for the Gembloux field trial. It should be mentioned that there is some uncertainty on the mass balance values given the uncertainty on some of the assumptions. The crop P offtake was only measured in the last 4 years, for the previous years this was based on crop yields and assumed plant P contents. If the soil density is 20% larger than the assumed density in this study (1350 and 1500 kg $m^{-3}$), the mass balance increases to 77%. It is not clear if including soil layers below 90 cm depth could further improve the mass balance. Deeper P leaching is not likely, since no significant differences in P-AL or P-AR between the treatments were observed in the 50 – 90 cm layer. However, P upward transport by plants from layers below 90 cm depth is a possible explanation for the larger P-AR in the P0 treatment than expected from the soil P balance and the P-AR contents of the P1 treatment. Moreover, the subsoil contribution to P delivery to crops increases at lower topsoil P content (Kautz et al., 2013). If the crop P export was estimated correctly for the Gembloux field trial, about one quarter of the crop P export was not accounted for by P-AR changes in the 0 – 90 cm layer and should originate from soil layers deeper than 90 cm. This is not unlikely since the Gembloux rotation is dominated by sugar beet and cereals, which are crops with some roots well below 1 m (Brown et al., 1987; Thorup-Kristensen et al., 2009).

An alternative explanation for both the negative mass balance gap for the P0 treatment and the positive mass balance gap in the P2 treatments in the Gembloux field trial is the lateral movement of P by tillage. By transect measurements and dispersion models it was shown for four Danish P field trials that 14 to 18% of net added P had left the 108 – 135 $m^2$ plots after 15 to 16 years of lateral dispersion due to tillage practices (Sibbesen et al., 2000). In the Gembloux field trial, the same ploughing direction was used for 51 years, with alternating throw side every year. The plot replicates with smaller P-AR values compared to the treatment average were more often located next to zero P fertilizer plots (8 out of 12 cases). This implies a possible lateral movement effect of the low P soil from the zero P-plots to adjacent plots. However, there was no clear lateral movement effect of the high P plots (P2) on the P-AR values of the adjacent plots.

### 4.2. Explaining changes in P-AL

In contrast to most other studies relating changes in soil P to cumulative soil P balances, we used data from a large number of field trials (33) and with a large range in cumulative soil P balance (−1200 to +2500 kg P $ha^{-1}$). Analyzing smaller time steps and smaller soil P balances gives lower correlations, possibly explained by analytical bias or sampling error (van der Bom et al., 2019a). We only included the largest time step for each treatment of the field trials in our dataset to avoid autocorrelation between the data.

The integration of 33 contrasting field trials allowed us to analyze the effect of several variables on the change in soil P (Eq. (4)). The positive effect of the soil P balance is as expected, but also other variables were found to have a significant effect on ΔP-AL. A significantly negative effect of the interaction between time and the cumulative soil P balance was observed: equal cumulative balances but longer periods lead to smaller ΔP-AL in a positive balance (more movement to the non-AL- extractable pool) and smaller ΔP-AL in a negative balance (more time for release to the AL-extractable P pool). This logically follows the two P pool concept, i.e. passage of time promotes movement of P out of the extractable pool in a positive balance and promotes weathering or mobilization into the extractable pool under P depletion scenarios (Smolders et al., 2020). There was no significant main effect of time in our model (Eq. (4)), so the effect of time only emerges in combination with large net P balances. Similarly to our study, a smaller slope of Olsen P versus soil P balance was observed for a time period of 20 years compared to 6 years of field trial duration (van der Bom et al., 2019b). However, Messiga et al. (2010) found in one field trial that the relationship between Olsen P and the cumulative soil P balance was not affected by duration of the experiment. They also found equal slopes for negative and positive soil P balances, similarly to our study. In contrast, Messiga et al. (2015) found smaller slopes at negative soil P balances than above certain inflection points.

We found a significant effect of the initial P-AL on the change of P-AL, both as single term and in interaction with the cumulative soil P balance. Both with and without taking other significant variables into account, the effect of the initial P-AL as single term was significantly negative. This was also observed for soil P extracted with acidic ammonium acetate buffer at pH 4.65 (Ekholm et al., 2005). High soil P contents are mostly related to high P fertilizer additions in the past, which can move from AL-extractable to AL-non-extractable fractions over time, as was also observed in the lab experiments and field experiments (van der Salm et al., 2017). In addition, there is a significantly positive effect of the interaction between P-$AL_{ini}$ and the cumulative soil P balance on the change of P-AL. This indicates that the changes in soil P at a certain soil P balance, both negative and positive, will be more pronounced at higher P-$AL_{ini}$, i.e. the slope of the ΔP-AL versus P balance line (Fig. 3A) is larger. This was also observed for other soil P tests (Dodd et al., 2012; Ekholm et al., 2005; Johnston et al., 2014; Schulte et al., 2010). A possible explanation is the higher P-AL/P-AR ratio at higher P-AL values, as was also observed for the Ath and Gembloux field experiments individually (Tables S2 and S3). Phosphorus removal from or addition to the soil will, therefore, be seen more clearly in the measured P-AL fraction at high P-AL compared to the situation where P-AL comprises only a small fraction of the total soil P amount. The buffering capacity of a soil to changes in P-AL is smaller at high P-AL, similarly to the levelling off of sorption isotherms at high P loading (Edmeades et al., 2006; Ehlert et al., 2003; Indiati and Sharpley, 1997). In contrast, the lab experiment with Gembloux samples did not confirm that trend, i.e. there were no significant differences in ΔP-AL between the samples with contrasting P-$AL_{ini}$. The effect of P-$AL_{ini}$ on ΔP-AL is negative alone and positive in combination with the P balance: the overall effect of P-$AL_{ini}$ on ΔP-AL (Eq. (4)) is negative as long as the cumulative soil P balance is lower than 809 kg P $ha^{-1}$, which is mostly the case.

