

# Testing soil phosphorus in a depleting P scenario: an accelerated soil mining experiment

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## Summary

The reduced use of phosphorus (P) fertilizer in fertile soil has reverted the P balance to negative in some regions. It is unclear how long current soil P stocks will ensure adequate P supply to crops. In addition, it is unknown if current soil tests for available P describe bioavailable P adequately in soil where P is becoming depleted. We set up an accelerated soil P mining test to address these questions. Perennial ryegrass (*Lolium perenne*, Melpetra tetra) was grown for 2 years in a greenhouse on 5-cm-deep soil layers of eight contrasting soils with periodic grass clipping. Each soil was split into four fertilizer treatments (i.e. no P (–P) and adequate P (+P)) and two nitrogen levels, the latter to alter the rate of P uptake. The long-term P mining induced P-related yield losses in seven of the 16 soil treatments. The cumulative uptake of shoot P at which yield loss started to exceed 10% (–P versus +P) varied over a small range of 37–74 mg P kg<sup>–1</sup> soil among the soils. This critical cumulative P uptake (CCP) was related to the soil P content prior to mining measured by five soil P tests (ammonium oxalate, ammonium lactate (AL), Olsen P, 0.01 M CaCl<sub>2</sub> and the diffusive gradient in thin film technique (DGT)); the largest R<sup>2</sup> values were observed for P-AL (R<sup>2</sup> = 0.72) and P-DGT (R<sup>2</sup> = 0.73). However, none of the tests was diagnostic for yield loss during the depletion period. Increased N supply accelerated growth and rates of P uptake and decreased the CCP by a factor of 1.7 on average, illustrating the effect of the rate of biomass production. The CCP values obtained in the treatment with reduced N fertilizer application are likely to be the most relevant for the field and suggest that current stocks allow adequate P supply for arable crops for 3–8 years under zero P application (0–23 cm) in soils similar to those tested. The lack of a successful diagnosis for P deficiency during this depletion experiment calls for further calibration of soil tests for available P in the field.

## Highlights

- The availability of legacy P in well-fertilized soil was evaluated with a P mining pot trial
- 10% loss of crop growth occurred when soil P was depleted by 37–74 mg P kg<sup>–1</sup> soil
- Accelerated plant growth with increased N supply decreased total P uptake beyond which P deficiency occurs
- In a depletion scenario, current soil P tests are not diagnostic but they can be used for prediction

## Introduction

Global phosphorus (P) fertilizer consumption quadrupled between 1961 and 2014, to increase crop production to feed the growing world population (IFADATA, 2016). The imbalance between the cumulative P input and the crop P uptake caused surpluses of P

in soil (residual or legacy P) during the last few decades in east Asia, western and southern Europe, the coastal USA and southern Brazil (Macdonald *et al.*, 2011; Lun *et al.*, 2017). On a smaller scale, there are inter-regional spatial distributions of P surpluses (Macdonald *et al.*, 2011) because of the variation in intensity of agricultural management and animal density. Two concerns have prompted the demand for a more sustainable use of P fertilizers: the non-renewability of P resources (Cordell *et al.*, 2009) and

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environmental problems from the eutrophication of surface waters (Smith, 2003). The reduction in rates of P application subsequently enforced a decrease in the average soil P balance in the EU-28 (countries of the European Union) from 5.2 kg P ha<sup>-1</sup> year<sup>-1</sup> in 2000–04 to 1.9 kg P ha<sup>-1</sup> year<sup>-1</sup> in 2010–14, and in some countries (Italy, Sweden, Slovakia and the Czech Republic) this decrease resulted in a shift from a positive to negative soil P balance (Eurostat, 2017). In Flanders (Belgium), stricter fertilizer regulations have been imposed since 2015, aiming for a negative soil P balance for soils with a high soil P status until the soil P content reaches a range of target values (Manure Action Plan, 2015).

A negative soil P balance leads to decreasing soil P stocks, which might entail a loss of agricultural productivity in the long term. This depends critically on how these decreasing stocks change P availability in the soil, which in turn is determined by two factors. The first is the intensity (I) of P in the soil (i.e. the amount of P in the soil solution that is readily available for plant uptake). The second is the phosphate buffer capacity (PBC); that is, the resistance of the soil solution P to change when P is added (fertilization) or removed from the soil (crop uptake) (Beckett & White, 1964; Holford, 1997). In the latter case, the soil solution is replenished with P from the soil's solid phase (the quantity, Q) (i.e. the potentially available P). Phosphorus sorbed on the solid phase can be subdivided into a labile fraction, which rapidly replenishes the soil solution P, and a non-labile fraction that slowly replenishes the labile fraction (Lookman *et al.*, 1995; Sattari *et al.*, 2012). This causes asymmetric sorption–desorption processes (hysteresis effect): for the same concentration of P in the soil solution more P is sorbed during the removal of P from the soil system (crop uptake) than during application of P (fertilization) (Fox & Kamprath, 1970; Okajima *et al.*, 1983). The plant availability of P (and the risk of P leaching) is thus larger in an accumulation scenario than in a depletion scenario (Schoumans *et al.*, 2015). Consequently, it is unclear whether current soil P tests, calibrated with crop fertilizer responses during accumulation scenarios, still hold in these depletion scenarios.

The goal of this study was to determine the plant availability of residual P in a P depletion scenario and to verify whether current soil P tests can predict yield losses. This evaluation requires sufficient (accelerated) P mining to obtain yield losses for the evaluation of soil P tests. For accelerated P mining we applied no P; this extreme case was preferred because field data have shown that (modest) negative soil P balances, caused by insufficient application of P, induce yield losses only after several years or decades (Dodd & Mallarino, 2005; Messiga *et al.*, 2010; Verloop *et al.*, 2010; Vanden Nest *et al.*, 2015). In addition, we also aimed to quantify how long crop yield can be maintained without fertilization. This was studied by a 2-year accelerated soil P mining test in a glasshouse with eight Flemish agricultural soils using an approach similar to that of Koopmans *et al.* (2004b); that is, a shallow soil layer (here 5 cm) and continuous crop growth with periodic removal of the clippings and no addition of P, but all other nutrients added periodically. Yields of perennial ryegrass in the P mining (–P) treatment were compared with yields obtained with adequate available P (+P

treatment). This was carried out at two rates of nitrogen application to simulate two rates of plant growth, each inducing different rates of P mining that might reflect the different rates of growth in the field. The plant P content and plant available P (measured by five different soil P tests) were determined regularly, and at each harvest the biomass was determined. The soil tests were assessed on soil samples taken before and after the mining. The results of the plant and soil analyses were combined to evaluate the capacity of the soil P tests to predict the amount of available P before yield losses of more than 10% were observed based on the initial soil P values (predictive test) or to diagnose the yield responses after mining based on the final soil P values (diagnostic test).

## Materials and methods

A schematic overview of the materials and methods of the P depletion experiment is given in Figure S1 in Supporting Information.

### Soil sampling

Eight Flemish soils, seven arable and one grassland, were selected based on their texture (sand, sandy loam and loam), geographical location (across Flanders), pH and differences in P content from the database of the Soil Service of Belgium (Table 1). An overview of the methodology used to determine soil characteristics is given in the Supporting Information. Soil samples were taken as several subsamples ( $\geq 14$ ) from the plough layer (0–23 cm) on a cross at each location. Soil samples were air dried and sieved through a 4-mm mesh.

