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## Highlights

- Positive relation between relative forage yield and the P nutrition index (PNI)
- Critical PNI value of 92% separates P-limited and non-P limited grasslands
- Stronger relationship of PNI with Olsen P than with other soil tests
- Critical Olsen P stock for a target PNI of 92%: 12.9 kg P $ha^{-1}$

**Abstract**

Developing more sustainable forage systems requires efficient decision support tools for fertilization management. Soil phosphorus (P) tests have long been used as decision support tools for fertilization management but, more recently, plant nutrition indices using the P concentration of shoot biomass were developed to assess the P nutrition status of grasslands. The objectives of this study were to (i) evaluate the relationship between the phosphorus nutrition index (PNI) and the yield response to P fertilization (ii) analyze relationships between PNI and soil plant-available P (SPAP) indicators, and (iii) evaluate PNI assets for P diagnosis in forage system. Five long-term (≥ 9 years) grassland P fertilization experiments under different soil and climate environments in Canada, Switzerland, France and Romania were used. Three SPAP indicators were tested: $C_P$, the soil solution orthophosphate ions (oPion) concentration (mg P $L^{-1}$), Olsen P (mg P $kg^{-1}$), and, a process-based assessment (Qw + Pr) from the sum of oPion in the soil solution (Qw, mg P $kg^{-1}$) and diffusive oPion with time and $C_P$ (Pr, mg P $kg^{-1}$). PNI was calculated as sward P concentration divided by the critical P concentration.

. The cumulative effect of P fertilization resulted in a wide range of SPAP values. Overall, $C_P$ varied from 0.03-3.6 mg P $L^{-1}$, (Qw + Pr60min) from 6-52 mg $kg^{-1}$, and Olsen P from 4-40 mg $kg^{-1}$. The PNI varied from 48 %-94% in plots with no applied P, and from 83 %-121% in P-fertilized plots. A generally positive relationship between relative forage dry matter yield and PNI was established, with a critical PNI value of 92% that distinguishes P-limited and non-P-limited grassland nutrition. Positive relationships between PNI and the three SPAP indicators confirmed that the soil P status influenced the grassland P nutrition status. Critical values on a stock basis for a target PNI value of 92% were similar for Olsen P (12.9 kg P $ha^{-1}$) and (Qw + Pr60min) (13.5 kg P $ha^{-1}$). This study opens perspectives for P diagnosis improvement in forage systems.

Keywords: phosphorus, grassland, long-term field experiment, phosphorus nutrition index, soil plant available P, Olsen P

## 1. Introduction

Grasslands provide an essential source of cattle feed, and the development of more sustainable agricultural systems tends to increase their contribution to livestock production systems (Carrère et al., 2020). In a given environment, forage production is determined largely by the amounts of nutrients supplied by soil reserves and/or organic or mineral fertilizers applied by farmers. The aim of phosphorus (P) fertilization is to meet crop requirements, which are determined largely by the nitrogen (N) supply (Bélanger et al., 1989; Schellberg et al., 1999; Griffin et al., 2002; Valkama et al., 2016). Environmental and economic concerns require developing more sustainable forage systems. Excess P threatens the integrity of terrestrial and aqueous ecosystems (Sharpley and Menzel, 1987; Janssens et al., 1998. Ceulemans et al., 2011), while readily available and high-quality reserves of phosphate rocks will be exhausted in the medium term (Cordel et al., 2011).

Developing more sustainable forage systems requires effective decision support tools for fertilization management. To this end, plant- and soil-based indicators with threshold values for assessing nutrient status and managing fertilizers have been developed. Nutrient concentration ratios in plant tissues were developed as assessment tools for alfalfa (*Medicago sativa* L.; Walworth et al., 1986) and perennial ryegrass (*Lolium perenne* L.) (Bailey et al., 1997; Bailey et al., 2000). These ratios are also used for natural ecosystems to determine whether biomass production in terrestrial plant communities is N- or P-limited or co-limited by both nutrients (Güsewell, 2004). Soil P tests inform on soil plant available P (SPAP) and provide response thresholds useful in decision support tools for fertilization management (Schulte and Herlihy, 2007; Reinjeveld et al., 2010). However, both plant and soil tests remain of limited general value. Ratios that indicate of N or P limitation vary according to the type of ecosystems. For instance, they are much lower in upland grasslands than in wetlands (Mamolos et al., 2005; Craine et al., 2008). Similarly, there is no agreement on a universal soil P test likely to provide,

84 for a given crop or grassland sward, a single threshold value regardless of the soil type (Schulte
85 and Herlihy, 2007; Jordan-Meille et al., 2012).
86 More recently, significant advances have been made with the development of innovative
87 assessment tools. The approach of nutrition indices based on the nutrient concentration of shoot
88 biomass allows grassland P nutrition status to be assessed during growth (Duru and Ducrocq,
89 1997). For P, this approach is more reliable than those based on a single critical concentration
90 since it considers changes in nutrient concentration as a function of sward biomass
91 accumulation and concentration of other nutrients (Duru and Ducrocq, 1997). The P nutrition
92 index (PNI) is an effective tool for P fertilization management in grasslands since it assesses
93 the sward P nutrition status well during growth and can verify and validate fertilization
94 practices *a posteriori* (Thélier-Huché et al., 1999). The PNI is adequate for the interpreting of
95 the effect of plant P nutrition status on plant growth in grasslands managed at different
96 intensities (Liebisch et al., 2013); as well, PNI provides appropriate plant nutrients status
97 evaluation at the interface between agricultural land and saline wetlands in protected saline
98 habitats (Luna et al., 2019). At an ecosystem level, the N nutrition index (NNI) and PNI
99 provide appropriate evaluation of the functional response of species and communities to
100 fertility gradients induced by practices (Garnier et al., 2007; Lavorel et al., 2009).
101 At the same time, Morel (2002) developed a mechanistic model based on the assumption
102 that the SPAP pool represents the sum of the amount of orthophosphate ions (oPion) in the soil
103 solution (Qw) and the amount of soil P that can diffuse from the soil to the solution over time
104 ($P_r$). This model assumes that (i) diffusion of oPion at the solid-to-solution interface of soils is
105 quantitatively the dominant process in plant P nutrition and (ii) depletion of oPion
106 concentration at the root surface, due to absorption, creates a gradient of oPion concentration
107 between the root surface, the soil solution, and the soil solid phase. This gradient is the driving
108 force behind the flux of diffusive oPion from the soil solid phase to the soil solution. A general

model, based on a Freundlich kinetic equation, was developed to calculate $P_r$ as a function of the oPion concentration in soil solution ($C_P$) and time (Morel, 2002). This model correctly simulated the changes in SPAP in the 0-5 cm soil horizon oven seven years from a long-term grassland experiment with contrasting P fertilization regimes (Stroia et al., 2007). In the present study, we examined the abilities of $C_P$ (Morel et al., 2000), Olsen P (Olsen et al., 1954), and the sum (Qw + Pr) to assess SPAP in grasslands. Both $C_P$ and Olsen P are used around the world for this purpose (Jordan-Meille et al., 2012; Ziadi et al., 2013).