Over the 33 field trials, no significant effect of soil texture (or interaction terms) on ΔP-AL was found. Also no other soil characteristics were found to have a significant effect on the change of soil P. Other studies also found no or limited effects of soil texture, pH, OC, clay fraction and parent material on the change of soil P (Coad et al., 2014; Dodd et al., 2012; Schulte et al., 2010). However, several studies reported that Fe and Al contents strongly affect the soil P buffer capacity and, hence, the potential to fix or mobilize P (Allen et al., 2001; Burkitt et al., 2002; Herlihy and McGrath, 2007).

### 4.3. Relevance for fertilizer advice

The results from the 33 field trials allowed us to estimate the general relationship between changes in P-AL and soil P balances (Eq. (4)). After validation, this relationship could be used for long-term predictions of soil P dynamics and formulating agro-environmental policies. The required time span and/or the required soil P balance for increasing or decreasing soil P-AL to agro-environmental target P-AL values, i.e. from an ecological and economical point of view, can be estimated from the model obtained. For example, the time required to reduce the average P-AL of 300 mg P $kg^{-1}$ in Flanders, measured by the Soil Service of Belgium, to the upper limit of the target range, 180 mg P $kg^{-1}$, as defined by legislation in Flanders is 13 to 22 years of P mining (-40 to −25 kg P $ha^{-1}$ $y^{-1}$). Crops with high P demand rate per unit of root area (e.g. maize) can start experiencing yield reductions when P-AL values drop below 180 mg P $kg^{-1}$ under prolonged negative soil P balances (Smolders et al., 2020). Critical P-values depend upon crop species. Crops with lower P demand rate (e.g. wheat) can cope well with relatively low P-AL values.

The cumulative soil P balance measurements are not always available to model the required input because yield data and/or crop P contents are sometimes not measured. In 63 of the 265 cases, we had to assume crop P export values, but varying these values to −30% and +30% of the assumed numbers gave very similar models. As an alternative, P input data, i.e. P application by fertilizer or organic amendments, can be used. Alvarez and Steinbach (2017) showed that the change in Bray-P could be predicted from the P input data only. Surprisingly, we found a slightly better fit ($R^2$ increased from 0.91 to 0.92) for the model with cumulative P input instead of cumulative P balance as the explanatory variable. This means that the model is rather insensitive to the exact P offtake by crops and good estimations can be done with P input data alone.

Once target soil P values are reached, a steady state of this soil P is desirable. The model for the annual change in P-AL (Eq. (5)) showed that the annual soil P balance to maintain the soil fertility constant has to be slightly positive to keep P-AL values in steady state. Table 1 shows that the required annual soil P balance is larger when the target P-AL is larger, as was also observed for a similar P extraction method by Ekholm et al. (2005). Often equilibrium P fertilizer use, i.e. zero P balance, is recommended in fertilizer advice and legislation (Jordan-Meille et al., 2012). Sometimes small P surpluses are advised for soil P maintenance as in Sweden (Djodjic et al., 2005). In the Netherlands it is advised to take an ‘inevitable P loss’ of 9 kg P $ha^{-1}$ $y^{-1}$ into account when calculating P fertilizer doses (de Haan and van Geel, 2013).

## 5. Conclusion

Data from lab experiments, greenhouse trials and 33 field trials with contrasting soil P balances allowed us to study soil P dynamics. Changes in P-AL are mainly related to the cumulative soil P balance, but the initial P-AL and the time span also affect the changes in P-AL over time, whereas soil characteristics are of minor importance. Maintaining a steady critical P-AL for crops requires slightly positive net soil P balances. Such information from the derived models is useful for long term predictions of soil P changes, for environmental and agronomic purposes and policy.

A large part of the added or withdrawn P did not end up in or originate from the P-AL fraction in the topsoil. Lab and greenhouse experiments show that such closed systems are more successful in closing the P mass balance, however also here considerable amounts of P are not recovered (only 68% mass balance). The smaller P-AL mass balance of the field experiments (27%) was improved by considering the *aqua regia* soluble P, thereby including non-AL-extractable P, and P in soil layers below the plough layer as P can migrate vertically by deep tillage, leaching and P transport by plant uptake. The mass balance of field experiments that are still not closed after including deeper soil layers and *aqua regia* soluble P, are possibly explained by upward P transport by plants from deeper soil layers, lateral P movement between trial plots due to soil movement by tillage practices and the partial involvement of occluded P (non *aqua regia* soluble) in P dynamics.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

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