### Phosphorus depletion experiment

The depletion experiment was carried out in a glasshouse. Temperature was kept between 19 and 22°C during daytime (06.00–19.00 hours) and between 16 and 17°C during the night. There was no cooling system; therefore, in summer the maximum temperature in the glasshouse was not controlled and could increase above 22°C. It was limited to the outdoor temperature minus 2°C by air circulation through the roof windows and by keeping the relative air humidity at 65%. In winter, additional photosynthetically active radiation (25 W m<sup>-2</sup>) was provided between 08.00 and 20.00 hours, when the natural light intensity outside decreased below 250 W m<sup>-2</sup>.

For each soil, two levels of P (no P (–P) and P addition (+P); the latter was the reference treatment) were imposed. These two treatments received two rates of N (limited N (–N) and more than sufficient N (+N) fertilizer), i.e. four treatments in total). The purpose of the N treatments was to simulate two rates of crop growth (and thus two rates of crop P demand), which tested the rate of P removal on the critical cumulative P uptake beyond which grass yield declines. All treatments received an initial fertilizer application of 34 mg Mg kg<sup>-1</sup> soil as MgSO<sub>4</sub>·7H<sub>2</sub>O and 69 mg K kg<sup>-1</sup> soil as K<sub>2</sub>SO<sub>4</sub>. The +N treatments received an initial dose of 82 mg N kg<sup>-1</sup> soil as a mixture of NH<sub>4</sub>NO<sub>3</sub> (50% N) and

**Table 1** Overview of the soil characteristics determined on the initial soil samples of the -N-P treatment. The pH is measured in the 0.01 M CaCl<sub>2</sub> extract

Soil number	P-Ox / mg kg <sup>-1</sup>	P-AL / mg kg <sup>-1</sup>	P-Olsen / mg kg <sup>-1</sup>	P-CaCl <sub>2</sub> / mg kg <sup>-1</sup>	P-DGT / µg l <sup>-1</sup>	Fe-Ox / mg kg <sup>-1</sup>	Al-Ox / mg kg <sup>-1</sup>	pH	OC / %	Texture	Arable / grassland
1	670	310	66	4.9	320.0	2650	470	5.7	1.2	Light loam	Arable
2	470	280	80	3.6	253.0	2610	450	7.1	1.3	Sandy loam	Arable
3	390	120	42	1.5	74.2	5490	380	6.3	1.2	Sandy loam	Arable
4	490	150	45	1.8	55.6	11 120	990	6.2	3.0	Fine sand	Arable
5	450	200	89	5.9	88.7	370	860	4.9	1.2	Sand	Arable
6	690	110	44	1.8	84.7	6390	700	5.4	1.7	Fine sand	Arable
7	247	120	35	1.9	86.3	1680	640	7.2	1.0	Loam	Arable
8	537	150	59	4.0	114.0	5610	850	5.3	3.4	Loam	Grassland

Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O (50% N), and +P treatments were initially fertilized with 27 mg P kg<sup>-1</sup> soil as KH<sub>2</sub>PO<sub>4</sub>. Soil samples were placed on a filter cloth in trays (41 cm × 38 cm, filled with 5 cm soil) and deionized water was added to give 94–109% of their field capacity (determined at 9.8 kPa). Total soil wet weight varied between 8.8 and 11.2 kg soil per tray depending on the soil. Four replicates (trays) were prepared per soil and treatment (8 × 4 × 4 = 128 trays); all trays had the same initial amount of wet soil.

After 1 week of incubation of the soil in the trays, the initial available P content of every treatment was determined by the five different soil P tests, and perennial ryegrass (*Lolium perenne*, Melpetra tetra) was sown with a density of 0.43 mg cm<sup>-2</sup> (day 0). During the following months, the seeding density was increased several times to ensure more dense grass. After more than 1 year (day 403) the pot trial was re-started on the already mined soil samples because plant growth ceased for reasons unknown, probably related to persistent moss growth. The grass mat was removed, the soil was sieved again, placed in trays (two replicates left, 64 trays), and perennial ryegrass was sown again. A complete overview of the seeding history is given in the Supporting Information. Final harvest was after 535 days for the +N treatment and after 667 days for the -N treatment, to allow more pronounced differences in crop yield between the P treatments to be established.

Sprinklers provided deionized water for the plants based on temperature and air humidity (computer controlled). Weekly, weeds were removed, the trays were weighed and water was added manually until the initial weight was reached. Soils were fertilized with N, P (only the +P treatment), K, Ca and Mg after every two harvests (except for N: each harvest) based on the nutrient withdrawal by grass in the previous harvests. The nutrient withdrawal was compensated for each soil and treatment by equilibrium fertilization (fertilizer was applied equal to crop uptake) based on the biomass produced and the optimal dry-weight-based plant content of these nutrients, 36 g N kg<sup>-1</sup>, 36 g K kg<sup>-1</sup>, 3 g Mg kg<sup>-1</sup>, 4 g P kg<sup>-1</sup> and 4 g Ca kg<sup>-1</sup> dry biomass (Reuter & Robinson, 1997), or twice the equilibrium fertilization (Table S1 in File S1). Compensation for the crop nutrient offtake per harvest was on average 6.6 mg P kg<sup>-1</sup> soil (+P), 30.6 mg N kg<sup>-1</sup> soil (-N) and 62.3 mg N kg<sup>-1</sup> soil (+N). Micronutrients (copper, zinc, manganese, boron and molybdenum) were added on days 101 and 498. More detailed information on fertilization is given in the Supporting Information.

#### Plant analysis and soil P tests

The grass was harvested at 21, 43, 70, 94, 121, 148, 178, 204, 227, 259, 287, 336, 365, 400, 441, 462, 477, 494, 511, 535 (final harvest +N), 557, 584, 612, 641 and 667 (final harvest -N) days after seeding (DAS). After drying the plant samples for 72 hours at 70°C, the biomass and shoot P content were determined (Supporting Information). Only plant samples at 21, 43, 94, 148, 204, 259, 336, 511, 535 and 667 DAS were analysed; shoot P content for other harvests was derived by interpolation. Because P fertilization varied during the experiment, this interpolation might result in biased shoot P contents. However, we expect this deviation to be small and irrelevant for our results because the cumulative uptake of +P was not used in any conclusion.

The initial (day 0) and final soil samples (+N, day 535; -N, day 667) were dried for 72 hours at 45°C, passed over a 2-mm sieve and crushed. Their soil P content was determined by five soil P tests: phosphorus extraction with ammonium oxalate (Ox) (Schwertmann, 1964), extraction with ammonium lactate and acetate at pH 3.75 (AL) (Egnér *et al.*, 1960), extraction with 0.5 M NaHCO<sub>3</sub> at pH 8.5 (Olsen) (Olsen *et al.*, 1954), extraction with 0.01 M CaCl<sub>2</sub> (CaCl<sub>2</sub>) (Houba *et al.*, 2000) and the diffusive gradient in thin film technique (DGT) (Zhang *et al.*, 1998). The first three tests measure the potentially available P pool (P quantity, Q-tests) of the soil, whereas the last two measure the immediately available P pool (P intensity, I-tests). The CaCl<sub>2</sub>, AL and Olsen extraction were included because these tests are used as official procedures to determine soil available P, the oxalate extraction was included as an extreme Q-test and the DGT technique was included because of its promising results in predicting plant growth on tropical and Australian soils in scientific studies (Jordan-Meille *et al.*, 2012; Six *et al.*, 2013; Speirs *et al.*, 2013). A summary of the soil P test methodologies and abbreviations is given in Table 2, and soil P test procedures are described in the Supporting Information.