The objectives of this study were to: (i) evaluate the relationship between PNI and the yield response to P fertilization over a range of soil types and climate conditions; (ii) analyze relationships between PNI and three SPAP indicators in order to compare their abilities to assess the soil P status and (iii) evaluate PNI assets for P diagnosis in forage system. The study relied on five long-term experiments that measured the response of forage yield to P fertilization under contrasting environments representative of grassland ecosystems in Canada, Switzerland, France and Romania. The four sites offered the opportunity to explore large gradients in soil P fertility caused by cumulative effects of P fertilization over several years.

## 2. Materials and Methods

### *2.1. Overview of the five sites*

Five long-term grassland experiments at five sites across Europe and North America were used (Table 1). At each site, a control treatment without P fertilization was compared to one or more P fertilization treatments. Sites differed primarily in the duration of the experiment, soil and climate characteristics, and species composition (Table 1; Table 2).

LÉVIS, CANADA (CA-LEV). A grassland experiment, sown with timothy (*Phleum pratense* L. cv. Champ), was established in 1998 at Lévis, Canada (Table 1). The experimental design was a split plot, with four application rates of P fertilizer as triple super phosphate [0 (P0), 15

(P15), 30 (P30), and 45 (P45) kg P $ha^{-1}$] assigned to main plots, and four application rates of N fertilizer as calcium ammonium nitrate [0 (N0), 60 (N60), 120 (N120), and 180 (N180) kg N $ha^{-1}$] assigned to subplots. Experimental treatments were replicated in four blocks. For this study, plots that received the four P application rates and 120 kg N $ha^{-1}$ were selected within the experimental setup. From 1999-2006, fertilizers were applied each year before the start of growth in the first week of May. Potassium (K) as KCl was applied at 84 kg K $ha^{-1}$ as the same time as P and N to ensure that K did not limit plant growth.

LES VERRIÈRES, SWITZERLAND (CH-LES). A permanent grassland experiment was established in 1993 on a Cambisol at Les Verrières, Switzerland (Table 1), with a mixture of red fescue (*Festuca rubra* L.), common bent (*Agrostis capillaris* L.), and orchard grass (*Dactylis glomerata* L.) (Table 1). The experiment consisted of four application rates of P [0 (P0), 9 (P9), 17 (P17), and 26 (P26) kg P $ha^{-1}$] in plots arranged in a randomized complete-block design with three replicates. P was applied each year in a single application as triple super phosphate in October. K was applied as KCl in a single application in October at different rates according to the P treatment: 0, 29, 58 and 116 kg K $ha^{-1}$ for treatments P0, P9, P17, and P26, respectively. N was applied at a rate of 25 kg N $ha^{-1}$ as ammonium nitrate in all treatments once a year after the first cut.

ERCÉ (FR-ERC) AND GRAMOND (FR-GRA), FRANCE. An experiment was conducted at Ercé and Gramond, France, on permanent multi-species grasslands (Table 1). The experiment was established in 1999 at Ercé and in 1998 at Gramond. At both sites, the experiment consisted of two rates of P fertilizer [0 (P0) and 50 (P50) kg P $ha^{-1}$] applied each year in February as triple super phosphate on plots arranged in a randomized complete-block design with four replicates. N was applied as ammonium nitrate at rates of 100 kg N $ha^{-1}$ in February and 60 kg N $ha^{-1}$ after the first cut. K as KCl was applied at 200 kg K $ha^{-1}$ at the same time as P and N to ensure that K did not limit plant growth.

158 DÂMBOVICIOARA, ROMANIA (RO-DÂM). An experiment was established in 1963 on a
159 permanent grassland in the Southern Carpathians Mountains in the Rucar-Bran-Dragoslavele
160 corridor, Romania (Table 1). The grassland had a mixture of *F. rubra* and *A. capillaris*. The
161 experiment consisted of two rates of P fertilizer [0 (P0) and 33 (P33) kg P $ha^{-1}$] applied in
162 autumn each year as super phosphate on plots arranged in a randomized complete-block design
163 with four replicates. N was applied as ammonium nitrate at 100 kg N $ha^{-1}$ in early spring and 50
164 kg N $ha^{-1}$ after the first cut. Potash salt was added at a rate of 108 kg K $ha^{-1}$ once a year in
165 autumn to ensure that K did not limit plant growth (Ciubotariu et al., 2002).
166 More details on the five long-term experiments can be found in Bélanger et al. (2008) and
167 Bélanger and Ziadi (2008) for CA-LEV, in Jeangros and Sinaj (2018) for CH-LES, in Stroia et
168 al. (2007) for FR-ERC and FR-GRA, and in Ciubotariu et al. (2002) for RO-DÂM.

169 *2.2. Yield and nutrition index determination*

170 Dry matter (DM) yield and nutrient concentration were measured at the end of the first
171 growth cycle (Table 2), a period of the year when growth is rarely limited by water. Forage
172 production in this first growth cycle represented 40%-70% of the average total annual
173 production, depending on the site and treatment. All plots were harvested again during the rest
174 of the growing season, but no measurements were taken. DM yield was measured by cutting an
175 area of at least 1$m^2$ to a height of 5-cm. A fresh sample of ca. 300-500 g was taken, dried at 55
176 °C for 48 h, and ground.
177 At CA-LEV, dried and ground (1 mm) forage samples (0.1 g) were wet-digested with 1.5
178 mL $H_2SO_4$-$H_2SeO_3$ and 2.0 mL $H_2O_2$ (Isaac and Johnson, 1976). N and P concentrations were
179 measured by colorimetry using an automated continuous-flow injection analyzer (QuickChem
180 8000 FIA+ analyzer; Lachat Instruments, Loveland, Colorado, USA) with the salicylate-

nitroprusside procedure for total N (method 13-107-06-2-E) and the vanadomolybdate reaction for total P (method 15-301-3).

At CH-LES, concentrations of P and N in plant tissues were determined in samples that had been oven-dried (55°C for 72 h) and ground in a Retsch rotor. Total N was determined after combustion using the Dumas method (Masson et al., 2010), while total P was determined by radial ICP-AES (Varian Vista RL Simultaneous) after incineration (480°C for 5 h) and solubilization in hydrofluoric acid (Masson et al., 2010).

At FR-ERC, FR-GRA, and RO-DÂM, concentrations of P and N in plant tissues were determined in dried and ground (0.5 mm) samples. Total P was determined after wet digestion in $H_2SO_4$-$H_2O_2$ with ceruleomolybdic blue colorimetry (Murphy and Riley, 1962). Total N concentration was determined with a CN gas analyzer (LECO Corporation, St Joseph, Michigan, USA). When present in samples, legumes were sorted by hand and excluded before analysis, as recommended by Jouany et al. (2004).

The NNI, expressed as a percentage, was calculated as the sample N concentration ($N_{measured,}$ mg $g^{-1}$ DM ) divided by the critical N concentration ($N_{critical,}$ mg $g^{-1}$ DM) which was estimated from the critical N-dilution curve (Lemaire and Gastal, 1997), as follows:

$$NNI = N_{measured}/N_{critical} \times 100 \quad (1)$$

$$N_{critical} = 48 \text{ (shoot DM)}^{-0.32} \quad (2)$$

with $N_{measured}$ and $N_{critical}$ expressed in mg $g^{-1}$ DM and shoot DM in t $ha^{-1}$.

The PNI and KNI, expressed as a percentages, were calculated as the sample P ($P_{measured,}$ mg $g^{-1}$ DM) or K ($K_{measured,}$ mg $g^{-1}$ DM) concentration divided by the critical P ($P_{critical,}$ mg $g^{-1}$ DM) or K ($P_{critical,}$ mg $g^{-1}$ DM) concentration.

$$PNI = P_{measured}/P_{critical} \times 100; \quad (3)$$

$$KNI = K_{measured}/K_{critical} \times 100; \quad (4)$$

$P_{critical}$ and $K_{critical}$ were estimated from Duru and Ducrocq (1997), as follows:

$$P_{critical} = 1.50 + 0.065 \times N_{measured} \quad (5)$$

$$K_{critical} = 1.6 + 0.525 \times N_{measured} \quad (6)$$

with $P_{measured}$ and $P_{critical}$ expressed in mg $g^{-1}$ DM.

*2.3. Grassland vegetation characterization*

At the four permanent multi-species grassland sites (CH-LES, FR-ERC, FR-GRA, and RO-DÂM), species composition was measured at the beginning of the experiments using exhaustive sorting of handfuls of vegetation, as described by De Vries and De Boer (1959). At least four species contributed 80% of community biomass (Table 1). Grasslands species compositions did not significantly change between control and fertilized plots for the whole period tested.

*2.4. Soil physical and chemical properties*

Before the start of the experiment at each site, soil samples were taken air-dried, sieved (2 mm) and stored at ambient temperature before analysis (Table 1). Soils were analyzed using the Swiss national reference methods for CH-LES (FAL et al., 2004) and the method recommended in the province of Quebec for CA-LEV, as reported by Bélanger and Ziadi (2008). For FR-ERC, FR-GRA and RO-DÂM sites, the soil samples were analyzed by the *Laboratoire d'Analyses des Sols* of the National Research Institute for Agriculture, Food and Environment (INRAE, 62000 Arras, France) using French standards (Afnor, 1994), as reported by Stroia et al. (2007).

228 SPAP was determined from existing recommendations in each country. The Olsen
229 procedure (Olsen et al., 1954) was used at the FR-ERC, FR-GRA, and RO-DÂM sites. A
230 ammonium acetate-EDTA mixture was used at the CH-LES site (Demaria et al., 2005), and
231 Mehlich-3 extractable P was used at the CA-LEV site (Tran and Simard, 1993).
232

233 *2.5 Soil plant available P*

234 Soil samples were taken in each replicate plot of each treatment at one date (Table 2). The
235 soil samples were air-dried, sieved (2 mm), and stored at ambient temperature before analysis.
236 Three methods were used to estimate SPAP. Two of them were laboratory tests based on soil /
237 solution-extraction procedures. $C_P$, which corresponds to the pool of immediately available P,
238 was measured using 1g of soil in 10 mL of distilled water (Morel et al., 2000). This test was
239 performed for soils from all sites, except RO-DÂM (Table 2). The Olsen P (Olsen et al., 1954)
240 represents the pool of readily extractable P. It was measured using a mass of 1 g of soil in 20
241 mL of 0.5M sodium bicarbonate solution (pH=8.5). This test was performed for soils from all
242 sites, except CA-LEV and RO-DÂM (Table 2). Both tests are suitable for a wide range of soil
243 pH and widely used for soil testing and fertilizer recommendations (Jordan-Meille et al., 2012;
244 Ziadi et al., 2013; Zehetner et al., 2018; Johnston et al., 2019).

245 We also tested the functional- and process-based approach previously developed for soils
246 with annual crops (Morel et al., 2014; Messiga et al., 2015) and perennial forages (Stroia et al.,
247 2007; Messiga et al., 2012), and for rivers sediments (Némery et al., 2005). The model assumes
248 that (i) roots absorb oPion only from the soil solution and (ii) this absorption generates a
249 gradient of oPion concentration between the soil solid phase and solution that drives the oPion
250 diffusion (Barbier et al., 1971; Barber, 1984). This method provides an experimental data-set
251 within a few hours for parameterizing of the function that describes the total amount of oPion

that can diffuse (Pr, mg P kg-1 soil) over time (t, minutes) and the soil-solution oPion concentration (CP, mg P L-1 solution). This equation, the Freundlich kinetic equation, is as follows:

$$Pr = v \times C_P^{w} \times t^{p} \text{ with } Pr < PrLIMIT \quad (7)$$

where v is the value or Pr at time t = 1 min and $C_P$ = 1 mg P $L^{-1}$, w is the non-linear increase in $P_r$ as a function of $C_P$, and p is the non-linear increase in $P_r$ as a function of time (t). Parameters v, w and p are specific to soil at each site. The value of $P_{rLIMIT,}$ which cannot be determined experimentally, is estimated to be lower than the soil inorganic P content.

The related experiments were performed for soils from all sites except RO-DÂM (Table 2), by combining studies of sorption-desorption in soil suspensions in which isotopic dilution kinetics reached a steady-state for a few hours, as described by Stroia et al. (2007), Messiga et al. (2012), and Morel et al. (2020; this issue). In this approach, SPAP equaled $Q_w + P_r$. $Q_w$ was calculated by multiplying $C_P$ by the solution-to-soil ratio (solution volume /soil mass).
In order to better appreciate the amount of soil plant available P and compare the different sites-treatments we expressed both Olsen P and the amount of diffusible P ($Q_w + P_{r)}$ as kg P $ha^{-1}$ by using soil dry matter (Table 1).

*2.6. Data analysis*

All statistics were performed with Statgraphics Centurion XV (version 15.2.06, StatPoint, Inc., Herndon, Virginia, USA). Analyse of variance was performed for each treatment and site for PNI, $C_P$, (Qw + Pr60min) and Olsen P. Statistical significance ($P$<0.05) was tested using a Fisher's exact test.

The response to P fertilization was characterized by calculating the relative DM yield for each combination of site and year (Colwell, 1963), as follows:

Relative DM yield = (DM yield in control plots / maximum DM yield) × 100 (8)

The maximum DM yield was the yield of P-fertilized plots at sites with only one P rate (FR-ERC, FR-GRA, and RO-DAM) or the yield of plots that received the highest P rate at sites with several P rates (CA-LEV and CH-LES). To describe the relationship between the relative DM yield and PNI, a linear-plateau model was used, as follows:

Relative DM yield = a + b × PNI if PNI < critical PNI (9)

Relative DM yield = plateau of relative DM yield if PNI ≥ critical PNI (10)

where a and b are fitted parameters.

The linear-plateau model was calculated by minimizing the residual sum of squares between the observations and regression estimates (least square) using Microsoft® Excel's Generalized Reduced Gradient nonlinear solver.