#### Statistical analysis

Data analysis was performed with the statistical program JMP Pro 12 (SAS, Cary, NC, USA). First, the final relative yield (RY) (i.e. the yield in the -P treatment relative to the average yield in the +P treatment (average of the last two harvests to reduce variance; +N, harvest 19 + 20; -N, harvest 24 + 25)), was plotted

**Table 2** Summary of the soil P test methodologies (extracted from Nawara *et al.*, 2017)

Soil P test				Solid:liquid ratio / g:ml	Extraction time / minutes	Phase separation	P analysis	Reference
Full name	Abbreviation	P measured	Extracting solution					
Oxalate extraction	Ox	P-Ox	0.2 M (COONH <sub>4</sub> ) <sub>2</sub> 0.14 M (COOH) <sub>2</sub> ± pH 3	1:20	120	2.5 µm filtration	ICP-OES	Schwertmann (1964)
Ammonium lactate extraction	AL	P-AL	0.1 M NH <sub>4</sub> -lactate 0.4 M acetic acid pH 3.75	1:20	240	Centrifugation <sup>a</sup>	ICP-OES	Egnér <i>et al.</i> (1960)
Sodium hydrogen carbonate extraction	Olsen	P-Olsen	0.5 M NaHCO <sub>3</sub> , pH 8.5	1:20	30	Centrifugation	Colorimetry	Olsen <i>et al.</i> (1954)
Calcium chloride extraction	CaCl <sub>2</sub>	P-CaCl <sub>2</sub>	0.01 M CaCl <sub>2</sub>	1:10	120	Centrifugation	ICP-MS	Houba <i>et al.</i> (2000)
Diffusive gradient in thin film technique	DGT	P-DGT	Ferrihydrite-based binding gel	Saturation	4–48 hours	Diffusion through a 0.45-µm filter membrane	ICP-MS	Zhang <i>et al.</i> (1998)

<sup>a</sup>10 minutes at 1830 g.

as a function of the final available P measured by each soil P test (Ptest). A Mitscherlich model was fitted by nonlinear regression to the data (all N and all P treatments) (Equation (1)):

$$RY = b_0 + (100 - b_0) \times (1 - \exp(-b_1 \times P_{\text{test}})), \quad (1)$$

where  $b_0$  is the RY (%) at  $P_{\text{test}}=0$  and  $b_1$  is the slope of the model. The maximum yield (100%) is approached asymptotically with increasing  $P_{\text{test}}$  concentration; at  $P_{\text{test}}=1/b_1$ , RY is  $b_0 + 0.63 \times (100 - b_0)\%$ .

In addition, the RY of the grass was plotted against time per soil and per N treatment for the  $-P$  treatment following Equation (2):

$$RY = \frac{100}{1 + \frac{1}{9} \times \left(\frac{t}{t_{90}}\right)^n}, \quad (2)$$

where  $t_{90}$  is the time to reach an RY of 90% (arbitrarily defined cut-off) and  $n$  determines the slope of the function; increasing values of  $n$  steepen the slope. Figure 4, a conceptual figure, illustrates the meaning of  $t_{90}$ ; this value was converted to the critical cumulative P uptake (CCP) by interpolation of the cumulative P uptake of the two harvest times around  $t_{90}$ . The plot of RY against time (and the curve fitting Equation (2)) is given in the Supporting Information, Figure S2, for all soils for which the CCP was determined.

The shoot P content was plotted as a function of cumulative P uptake (Pupt) for each N treatment by an exponential decreasing function (Equation (3)):

$$\text{Shoot P content} = 0.15 + b_2 \times e^{-b_3 \times \text{Pupt}}, \quad (3)$$

where  $0.15 + b_2$  is the initial shoot P concentration,  $b_3$  is the slope of the model and the minimal shoot P content (defined in this study as 0.15%, based on data from Reuter & Robinson, 1997) is reached asymptotically with increasing Pupt.

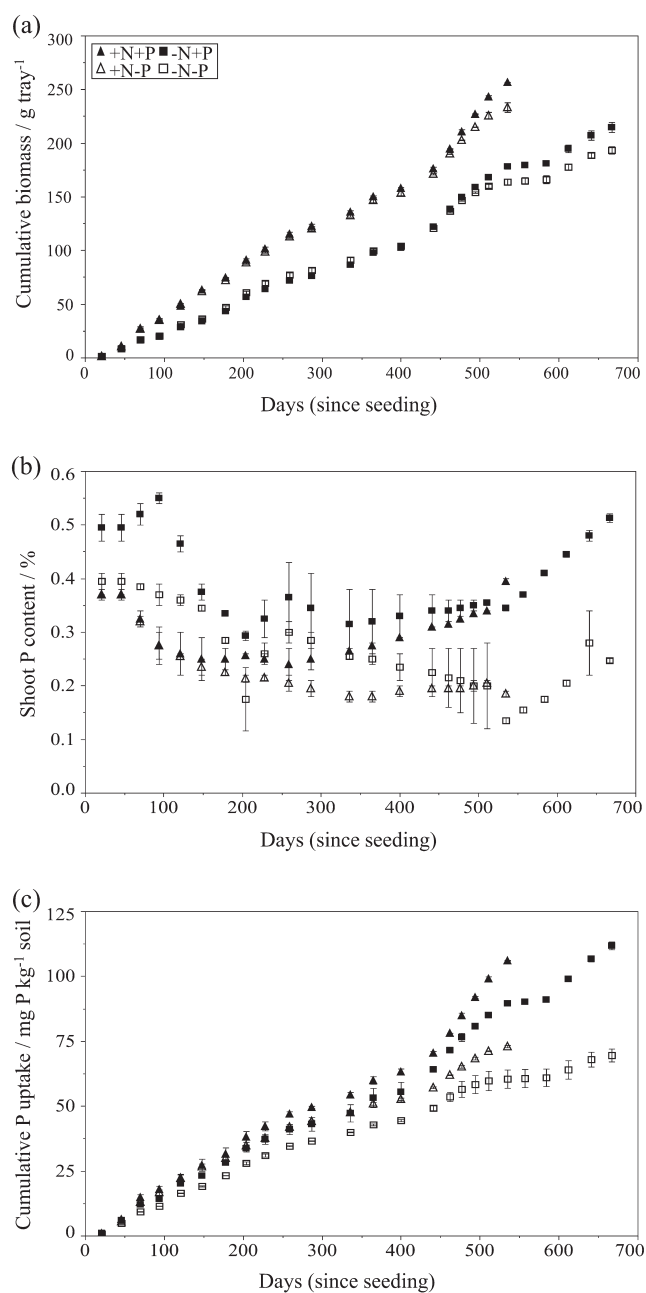
## Results

### Soil samples

The initial soils from the  $-N-P$  treatment varied in texture (sand-loam), pH-CaCl<sub>2</sub> (4.9–7.2), organic carbon content (1.0–3.4%) and initial P content (P-AL, 110–310 mg P kg<sup>-1</sup> soil) (Table 1). Five out of eight soils had P-AL values below the target range for grasslands imposed by the Flemish government (190 < P-AL ≤ 250 mg kg<sup>-1</sup>, 0–6-cm depth), which is the range of soil P values where fertilization advice is based on the crop's requirement (Manure Action Plan, 2015). In the UK, target Olsen P values for grassland, arable and forage crops range from 16 to 25 mg l<sup>-1</sup> (Defra, 2010), which is 12–19 mg kg<sup>-1</sup>, assuming an average bulk density of 1300 kg m<sup>-3</sup>. This is much smaller than the initial Olsen P values from the soils in this study (35–89 mg kg<sup>-1</sup>). The initial soil P values in this study were also larger than the average critical P values for arable crops (RY = 95%) determined by Nawara *et al.* (2017) (73 mg kg<sup>-1</sup> for P-AL, 19 mg kg<sup>-1</sup> for P-Olsen and 1.7 mg kg<sup>-1</sup> for P-CaCl<sub>2</sub>) on long-term field trials in Europe, except for the P-CaCl<sub>2</sub> of soil 3, which is just below this critical value.