## 3. Results

### *3.1. Forage yield response to the grassland P nutrition status*

DM yield response to P fertilization varied greatly among sites. P fertilization increased DM yield significantly in 2 out of 4 years at CH-LES, 1 out of 3 years at RO-DÂM, and 3 out of 9 years at FR-ERC, but had no effect at CH-LEV or FR-GRA. On control plots (P0), the mean PNI among years was 87% at CA-LEV and 94% at FR-GRA; these values were representative of adequate P nutrition for growth. Consequently, mean relative DM yields among years were high, with values of 98% at CA-LEV and 96% at FR-GRA, and remained constant even when the PNI increased due to P fertilization (Fig. 1a). For the three sites with a positive response to P fertilization, however, the PNIs on control plots were less than 80%, with mean values among years of 61% at CH-LES, 66% at FR-ERC, and 48% at RO-DÂM. These values indicated that grassland growth was P-limited (relative DM yields of 77%, 80%, and 57%, respectively) (Fig. 1a). The linear plateau model satisfactorily described the relationship between relative DM yield and PNI for the five sites (Fig. 1a). The critical PNI value, corresponding to the inflection

300 point of the linear-plateau curve, was 92%. Below this threshold, the increase in DM yield was
301 proportional to that in PNI (Fig. 1). We observed a very significant positive association
302 between the average values of PNI and RDMY for each combination site-treatment (p=9.88e-
303 05, $R^2$=0.88), despites a moderate sample size (nine site-treatment combinations).

304 Nevertheless, this association is not observed within each site-treatmenent (Fig. 1b); indeed, the
305 sign of the slope of the regression slope of PNI on relative DMY is negative in five out of nine
306 site-treatment combinations; moreover, if one limits themself to significant associations
307 ($p<0.05$), regression slope is negative for two out of three site-treatment combinations, and
308 positive for one out of three.
309

310 *3.2 Soil plant available P*

311 The $C_P$ values varied among the fertilization treatments, with the control treatment (P0)
312 having significantly lower $C_P$ than the P-fertilized treatments at each site (Table 4). At sites
313 where several P fertilizer rates were applied (CA-LEV and CH-LES), $C_P$ was highest at the
314 highest P rate.

315 For control treatments, mean $C_P$ varied from 0.042 mg $L^{-1}$ at FR-ERC to 0.38 mg $L^{-1}$ at FR-
316 GRA and between 0.18 mg $L^{-1}$, at FR-ERC, and 3.55 mg $L^{-1}$ at FR-GRA for fertilized
317 treatments (Table 4).

318 As for Cp, mean Olsen-P always differed significantly between control and fertilized plots;
319 at CH-LES, site Olsen-P was highest for the highest fertilizer rate. For control treatments, mean
320 Olsen-P varied from 10.3 mg $kg^{-1}$ at FR-ERC to 14.5 mg $kg^{-1}$ at FR-GRA and, from 10.3 mg
321 $kg^{-1}$ for FR-ERC to 71.6 mg $kg^{-1}$ at FR-GRA for fertilized treatments (Table 4).

322 Data obtained from sorption-desorption and isotope dilution procedures showed that, for
323 each site, the total amount of diffusive oPion in soils (Pr) increased as $C_P$ and time increased
324 (Fig. 3); the magnitude of the response differed among sites. For similar $C_P$ ranges of less than
325 1 mg P $L^{-1}$, Pr measured in the FR-ERC soil was twice that measured in the CA-LEV soil,
326 regardless of the time (t) (Fig. 3). The entire dataset served to estimate the parameters of Eq. (7)
327 (Table 3). For each site, except RO-DÂM, the entire $C_P$ dataset (Table 2; Table 4) and the
328 parameter estimates of the Freundlich kinetic equation (Table 3) were used to calculate (Qw +
329 Pr) values for increasingly long periods of diffusion. For each value of t, a general relationship
330 was built between (Qw + Pr) and PNI (data not shown); the best fit was obtained for a diffusion
331 time t of 60 minutes (Pr60min) (Fig. 2c).
332 Like $C_P$, the mean amount of diffusive P (Qw + Pr60min) varied at each site with treatments
333 (Table 4). When several P fertilizer rates were applied (CH-LES and CA-LEV), the maximum
334 (Qw + Pr60min) was measured for the highest P fertilizer rate. For each site, differences
335 between control and fertilized plots were always significant.
336 For control treatments, mean (Qw + Pr60min) varied from 18.8 mg $kg^{-1}$ at CH-LES to 22.3
337 mg $kg^{-1}$ at CA-LEV and, from 21.5 mg $kg^{-1}$ for CH-LES (P1) to 84.3 mg $kg^{-1}$ at FR-GRA for
338 fertilized treatments (Table 4).
339

340 *3.3. Relationship between P nutrition index and soil plant available P*
341 As for SPAP, the over years cumulative effects of P fertilization regimes induced a wide
342 range of PNI at each site (Table 4; Fig. 2). Each SPAP tested generally displayed a positive and
343 significant ($P<0.05$) relationship with PNI. Thus, any increase in soil P status improved
344 grassland P nutrition status (Fig. 2 a, b and c). Compared to considering only $C_P$ (Fig. 2a),
345 considering the pool of diffusive P (Fig. 2b) as well improved the reliability of the PNI
346 response curve in the 0-5 cm soil horizon: $R^2 = 0.65$ ($P< 0.05$) for $C_P$ and $R^2 =0.70$ ($P< 0.05$)

347 for Qw + Pr60min. We obtained a similar response pattern for the relationship between the
348 Olsen P stock in the 0-5 cm soil horizon (kg $ha^{-1}$) and PNI with $R^2$=0.85 (P<0.05) (Fig. 2b).
349 The threshold of 92% for PNI (Fig. 1b) served to calculate the critical SPAP values which
350 value were 13.5 kg P $ha^{-1}$ and 12.9 kg $ha^{-1}$ for, respectively (Qw + Pr60min) and Olsen P.
351

352 **4. Discussion**

353 *4.1. Relationship between relative dry matter yield and P nutrition Index*

354 A general relationship between relative DM yield and PNI was obtained by combining data
355 sets from five long-term experiments at contrasting sites with different response patterns to P
356 fertilization (Fig. 1 a). For CA-LEV and FR-GRA, no significant yield response to P
357 fertilization was observed throughout the experiments. At both sites, mean PNI values greater
358 than 80%, for control treatment plots, indicated that P did not limit growth (Duru and Ducrocq,
359 1997); consequently, mean relative DM yields remained close to 100% and did not vary. At
360 both sites, soil reserves provided enough P to meet grassland requirements and to maintain
361 optimal sward P nutrition for nine years at CA-LEV and seventeen years at FR-GRA. At CH-
362 LES, FR-ERC, and RO-DÂM, PNI values less than 80%, for control treatment plots, indicated
363 that growth was P-limited (Duru and Ducrocq, 1997). Soil reserves did not provide enough P to
364 meet grassland requirements for optimal growth and, consequently, a significant yield response
365 to P addition was observed. At CH-LES site, with four P rates tested, we observed a similar
366 response pattern to P; PNI increased in proportion with fertilizer rate between 0 and 26 kg P $ha^{-1}$,
367 and, consequently, relative DMY. At that site, relative DM response cannot be exclusively
368 ascribed to P nutrition since fertilization rates differed in both P and K amounts. We cannot
369 exclude the hypothesis that relative DMY responded to K as well; however, K impact should be
370 limited as reported by Duru (1992).

371 At the start of the experiments, the initial SPAP value exceeded the critical value of 25 mg
372 Olsen P $kg^{-1}$ at FR-GRA (Table 1) identified by Poulton et al. (1997). The experimental site of
373 CA-LEV was chosen because of its expected positive response to P fertilization (Bélanger and
374 Ziadi, 2008). Its soil (Mehlich-3 extraction; 0-15 cm) contained 54 kg available P $ha^{-1}$ (Table
375 1) and was considered poor in P according to local recommendations (Conseil des Productions
376 Végétales du Québec, 1996). During the experiment at FR-GRA, SPAP measured in the P0
377 treatment remained above the critical value despite large negative annual P budget (-35 kg $ha^{-1}$;
378 Stroia, 2007). At CA-LEV, SPAP measured in the P0 and P15 treatments first decreased and
379 then remained relatively stable over time, despite negative annual P budgets (-5 and -20 kg $ha^{-1}$,
380 respectively) (Messiga et al., 2015). At FR-ERC and CH-LES, SPAP values laid below 25 mg
381 Olsen P $kg^{-1}$ at the start of the experiments, and they decreased over time as cumulative P
382 budgets became more negative (Messiga et al., 2015).
383 For similar mean annual P outputs and budgets (Messiga et al., 2015), soils were less
384 resilient at FR-ERC than at CA-LEV and FRA-GRA, where productivity was not affected after
385 not applying P for nine and seventeen years, respectively. These results confirmed that the
386 long-term impact of stopping P fertilization on grassland productivity varied greatly for
387 European upland grasslands (Marriott et al., 2004) and that legacy P can contribute greatly to
388 grassland nutrition (Sattari et al., 2012).
389 The significant relationship between relative DM yield and PNI demonstrated that PNI
390 captures differences in DM yield between P fertilized and non-fertilized plots and that, under P
391 limitation, the decrease in DM yield is directly proportional to the decrease in the grassland P
392 nutrient status measured with PNI. The overall critical PNI value, corresponding to the
393 inflection point of the linear-plateau curve, was 92%. This value was close to PNI = 100%
394 which theoretically represents the boundary between P-limited and non-P-limited growth
395 conditions according to Thélier-Huché et al. (1999). The difference between this threshold

396 (92%) and the statistical value (100%) given by Duru and Thélier-Huché (1997) and Thélier-
397 Huché et al. (1999) is likely due to the smallest dataset that they used to determine the critical
398 P-dilution curve or to establish the relationship between relative yield and PNI.
399 This study provides the first evidence that the relative DM yield of a P-limited sward, , is a
400 direct function of its P status assessed with PNI, within different site-treatments. These results
401 validate the PNI approach as an adequate tool for *a posteriori* assessment of grassland P
402 nutrition. They extend conclusions of studies conducted on grasslands at local scales (Duru et
403 al., 1993; Duru and Ducrocq, 1997; Duru and Thélier-Huché, 1997; Liebisch et al., 2013).
404 Under P-limited nutrition status, relative DM yield was also shown to be a direct function of
405 the PNI in canola (*Brassica napus* L.) (Cadot et al., 2018) and maize (*Zea Mays* L.) (Cadot et
406 al., 2018; Gagnon et al., 2020).
407 While we observed a high positive association between average PNI and average relative
408 DM measured within the nine site-treatment clusters (Fig. 1a), a similar relation was not
409 observed for each site-treatment; relationships were positive for some site-treatments and
410 negative for others (Fig. 1b). We considered that there were variables, other than PNI, that
411 came into account to explain inter annual relative DM variability for a given site-treatment.
412 Since interactions between N and P nutrition control forage response to fertilization (Duru and
413 Thélier 1997), we tested to what extend NNI and KNI, for CH-LES site, could explain relative
414 DM yield variability between years.
415 For each CA-LEV and FR-GRA treatments, there was no significant relationship between
416 NNI and PNI, between relative DM and NNI; including NNI as a co variable did not improve
417 none of the model obtained with PNI (Fig. 1b). No significant relationship was obtained either
418 when testing the relationships for the three CA-LEV data sets pooled together.
419 At site level, these results confirmed that for both sites and treatment optimal N and P
420 nutrition allowed optimal biomass production. On the other hand, negative relationships

421 between relative DMY and PNI at CA-LEV P0 and CA-LEV P1 (Fig. 1b) could result from P
422 luxury consumption and accumulation which increased as relative DM decreased.
423 For CH-LES, there was no significant relationship between NNI and PNI, relative DMY
424 and NNI and between relative DMY and KNI for any of the treatments. Including NNI or KNI
425 as co variable did not improve the model between relative DM and PNI for CH-LES P0 (Fig.
426 1b). Finally, when pooling the three CH-LES data sets, we got a significant relationship
427 between relative DMY and NNI ($R^2$=0.44; p=0.018); best fit was obtained with PNI as a single
428 variable ($R^2$=0.65; p=0.0016). This result demonstrated that, under P and N limited conditions,
429 inter annual relative DM variability was better explained by PNI than NNI.
430 For FR-ERC, there was a significant relation between NNI and PNI ($R^2$=0.49; p=0.0353)
431 which confirmed that, under P limitation, improving P nutrition has a positive and significant
432 effect on herbage nutrient status as reported by Duru and Ducrocq (1997). Introduction of NNI
433 as a co variable with PNI (Fig. 1b) improved the relationship ($R^2$=0.76; p=0.0135). Finally, best
434 fit of the data was obtained with a simple linear model with NNI ($R^2$=0.75; p=0.0024. Under P
435 limited and non N limited conditions, NNI explained better variations in relative DM than PNI;
436 grassland response to P limitation varies according to NNI. For a given PNI, relative DM is
437 higher, the higher NNI.
438 The results showed that P effect on relative DM was different according to the grassland
439 nutritional status. At CH-LES site, with both N and P limitation, increasing PNI improved the
440 sward efficiency for N conversion in biomass. P supply has a direct impact on forage growth.
441 On the opposite, at FR-ERC site, where P was limiting growth but not N, increasing PNI
442 improved sward N nutrition status and consequently relative DM.
443 These results confirmed at multi annual scale that interaction between N and P controls forage
444 response to P fertilization (Duru and Ducrocq, 1997; Jouany et al (2011).
445

446 *4.2. Relationship between P nutrition index and soil plant available P*

447 Positive relationships between PNI and each SPAP indicator (Fig. 2) indicates that the plant
448 nutritional status of grassland swards increases with increasing soil P status. This result extends
449 the results of studies conducted at a regional scale by Duru (1992) to a wider range of soil and
450 climate conditions. The response curve between PNI and each SPAP indicator could be used to
451 determine the critical SPAP value required to obtain a PNI value of 92% (*i.e.* the PNI needed to
452 reach maximum relative DM yield) (Fig. 1a). When considering all sites except RO-DÂM
453 together, the critical $C_P$ value was 0.26 mg P $L^{-1}$ (Fig. 2a), but it varied among sites, from a
454 minimum of 0.12 mg P $L^{-1}$ at FR-ERC to a maximum of 0.50 mg P $L^{-1}$ at FR-GRA. This
455 variability was due to large differences among sites in the ability of the soil solid phase to
456 supply the soil solution with oPion, which is controlled by soil physicochemical characteristics
457 (Table 1). The FR-ERC soil, with a clay-loam texture and high P-buffer capacity due to high
458 contents of Fe and Al oxide, and hydroxide (Stroia et al., 2007), had the lowest critical $C_P$
459 value. In contrast, the sandy soil at FR-GRA had a low P-buffer capacity and thus the highest
460 critical $C_P$ value (Stroia et al., 2007). Morel et al. (2021) reported similar soil-specific
461 responses of maize to P, measuring the lowest critical $C_P$ in Fe and Al oxide-rich soil and the
462 highest critical $C_P$ in sandy soil.