### Plant growth and P uptake

Figure 1 shows the cumulative biomass, the shoot P content and the cumulative P uptake as a function of time for soil 8 (random example). Final values for all soils are given in Table S2 (Supporting Information). The effect of N on the cumulative biomass (i.e. cumulative shoot dry weight) was larger than the effect of P. At day 535 (end of +N treatment) the cumulative biomass averaged over all soils differed by a factor of 1.5 between N treatments for the  $-P$  treatment, whereas it differed by about a factor of only 1.1 between the P treatments within the +N treatment. The final cumulative biomass (+N, day 535;  $-N$ , day 667) was significantly smaller (Student's *t*-test,  $P < 0.05$ ) for  $-P$  than +P treatments for six



**Figure 1** (a) Cumulative biomass (g tray<sup>-1</sup>), (b) shoot P content (%) and (c) cumulative P uptake (mg P kg soil<sup>-1</sup>) as a function of time (days after seeding (DAS)) for soil 8 for the four treatments: with (+) or without (-) P fertilization, large (+) or small (-) N fertilizer application. Error bars denote the standard error (day 0–400,  $n = 4$ ; day 400–end,  $n = 2$ ).

+N soils and for one -N soil (Table S2 in Supporting Information). The relative yield (RY) was calculated at the end of the mining test (i.e. the ratio of the yield in the -P treatment to the average yield in the +P treatment). For the +N treatment, five out of the eight soils had a significantly (Student's  $t$ -test,  $P < 0.05$ ) smaller yield for -P than +P, whereas this was not observed for the -N treatment (Table 3, Figure S3 in Supporting Information). The final

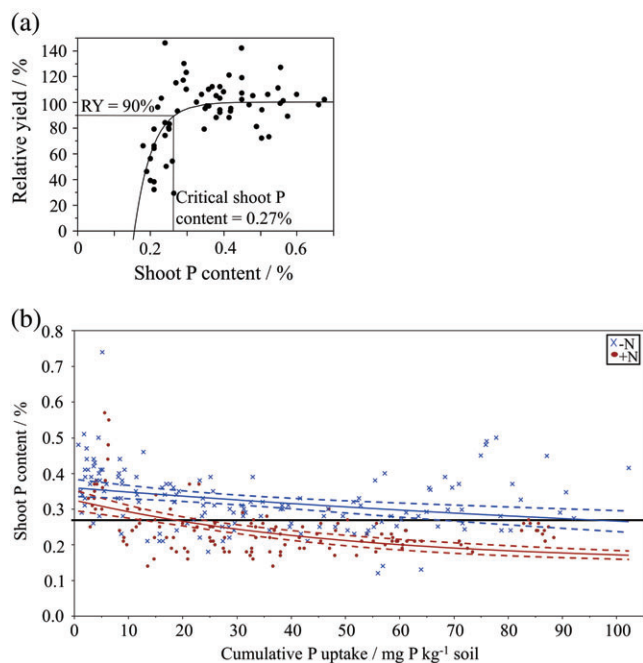
**Table 3** Mean relative yield (= (yield -P treatment / yield +P treatment)  $\times 100$ )  $\pm$  standard error ( $n = 2$ ) for each soil at the end of the pot trial. The relative yield was calculated as an average of the relative yields of the two last harvests to reduce variances (+N, days 535 and 667; -N, days 641 and 667). The initial P-AL value is the soil P value of the -N-P treatment at day 0

Soil	Relative yield / %		Initial P-AL / mg kg <sup>-1</sup>
	+ N (day 535)	-N day (667)	
1	84 $\pm$ 15	104 $\pm$ 12	310
2	81 $\pm$ 2 <sup>a</sup>	100 $\pm$ 13	280
3	126 $\pm$ 9	115 $\pm$ 2 <sup>a</sup>	120
4	64 $\pm$ 3 <sup>a</sup>	104 $\pm$ 5	150
5	38 $\pm$ 1 <sup>a</sup>	98 $\pm$ 1	200
6	70 $\pm$ 1 <sup>a</sup>	74 $\pm$ 7	110
7	66 $\pm$ 4 <sup>a</sup>	106 $\pm$ 5	120
8	62 $\pm$ 15	79 $\pm$ 3	150

<sup>a</sup>Relative yield of the two last harvests in the -P treatment is significantly (Student's  $t$ -test,  $P < 0.05$ ) different from that in the +P treatment.

RY data for all soils and treatments (two replicates) were collated and plotted against percentage shoot P content at the end of the experiment (Figure 2a). This plot indicates clearly that the relative yields decreased in response to a reduced shoot P content, and not to other factors. Comparing the plot of RY (%) as a function of shoot P content (%) at two different moments during the pot trial (Figure 2a, final harvest (+N, day 535; -N, day 667); Figure S4 in Supporting Information, harvest 4), illustrates that the shoot P content in the -P treatment decreased during the depletion experiment. The mean plant shoot P content at which RY = 90%, derived from the data in Figure 2(a), is 0.27% (standard error, 0.02%, based on a fit to 64 data points) and indicates the critical foliar P concentration. This corresponds to the critical P content for *Lolium perenne* in the literature, which varies between 0.20 and 0.38% (Reuter & Robinson, 1997). For the +N treatment, all final shoot P contents were below that critical value of 0.27% but there were no consistent significant differences in yield between +P and -P, probably because of the variation in responses (Table 3). Increasing N supply decreased shoot P content under P deficiency (Figure 1b), suggesting a dilution of P in the faster growing +N treatment.

The cumulative P uptake (Figure 1c) is the sum of P uptake after each harvest, where each uptake was calculated by multiplying the biomass per tray with the shoot P content relative to the air-dried soil mass per tray (mg P kg soil<sup>-1</sup>). For the -P treatment (day 535), the effect of N on the cumulative biomass (factor of 1.5 difference) was larger than the effect of N on the cumulative P uptake (factor of 1.1 difference). The cumulative P uptake was markedly affected by P treatment in all soils with +N treatment (day 535) and in seven of the eight soils with -N treatment (day 667) (Table S2 in Supporting Information; Student's  $t$ -test,  $P < 0.05$ ). The shoot P content of the -P treatment decreased with increasing cumulative P uptake (Figure 2b). Moreover, the cumulative P uptake where the critical foliar P (0.27%), and thus an RY of 90%, was reached was significantly different



**Figure 2** (a) Relative yield (RY) (last two harvests of each N treatment) as a function of the final shoot P content for all soils and treatments. Points are individual observations; the curve fits the Mitscherlich model. (b) Measured shoot P content as a function of the cumulative P uptake for the  $-P$  treatment. Points are individual observations; the lines fit an exponential decreasing function (Equation (3)) for the  $+N$  (dots) and  $-N$  (crosses) separately. The black horizontal line is the critical shoot P content (0.27%) at which RY equals 90%.