463 The stronger relationship observed between PNI and (Qw + Pr60min) compared to that with
464 $C_P$ alone (Fig. 2c) demonstrated that assessing SPAP as the amount of soil diffusive oPion,
465 captured the specific effects of soil type on P-ion mobility better than $C_P$ did. These results
466 generalized results obtained for annual crops by Morel et al. (2000; 2021), to grassland soils.
467 However, our study differed on two points. First, the time of diffusion (t) that minimized
468 variance in the relationship between PNI and (Qw + Pr) (Fig. 2c) was 60 minutes (Pr60min),
469 much shorter than 1360 minutes for annual crops (Morel et al., 2021). The critical (Qw +
470 Pr60min) value for grasslands was 13.5 kg P $ha^{-1}$ (Fig. 2c), twice as large as that for maize (7.9

kg P $ha^{-1}$; Morel et al. (2021)). These differences may be due to the soil depth considered when measuring the (Qw + Pr60min) stock: 0-5 cm for grasslands *vs*. the plowed layer (0-25 cm) for maize.

The assumption that maize obtains P from the plowed layer is reasonable since this layer contains 80% of maize root biomass (Li et al., 2017) and the soil horizons below it contribute little to crop P nutrition. This assumption is more questionable for permanent grasslands, in which soil horizon below 5 cm may contribute greatly to sward P nutrition. This is more likely to occur in non-fertilized grasslands with a soil with a low P status (Fort et al., 2016).

SPAP tests could be compared only for three sites where the three indicators were measured (CH-LES, FR-ERC, and FR-GRA; Table 2). Olsen P explained more of the variation in PNI ($R^2$ = 0.85; P <0.05; Fig. 2b) than the (Qw + Pr60min) stock ($R^2$ = 0.70; P<0 .05; Fig. 4a) or $C_P$ ($R^2$ = 0.66; P < 0.05; Fig. 4b) did. The $NaHCO_3$ extraction with the Olsen procedure represented soil-type-specific effects on oPion mobility better than $C_P$ (Fig. 2a) or (Qw + Pr60min) (Fig. 2c) did. These results for grasslands, however, differ from those for maize of Morel et al. (2000), who reported that (Qw + Pr) stock explained the yield response better than Olsen P for three contrasting soil types.

We hypothesize that the better fit observed for Olsen P is due to its ability to extract organic P, which is partly mineralized when measuring phosphate ions. This is particularly likely to occur when the test is performed for grassland soils rich in organic matter (Bowman and Cole, 1978). Tate et al. (1991) demonstrated that soil organic P pool in grassland soils can contribute greatly to grassland P nutrition. In contrast, the (Qw + Pr) stock includes only the inorganic P pool.

Expressed on a stock basis, the Olsen P critical value was 12.9 kg P $ha^{-1}$ (Fig. 2b) which was close to the (Qw + Pr60min) critical value of 13.5 kg P $ha^{-1}$ (Fig. 2c).

The comparison of response curves showed that, for both Olsen P and (Qw + Pr), correlations were stronger when the PNI was related to SPAP measured in the 0-5 cm soil horizon instead of the 0-10 cm horizon (data not shown). This result confirms that surface soil horizons, where high nutrient concentrations (Kidd et al., 2017) foster intensive exploitation and acquisition of resources by roots (Fort et al., 2016), contribute greatly to grassland P nutrition.

### *4.3. Strengths and limits of nutrient index approach for Phosphorus diagnosis in agro ecosystems*

Our results confirmed that PNI was a reliable indicator of the level of satisfaction of plant's P needs from soil. Over large soil P gradients, a significant relationship existed between grassland P nutrition status, evaluated with PNI, and SPAP, assessed according to conventional soil tests. Situations exist in agro systems where nutrition index approach could be used in association with soil analysis, or be an alternative to, when soil analysis is difficult to perform. Our study demonstrated that PNI presents some assets for improving P status diagnosis for permanent grasslands where soil analysis is difficult to perform. Taking a representative soil sample is tough because of nutrients stratification and non-uniform vertical repartition of nutrients (; this issue specially applies to P that is not mobile in soils (Messiga et al, 2013). Moreover, little soil references are available for permanent grasslands, compare to cropping systems, and thresholds responses based on soil analysis are rarely available. Likewise, nutrition indices could be implemented for P diagnosis in cropping system where non-uniform nutrients repartition makes soil sampling more tedious (no tillage, Conservation agriculture, precision farming) and where available soil thresholds and references are unsuitable..

In other situations, PNI allows specifying, or even correct, soil diagnosis as it is the case for CA-LEV site in this study. Although initial soil P status was diagnosed as limiting for growth, based on soil P test, our study demonstrated that forage P nutrition status was adequate on

control treatment plots and its conclusion questioned soil based diagnosis, or at least the method used.

On an other hand, this approach presents some limits since the 'critical' P concentrations (Eq. 4) that serves for PNI calculations (Eq. 3) were more an approximation of critical P concentration than critical concentration *sensu stricto* as defined in Justes et al. (1994). As a consequence, P critical value (Eq. 4) was higher and PNI lower than expected. This bias results in underestimation of forage P status diagnosis and, as a consequence, a risk of excess P supply in subsequent fertilization recommendations.

In order to improve the reliability of the nutrient index approach for P diagnosis, a more precise critical curve is necessary. In that purpose, the interaction between N and P must be investigated on P deficient grasslands, by combining different P and N doses in order to identify precisely the true critical P concentration.

## 5. Conclusions

This study of five long-term grassland experiments conducted on contrasting soil types and climates demonstrated that a direct and general relationship exists between PNI and the forage yield of the first growth cycle. Forage yield increased linearly as sward P nutrition status, assessed by the PNI, increased up to nearly 100%. This critical threshold differentiated P-limited growth from that with adequate P nutrition. Significant positive relationships between PNI and three SPAP indicators confirmed that the soil P status influences sward P nutrition status. The Olsen P extraction procedure provided the best fit with PNI, with a critical value of 12.9 kg $ha^{-1}$.

The study confirmed the potential of using plant analysis and nutrient indices for P diagnosis in forage systems and making fertilizer P recommendations. Our study highlights the utility of long-term fertilization experiments in which highly P-depleted soils (i.e. control plots with no P

545 applied) offer the opportunity to test grassland responses to P fertilization and set more reliable
546 and precise critical curves.

## Author contribution section

**Claire Jouany**: Conceptualization, Investigation, Formal analysis, Writing original draft, Writing - review and editing. **Christian Morel**: Conceptualization, Investigation, Formal analysis, Writing - review and editing. **Noura Ziadi**: Conceptualization, Investigation, Writing - review and editing. **Gilles Bélanger**: Conceptualization, Investigation, Writing - review and editing. **Sokrat Sinaj**: Investigation, Writing - review and editing. **Ciprian Stroia**: Investigation, Writing - review and editing. **Pablo Cruz**: Investigation, Writing - Review and Editing. **Jean-Pierre Theau**: Investigation, Writing - review and editing. **Michel Duru**: Writing - review and editing.