**Table 4** The decrease in available soil P ( $\Delta P$ , mg P kg<sup>-1</sup> soil) for the  $-P$  treatment measured by the different soil P tests between the start and the end of the soil mining pot trial and the corresponding cumulative shoot P uptake (mg P kg<sup>-1</sup> soil). Means and standard error ( $n = 2$ ) at day 535 ( $+N$ ) and day 667 ( $-N$ )

Soil	N	Day	Cumulative P uptake / mg kg <sup>-1</sup>	$\Delta P$ -Ox / mg kg <sup>-1</sup>	$\Delta P$ -AL / mg kg <sup>-1</sup>	$\Delta P$ -Olsen / mg kg <sup>-1</sup>	$\Delta P$ -CaCl <sub>2</sub> / mg kg <sup>-1</sup>	$\Delta P$ -DGT / $\mu\text{g l}^{-1}$
1	+	535	88 ± 1	118 ± 4 <sup>a</sup>	99 ± 6	17 ± 1 <sup>a</sup>	1.6 ± 0.2 <sup>a</sup>	136 ± 26
2	+	535	86 ± 1	114 ± 6 <sup>a</sup>	95 ± 3	35 ± 3 <sup>a</sup>	1.7 ± 0.1 <sup>a</sup>	120 ± 4
3	+	535	87 ± 1	143 ± 1 <sup>a</sup>	65 ± 1 <sup>a</sup>	19 ± 1 <sup>a</sup>	0.69 ± 0.06 <sup>a</sup>	37 ± 2
4	+	535	63 ± 1	167 ± 1 <sup>a</sup>	44 ± 1 <sup>a</sup>	13 ± 1 <sup>a</sup>	0.06 ± 0.04 <sup>a</sup>	-18 ± 16
5	+	535	60 ± 1	44 ± 28	54 ± 3	16 ± 13	1.8 ± 0.1 <sup>a</sup>	44 ± 3
6	+	535	62 ± 1	100 ± 21	42 ± 1 <sup>a</sup>	9.0 ± 1 <sup>a</sup>	0.06 ± 0.40 <sup>a</sup>	17 ± 6
7	+	535	57 ± 1	61 ± 4	50 ± 2	25 ± 1 <sup>a</sup>	1.2 ± 0.1 <sup>a</sup>	67 ± 1
8	+	535	73 ± 1	129 ± 2 <sup>a</sup>	79 ± 2	29 ± 1 <sup>a</sup>	1.5 ± 0.1 <sup>a</sup>	48 ± 1
1	-	667	91 ± 4	85 ± 4	53 ± 2 <sup>a</sup>	1.8 ± 0.1 <sup>a</sup>	0.48 ± 0.20 <sup>a</sup>	92 ± 6
2	-	667	87 ± 4	80 ± 2	73 ± 13	34 ± 1 <sup>a</sup>	2.2 ± 0.1 <sup>a</sup>	163 ± 1
3	-	667	70 ± 1	87 ± 3 <sup>a</sup>	62 ± 1 <sup>a</sup>	16 ± 1 <sup>a</sup>	0.78 ± 0.04 <sup>a</sup>	26 ± 13
4	-	667	91 ± 1	213 ± 15 <sup>a</sup>	49 ± 2 <sup>a</sup>	13 ± 1 <sup>a</sup>	0.65 ± 0.07 <sup>a</sup>	24 ± 2
5	-	667	66 ± 1	75 ± 18	27 ± 4 <sup>a</sup>	9.5 ± 0.3 <sup>a</sup>	2.8 ± 0.1 <sup>a</sup>	98 ± 8
6	-	667	58 ± 1	1.5 ± 61 <sup>b</sup>	25 ± 6 <sup>a</sup>	9.1 ± 0.1 <sup>a</sup>	0.69 ± 0.06 <sup>a</sup>	45 ± 1
7	-	667	63 ± 2	81 ± 3 <sup>a</sup>	59 ± 4	23 ± 1 <sup>a</sup>	1.3 ± 0.1 <sup>a</sup>	74 ± 2
8	-	667	70 ± 2	116 ± 3 <sup>a</sup>	63 ± 1	22 ± 2 <sup>a</sup>	2.0 ± 0.1 <sup>a</sup>	59 ± 6

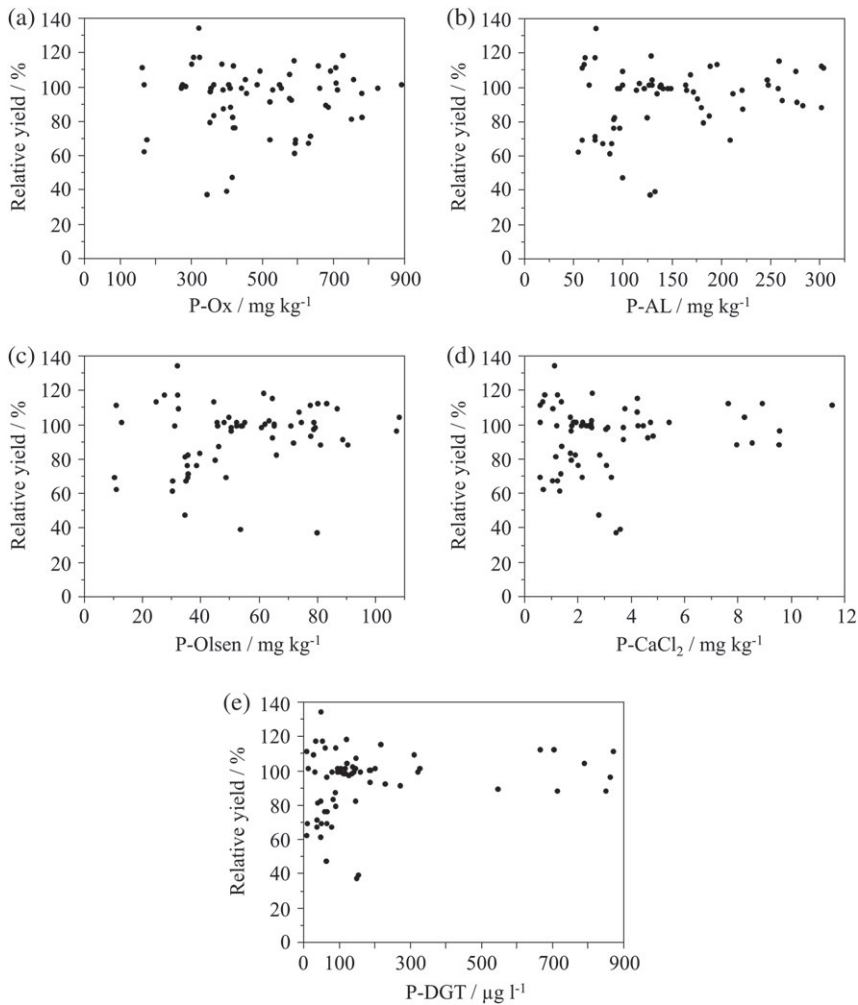
<sup>a</sup> $\Delta P$  is significantly different from the corresponding cumulative P uptake (two-sided Student's  $t$ -test,  $P < 0.05$ ). The comparison is not made for DGT (diffusive gradient in thin film technique) because of the different dimensions.

<sup>b</sup> $\Delta P$ -Ox of soil 6  $-N$  almost equals zero; this is probably because of a measurement error in one of the replicates (large standard deviation).