## Declaration of competing interest

The authors declare no conflict of interest of any kind that could have influenced the work reported in this paper.

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798 Table 1.
799 Climate, soil, and management characteristics of the five experimental sites; NA = no data available
800

| Site | CA-LEV | CH-LES | FR-ERC | FR-GRA | RO-DÂM |
|---|---|---|---|---|---|
| Country | Canada | Switzerland | France | France | Romania |
| Community | Lévis | Les Verrières | Ercé | Gramond | Dâmbovicioara |
| Location | 46°47' N 71°07' W | 46°54'N 6°29' E | 42°50' N 1°17' E | 44°16’ N 2° 22’ E | 45°24'N 25°14'E |
| Climate type (Köppen-Geiger) | Continental humid | Continental humid | Temperate humid | Temperate humid | Continental humid |
| Elevation (m) | 65 | 1100 | 660 | 607 | 1204 |
| Mean annual temperature (°C) | 4.0 | 5.8 | 12.7 | 11.0 | 4.3 |
| Mean annual rainfall (mm) | 692 | 1400 | 1079 | 960 | 895 |
| Soil horizon (cm) | 0-15 | 0-5 | 0-5 | 0-5 | 0-5 |
| Soil type (US taxonomy) | Fragihumod | Cambisol | Alfisol | Inceptisol | Rendollic eutrocryepts |
| Soil bedrock | Limestone | NA | Alluvium | Micashist | Limestone |
| $pH_{water}$ | 5.8 | 5.5 | 5.9 | 5.5 | 6.2 |
| Clay (g $kg^{-1}$) | NA | 290 | 251 | 214 | 663 |
| Loam (g $kg^{-1}$) | NA | 470 | 509 | 220 | 309 |
| Sand (g $kg^{-1}$) | NA | 240 | 250 | 566 | 28 |
| Total soil C (g $kg^{-1}$) | 26.0 | 40.6 | 55.2 | 36.8 | 195.5 |
| C: N ratio | NA | NA | 10.0 | 11.2 | 17.6 |
| Cation Exchange Capacity (cmol+$kg^{-1}$) | NA | NA | 18.6 | 9.7 | 38.8 |
| Exchangeable Ca (cmol+$kg^{-1}$) | NA | NA | 15.7 | 8.3 | 35.8 |
| Exchangeable Mg (cmol+$kg^{-1}$) | NA | NA | 1.6 | 1.0 | 1.9 |
| Exchangeable K (cmol+$kg^{-1}$) | NA | NA | 0.2 | 0.2 | 0.4 |
| Exchangeable Na (cmol+$kg^{-1}$) | NA | NA | 0.1 | 0.1 | 0.1 |

| | | | | | |
|---|---|---|---|---|---|
| Total soil P (g $kg^{-1}$) | NA | NA | 1.92 | 1.03 | 1.75 |
| Initial soil plant available P (mg $kg^{-1}$) | 24 | 16 | 6 | 44 | 14 |
| Extracting solution | Mehlich-3 | AA-EDTA | $NaHCO_3$, pH 8.5 | $NaHCO_3$, pH 8.5 | $NaHCO_3$, pH 8.5 |
| Reference method | Tran and Simard (1993) | Demaria et al. (2005) | Olsen et al. (1954) | Olsen et al. (1954) | Olsen et al. (1954) |
| Bulk density | 1.15 | 0.88 | 0.93 | 1.05 | NA |
| Soil (0-5 cm) dry matter (t $ha^{-1}$) | 450 | 438 | 465 | 525 | NA |
| Number of cuts per year | 2 | 2 | 4 | 4 | 2 |
| Mean optimum annual yield (t DM $ha^{-1}$) | 6.8 | 5.7 | 13.8 | 10.5 | 4.7 |
| Dominant species | *Phleum pratense* | *Agrostis capillaris* | *Lolium perene* | *Holcus lanatus* | *Festuca rubra* |
| | | *Festuca rubra* | *Cherophylum aureum* | *Anthoxanthum odoratum* | *Arrenatherum Elatius* |
| | | *Dactylis glomerata* | *Dactylis glomerata* | *Agrostis capillaris* | *Vicia cracca* |
| | | *Trifolium repens* | *Holcus lanatus* | *Rumex acetosa* | *Trifolium repens* |

Table 2.

Plant and soil sampling and analyses agenda at the five experimental sites. Soils were sampled from 0-5 cm, except to parameterize the Freundlich kinetic model for CA-LEV (0-15 cm); NA = no data available.

| | | Site and experiment period | | | | |
|---|---|---|---|---|---|---|
| | | CA-LEV | CH-LES | FR-ERC | FR-GRA | RO-DÂM |
| Analysis | Detail | 1999-2007 | 1991-2008 | 1999-2007 | 1998-2014 | 1964-2005 |
| Plants | Sampling year(s) | Every year | 1997, 2001, 2005, 2008 | Every year | Every year | 2002, 2003, 2004 |
| Soil $C_P$ | Sampling year(s) | 2006 | 2008 | 2007 | 2014 | NA |
| | Soil horizon (cm) | 0-5 | 0-5 | 0-5 | 0-5 | NA |
| Soil Olsen P | Sampling year | NA | 2008 | 2007 | 2014 | NA |
| | Soil horizon (cm) | NA | 0-5 | 0-5 | 0-5 | NA |
| Soil Freundlich kinetic | Sampling year | 2007 | 2008 | 2007 | 2007 | NA |
| model parameterization | Soil horizon (cm) | 0-15 | 0-5 | 0-5 | 0-5 | NA |

Table 3.

Mean (and standard error) estimates of parameters v, w and p of the Freundlich kinetic equation ($P_r = v \times C_P^{w} \times t^{p}$).

| Site | Soil sampling year | Soil horizon (cm) | v [a] | w [b] | p [c] |
|---|---|---|---|---|---|
| CA-LEV | 2006 | 0-15 | 6.95 (0.95) | 0.38 (0.05) | 0.371 (0.024) |
| CH-LES | 2008 | 0-5 | 6.54 (0.59) | 0.48 (0.05) | 0.428 (0.013) |
| FR-ERC | 2007 | 0-5 | 14.19 (0.46) | 0.44 (0.01) | 0.419 (0.006) |
| FR-GRA | 2007 | 0-5 | 9.62 (0.39) | 0.36 (0.02) | 0.273 (0.006) |

[a] v: total amount of the diffusive soil P after 1 minute when $C_P$ is 1 mg P $L^{-1}$.

[b] w: the increase in Pr as a function of $C_P$.

[c] p: the increase in Pr as a function of t.

Table 4.

Mean Phosphorus nutrition Index (PNI), soil solution oPions concentration (Cp), Olsen extracted Phosphorus (Olsen-P) and diffusive P (Qw + Pr60min) ; NA = no data available.