(Student's  $t$ -test,  $P < 0.05$ ) between both N treatments (no overlap of confidence intervals). This indicates the large effect of rate of plant growth ( $\sim N$  supply) on the point when yield losses of more than 10% were observed. The plant analysis confirmed that other nutrients (N, K, Mg and Ca) were not limiting in all treatments (Table S3 in Supporting Information).

#### Available soil P and its relation with crop growth

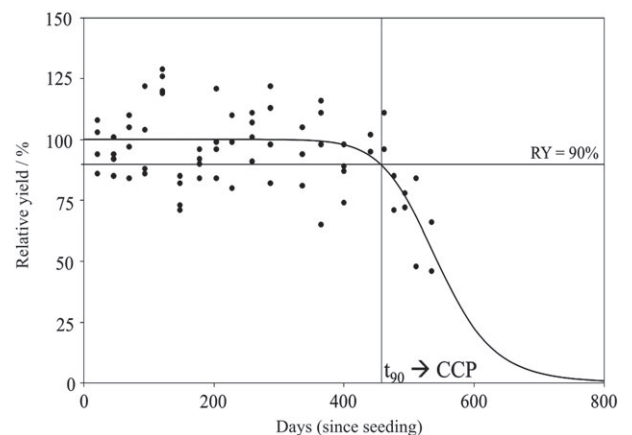
The available soil P, measured with the five different soil P tests, decreased with soil mining. Table 4 summarizes this decrease as the difference between the initial and final soil P test value ( $\Delta P$ ) for the  $-P$  treatment. The absolute values of  $\Delta P$  for AL and Ox corresponded best with the cumulative P uptake in the shoot. The relative plant yield at the final stage was plotted against the soil P test values at the final harvest for all N treatments (Figure 3). Overall, this relation was described poorly by the Mitscherlich model ( $R^2$ , 0.00–0.05), indicating that none of the soil P tests performed adequately in diagnosing yield losses from P deficiency in a depletion scenario. Nevertheless, for P-CaCl<sub>2</sub> and P-DGT, the data obeyed a biphasic pattern of responsive and non-responsive soils, and little yield response was observed above soil P test values of 3.8 mg kg<sup>-1</sup> for P-CaCl<sub>2</sub> and 160  $\mu\text{g l}^{-1}$  for P-DGT. Shoot P content responded clearly to P application (Figure 1b); therefore, shoot P uptake (product of yield and shoot P content) was analysed as a second index of bioavailable P. The final relative P uptake ( $-P$  relative to  $+P$ ) was plotted against the final soil P values (Figure S5 in Supporting Information); this relation was described better by the Mitscherlich model ( $R^2$ , 0.06–0.33, largest  $R^2$  for P-DGT) than



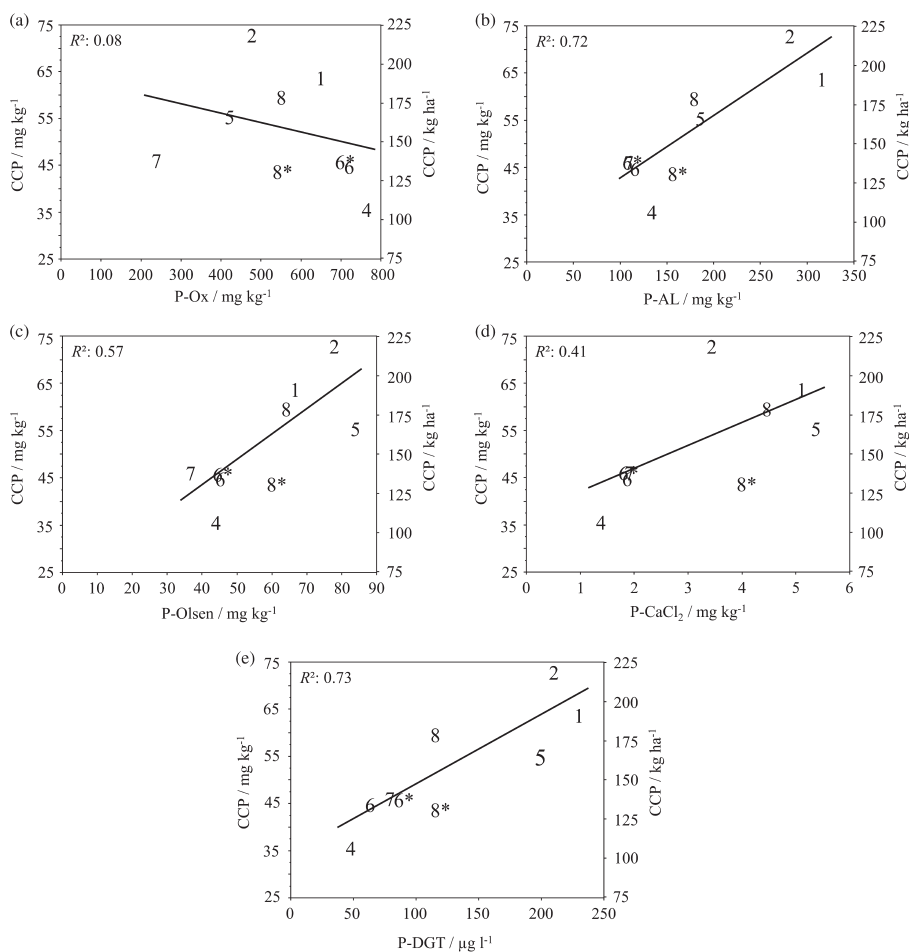
**Figure 3** Relative yield (average of last two harvests) as a function of the final soil P values measured by the different soil P tests for all N treatments. Points are individual observations. The curve describing the Mitscherlich model is not given because of its poor fit ( $R^2$ , 0.00–0.05). (a) P-Ox, (b) P-AL, (c) P-Olsen, (d) P-CaCl<sub>2</sub> and (e) P-DGT (diffusive gradient in thin film technique).

RY (Figure 3). Furthermore, for this relation a biphasic pattern was observed for the I-tests only (P-DGT and P-CaCl<sub>2</sub>).

The initial soil test values were related to the critical cumulative P uptake (CCP) to identify whether the point when RY decreases below 90% can be predicted. First, the relative yield as a function of time was plotted with Equation (2), as illustrated by Figure 4; the model parameters ( $n$ ,  $t_{90}$ ) are given in Table S4 in (Supporting Information). The model was fitted to all +N soils (except soil 3) and soils 6 and 8 of the –N treatment. Fitting was probably not possible for the other soils because RY had not decreased below 90% at the end of the experiment (Table 3). Under the experimental conditions in this study, the time taken for the relative yield to decrease to 90% ( $t_{90}$ ) varied by a maximum factor of 1.8 (minimum  $t_{90}$ , 288 days; maximum  $t_{90}$ , 514 days; mean  $t_{90}$ , 438 days) between all soils and N treatments. The associated CCP (Table S4, Supporting Information) varied by a factor of only two between all soils (37–74 mg kg<sup>-1</sup>). The  $R^2$  of CCP as a function of the initial soil P values ranged from 0.08 to 0.73, depending on the P test (Figure 5), indicating that some tests can predict the cumulative P uptake before the grass yield decreased by more than 10%. The smallest  $R^2$  was for P-Ox and the largest values were for P-DGT and



**Figure 4** Conceptual figure of the time to reach the critical cumulative phosphorus uptake (CCP) (example: soil 8 + N). The shoot yield of the –P soil is divided by that of the corresponding +P soil (= relative yield (RY)) at corresponding harvest time. The empirical curve enables determination of the time to reach 90% relative yield ( $t_{90}$ ). The CCP (mg P kg soil<sup>-1</sup>) at  $t_{90}$  is derived by interpolating the cumulative P uptake between the two harvest times enclosing the  $t_{90}$ .



**Figure 5** The critical cumulative P uptake (CCP, mg shoot P kg<sup>-1</sup> soil on the left y-axis, kg ha<sup>-1</sup> on the right y-axis; see Figure 4 for definition) for the -P treatments as a function of the initial soil P values. The line represents a linear function describing the data. (a) P-Ox, (b) P-AL, (c) P-Olsen, (d) P-CaCl<sub>2</sub> and (e) P-DGT (diffusive gradient in thin film technique). The numbers refer to +N treatments, -N treatments (\*) are included for soils 6 and 8.

P-AL. The initial P-Olsen values varied in proportion with CCP, and the average ratio of P-Olsen to CCP was 1.08. For the other tests the CCP was not proportional to the initial soil P values.

## Discussion

### Soil testing for available P in a mining scenario

This study was set up to test whether current soil P tests can determine the availability of soil residual P with P depletion. Numerous soil tests for available P exist (Jordan-Meille *et al.*, 2012; Six *et al.*, 2013; Speirs *et al.*, 2013) and have been calibrated during the last few decades (i.e. during periods of P build-up). A summary of the five tests used in this study and their recalibration to EU-wide field trials was given recently in Nawara *et al.* (2017). In a P-depleting scenario, the situation is different because the soil contains only residual P and its availability depends on the rates of P desorption (Koopmans *et al.*, 2004a). Soil P tests in this study were evaluated in several ways, for example adequate matching of the soil P mass balance, diagnosing P deficiency during mining or prediction of the total stock of available P (the cumulative P uptake, the CCP) until yield losses of more than 10% are observed.

The mass balance (Table 4) aspect related best to changes in P-AL and P-Ox. In absolute quantities,  $\Delta$ P-Ox was larger than the cumulative P uptake (except for four cases), whereas  $\Delta$ P-AL was smaller than the cumulative P uptake (except for three cases). This reflects the differences in P pools measured by these tests: P-AL is a more buffered method, whereas the aggressive ammonium oxalate extraction is a measure for total soil P. That  $\Delta$ P-Ox exceeds the cumulative shoot P uptake (+N treatment) can be partly explained by underestimation of the total cumulative P uptake because the P uptake by roots was not included. Alternatively, part of the extractable P at the start of the pot trial can become unavailable for the acid ammonium oxalate extraction because of a transformation to non-extractable P forms, for example because of the stronger binding and accumulation of P in inorganic forms or in organic material (Van der Salm *et al.*, 2017). For the +P treatment, the mass balance (calculated as P fertilizer application minus cumulative P uptake) was positive (i.e. more P had been added to the system than was taken up by the plant). In contrast, in this +P treatment  $\Delta$ P-Ox and  $\Delta$ P-AL were negative, suggesting that freshly added P was also transformed into P forms that were not extractable with the ammonium oxalate and ammonium lactate extraction. A similar gap between the mass balance and  $\Delta$  P values has been observed in



other pot trials for the oxalate (Koopmans *et al.*, 2004b) and Olsen extractions (Schelfhout *et al.*, 2015). In field studies, a decrease in soil P availability larger than the mass balance has also been observed (Gallet *et al.*, 2003; Van der Salm *et al.*, 2017). In the present study, the mass balance of the Olsen extraction was smaller than the cumulative P uptake. This indicates that in a depletion scenario the initial P measured by the Olsen extraction is a snapshot of the available P, which is replenished by other forms of P in the soil. This is also true for the P-AL, but to a lesser extent. The  $\Delta\text{P-CaCl}_2$  and  $\Delta\text{P-DGT}$  cannot be used to close the mass balance because the P in soil solution is replenished by P from the solid phase with long-term P mining.

The soil P tests performed poorly in diagnosing losses of crop yield from P deficiency (Figure 3). This negative result might be related to a too limited P deficiency to detect RY sensitively (average RY -P, 86%). The P test values of the final soil samples were still large when compared with the range of P values where yield losses were observed in Nawara *et al.* (2017). The P tests performed better in diagnosing the relative P uptake (than in diagnosing the RY), which is more sensitive to the availability of P but has less agronomic relevance than RY. The Q-test P-Ox performed the worst and the I-test P-DGT performed the best (Figure S5 in Supporting Information,  $R^2$  P-Ox = 0.06 and  $R^2$  P-DGT = 0.33). For the I-tests, data were scattered only in the relevant range of P (for example, 0–3.5 mg kg soil<sup>-1</sup> for P-CaCl<sub>2</sub>), whereas for Q-tests the data were scattered for almost the entire range of possible soil P values. These results indicate that in this modest P depletion scenario Q-tests underperformed in diagnosing P deficiency compared with the I-tests. However, it must be noted that the P concentrations in the small range were absent in this study (i.e. the conclusions should be considered with caution because of limited power (moderate P deficiency) to compare the tests). Conceptually, Q-tests might be less successful than I-tests in a P depletion scenario because replenishment of the soil solution by the solid phase is not fast enough to meet the plant's P demand, and only the immediately available P (i.e. the P in soil solution, quantified by I-tests) determines the crop yield. The increasing importance of I-tests in situations where plant available P is scarce, for example at small soil P concentration or large Fe and Al oxides concentrations, has been illustrated by the Q–I analysis in Nawara *et al.* (2017).

The third aspect of a suitable soil test in this depletion experiment is prediction of the point when the RY decreased below 90%. Soil tests performed well in predicting the critical cumulative P uptake based on their initial soil P content (Figure 5), except for oxalate extraction. This was not unexpected because it is well known that P extracted with this test does not relate well to plant available P. The largest  $R^2$  between the CCP and P test was for P-DGT and P-AL. The almost 1:1 relation between the CCP and initial P-Olsen values suggests that this test would be a good predictor of the stock of plant available P and of the number of cropping cycles before a yield reduction of 10% is observed. Despite the good mass balance fit of P-AL with plant P uptake (Table 4), the CCP did not increase in proportion with the initial P-AL. The initial P-AL values varied between 110 (soil 6) and 310 mg kg<sup>-1</sup> (soil 1), whereas CCP varied

by a factor of two only between the soils (34–74 mg kg<sup>-1</sup>). This suggests that yield losses for soil 1 occurred at a P-AL value larger than the initial one of soil 6 at which no yield losses were observed.

Taken together, the soil P tests related well to the initial soil P content with the CCP (predictive) but were not diagnostic in analysing RY in this depleting P scenario. The P-AL (Q-test) had an adequate mass balance, although it is not suitable as a diagnostic test, but showed a good relation with the CCP despite its non-proportionality. The Olsen test (Q-test) performed well in predicting the reserve of available P before yield losses of more than 10% were observed. The shoot P uptake analysis suggests that in this modest depletion scenario I-tests were more favourable in evaluating P availability.

### *The role of plant growth rate in critical soil P*

This experiment included two N treatments to simulate different rates of P demand. The +N stimulated growth for obvious reasons. Differences in the rate of potential P uptake between the N treatments were reflected by the total cumulative P uptake in +P, which was on average 107 mg P kg soil<sup>-1</sup> for the +N (535 days) and 100 mg P kg soil<sup>-1</sup> for the -N (667 days). Expressed per unit of surface area (8.4 kg dry soil tray<sup>-1</sup>, 0.1558 m<sup>2</sup> tray<sup>-1</sup>), this is 39 kg P ha<sup>-1</sup> year<sup>-1</sup> for +N and 30 kg P ha<sup>-1</sup> year<sup>-1</sup> for -N, which is only slightly smaller than the annual export of P by grass in Flanders (D'Haene & Hofman, 2015). However, there was still an accelerated mining of soil P compared to that in field conditions because the experiment used a 5-cm depth of soil only, whereas in the field P can be taken up from a larger soil volume.

The larger biomass and smaller shoot P content in the +N treatment compared with the -N treatment suggest a growth dilution of P with +N (Figure 1). This was observed for both P treatments, indicating that the rate of P supply was smaller than the plant's potential rate of uptake. The effect of N on cumulative P uptake (Figure 1c) is smaller than that of N on the cumulative biomass (Figure 1a). The data in Figure 2(b) show that the rate of growth affects total P uptake before reaching the critical foliar P (equivalent to CCP at RY = 90%), which was significantly different (Student's *t*-test,  $P < 0.05$ ) between both N treatments. From this figure an average CCP value of 95 mg kg soil<sup>-1</sup> across all soils can be determined by extrapolation for the -N treatment. The value for the +N treatment determined with this approach is smaller than those determined for each soil individually (Table S4 in supporting information, average CCP 55 mg P kg soil<sup>-1</sup>). This analysis showed that equal total P uptake in a soil by plants with varying rates of growth does not infer equal P deficiency and that the rate of biomass production also matters. Such a dynamic analysis suggests that the yield responses during mining cannot be defined uniquely by soil P tests that are static and not flux based, except for DGT.

This effect of N application and, therefore, of the rate of growth on CCP can be explained by conditions in which P supply to the plant is limited by the rate of desorption from solid phase P or by the rate of organic P mineralization. In such conditions, a flux-based (dynamic) analysis is more suitable to determine whether plant

uptake is limited by desorption or mineralization kinetics. Such a dynamic bioavailability index has been discussed previously by Koopmans *et al.* (2004a).

### Extrapolation to the field scale

The CCP for fast-growing plants (+N, Table S3 in Supporting Information) when converted to area-based values in the field (soil sampling depth Flanders: 0–6 cm grass, 0–23 cm arable crop, 1300 kg m<sup>-3</sup> density) is equivalent to 29–58 kg P ha<sup>-1</sup> in the 0–6-cm layer and to 111–222 kg P ha<sup>-1</sup> in the 0–23-cm layer. For the –N treatment (slower growing plants), the CCP derived from Figure 2(b) is equivalent to 74 kg ha<sup>-1</sup> for the 0–6-cm layer and to 285 kg ha<sup>-1</sup> for the 0–23-cm layer. Assuming an average annual P export in Flanders of 48 kg P ha<sup>-1</sup> for grasslands (D'Haene & Hofman, 2015) and assuming that the 0–6-cm layer is the rooted soil volume, it would take 0.6–1.5 years before a yield decrease of 10% was observed without any addition of P fertilizer, depending on soil type and the rate of plant growth. For arable crops with 34 kg P ha<sup>-1</sup> uptake (D'Haene & Hofman, 2015) and rooted soil volume of 0–23 cm, corresponding values are 3.3–8.4 years. This means that the critical time before yield losses are observed increases in proportion with the estimated rooting depth.

Most field studies on European soils did not find yield losses in the first 10 years with zero P fertilizer (Jaakkola *et al.*, 1997; Ellmer *et al.*, 2000; Gransee & Merbach, 2000; Rubæk & Sibbesen, 2000; Gallet *et al.*, 2003). Differences between field studies and the results obtained in this study might result from errors in the extrapolation, for example contribution from the subsoil (below the soil depths assumed above) in the field, which increases the time before yield reductions are observed. In addition, there are the effects of the rate of P removal on the CCP, or the artificial conditions of the accelerated experiment that might affect the small  $t_{90}$ -value, the small CCP and the large soil P content at  $t_{90}$ . For example, the soil volume to which plants had access (5 cm) in this study was smaller than in the field; in the pot trial of Koopmans *et al.* (2004b) plant growth was affected by the soil volume and a larger cumulative biomass was observed on soil of 10-cm depth than on soil of 5-cm depth in a P depletion scenario. The effects of rate of removal on CCP were large, as shown in Figure 2(b), indicating that the CCPs in the field are likely to be larger than the maximum values used here.

### Conclusion

This study has shown that the accelerated mining of P reduced soil P content and induced P deficiency in grass, which occurred faster and at a smaller total P uptake when N-application was larger. This illustrates that the rate of P removal in addition to total P uptake determines the point at which deficiency starts to occur. For the soils tested under zero P application, arable crops should have an adequate supply of P for 3 to 8 years (0–23 cm) depending on the initial soil P content and rate of biomass production.

The performance of the five soil P tests in this modest depletion scenario was evaluated by various criteria. For mass balance, P-AL

and P-Ox corresponded best with plant cumulative P uptake in a depletion scenario, whereas the other soil P tests provided a 'snapshot' of the available P that is replenished by non-extractable P pools with P removal. None of the five soil P tests was able to diagnose yield deficiency in this modest depletion set-up based on the final soil P values. In contrast, the initial soil P values related well to the CCP (i.e. the cumulative P taken up by the plants when P deficiency started, 37–74 mg kg<sup>-1</sup> depending on the soil and N treatment), indicating the predictive potential of the soil P tests. Further calibration of the soil P tests in the field with P depletion is needed to identify P deficiency successfully.

### Supporting Information

The following supporting information is available in the online version of this article:

#### Materials and methods

##### Soil characteristics

##### Change of seeding density

##### Fertilizer application

##### Methods of soil P testing (extracted from Nawara *et al.*, 2017)

**Figure S1** Schematic overview of the materials and methods of the P depletion experiment trial.

**Figure S2** Relative yield as a function of time for the soils for which the CCP was calculated.

**Figure S3** Yield difference between the –P and +P treatment for soils 4,5,7 and 8 on day 535 for the +N treatment and on day 667 for the –N treatment.

**Figure S4** (a) Relative yield as a function of the shoot P content for harvest 4 and (b) the final harvest (+N, day 535; –N, day 667). The shoot P content of the –P treatment (blue dot) decreases during the depletion experiment. Data are shown for harvest 4 instead of harvest 2 to exclude contribution of the seed P to the initial values.

**Figure S5** Relative P uptake (P uptake of –P relative to P uptake of +P) at the final harvest as a function of the final soil P values measured by the different tests. Points are individual observations, the curve describes the Mitscherlich model and is not given for P-Ox because of its poor fit ( $R^2 = 0.06$ ). (a) P-Ox, (b) P-AL, (c) P-Olsen, (d) P-CaCl<sub>2</sub> and (e) P-DGT.

**Table S1** Nutrient fertilizer application after each harvest expressed as the percentage of the biomass produced for which fertilizer compensation was calculated (for example, 100% = equilibrium fertilization). The optimal dry weight based plant content in ryegrass is also included in the calculation of fertilizer application. After day 535 (harvest 20) the +N treatment was stopped, at day 667 (harvest 25) the –N treatment stopped. The last nutrient compensation was after harvest 24.

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