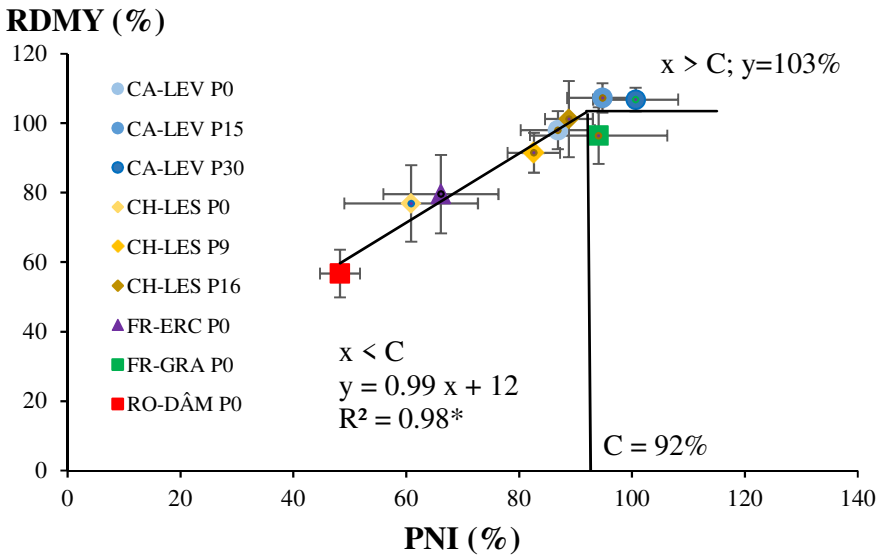
| Site | Treatment | PNI[1] (%) | | Cp mg P $l^{-1}$ | | Olsen-P mg P $kg^{-1}$ soil | | (Qw + Pr60min) mg P $kg^{-1}$ soil | |
|---|---|---|---|---|---|---|---|---|---|
| CA-LEV | P0 | 83 | a[2] | 0.3 | a | NA | NA | 22.3 | a |
| | P1 | 95 | b | 0.59 | ab | NA | NA | 31.5 | ab |
| | P2 | 101 | bc | 1 | bc | NA | NA | 41.3 | bc |
| | P3 | 106 | c | 1.37 | c | NA | NA | 49.2 | c |
| CH-LES | P0 | 51 | a | 0.18 | a | 12.5 | a | 18.8 | a |
| | P1 | 86 | b | 0.24 | ab | 16.7 | a | 21.5 | ab |
| | P2 | 90 | bc | 0.32 | b | 23 | b | 24.1 | b |
| | P3 | 99 | c | 0.34 | b | 32.3 | c | 25 | b |
| FR-ERC | P0 | 59 | a | 0.042 | a | 10.3 | a | 19.8 | a |
| | P1 | 102 | b | 0.18 | b | 45.3 | b | 39.4 | b |
| FR-GRA | P0 | 89 | a | 0.38 | a | 14.5 | a | 22 | a |
| | P1 | 127 | b | 3.55 | b | 71.6 | b | 84.3 | b |

[1] Measured on the soil sampling date (Table 2)

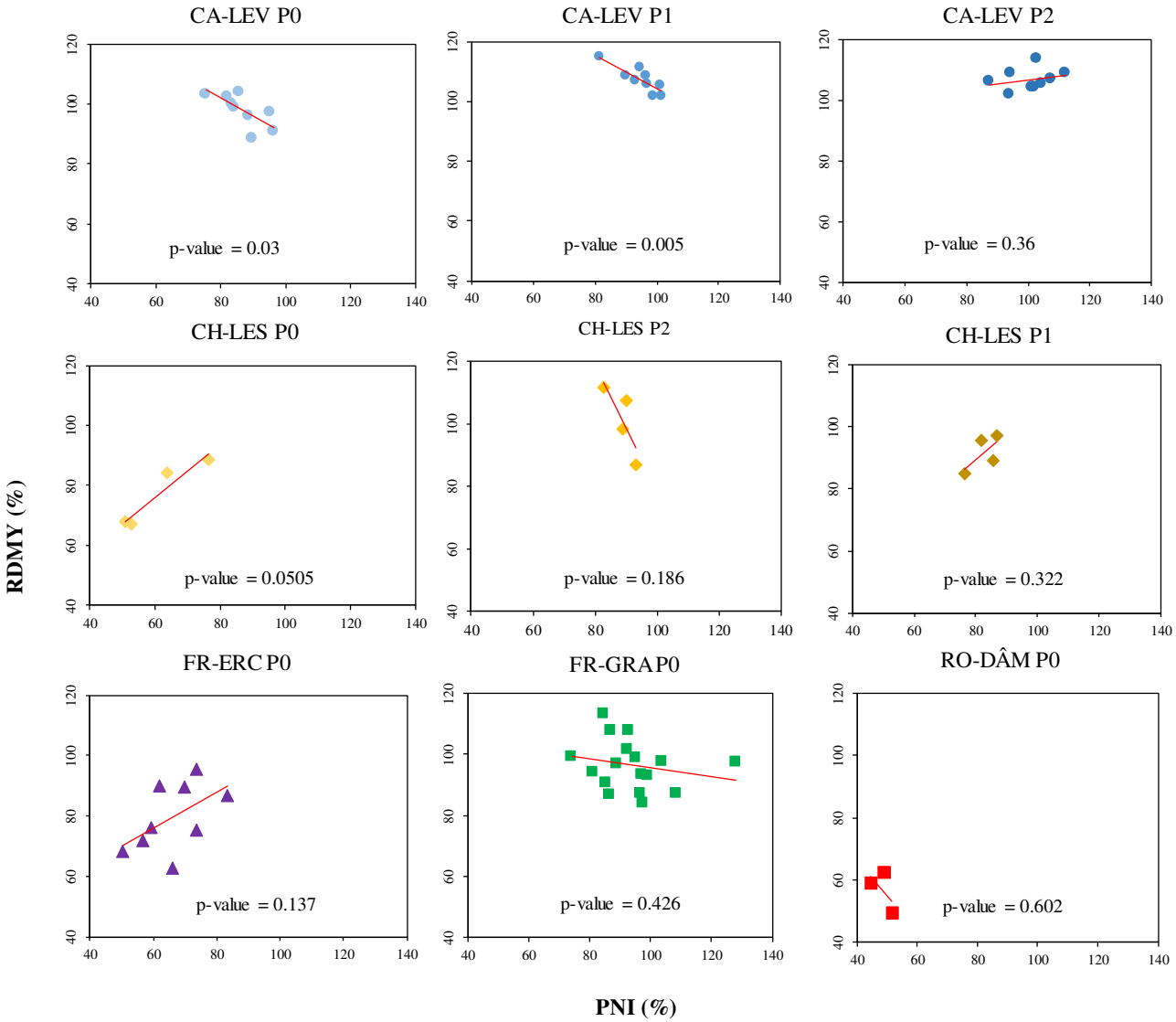
[2] Different letters in a given column for a given site indicate significant differences *(P<0.05)*

Fig. 1. Relationship between relative dry matter yield (RDMY) and Phosphorus nutrition index (PNI) a) the average RDMY as a function of the average PNI, with averages computed over all years for each site-treatment combination; C represents the critical PNI (%) b) RDMY measurements as a function of PNI measurements, for each site-treatment combination. The line corresponds to the linear regression of PNI on RDMY, and *p* is the Fisher p-value.

a



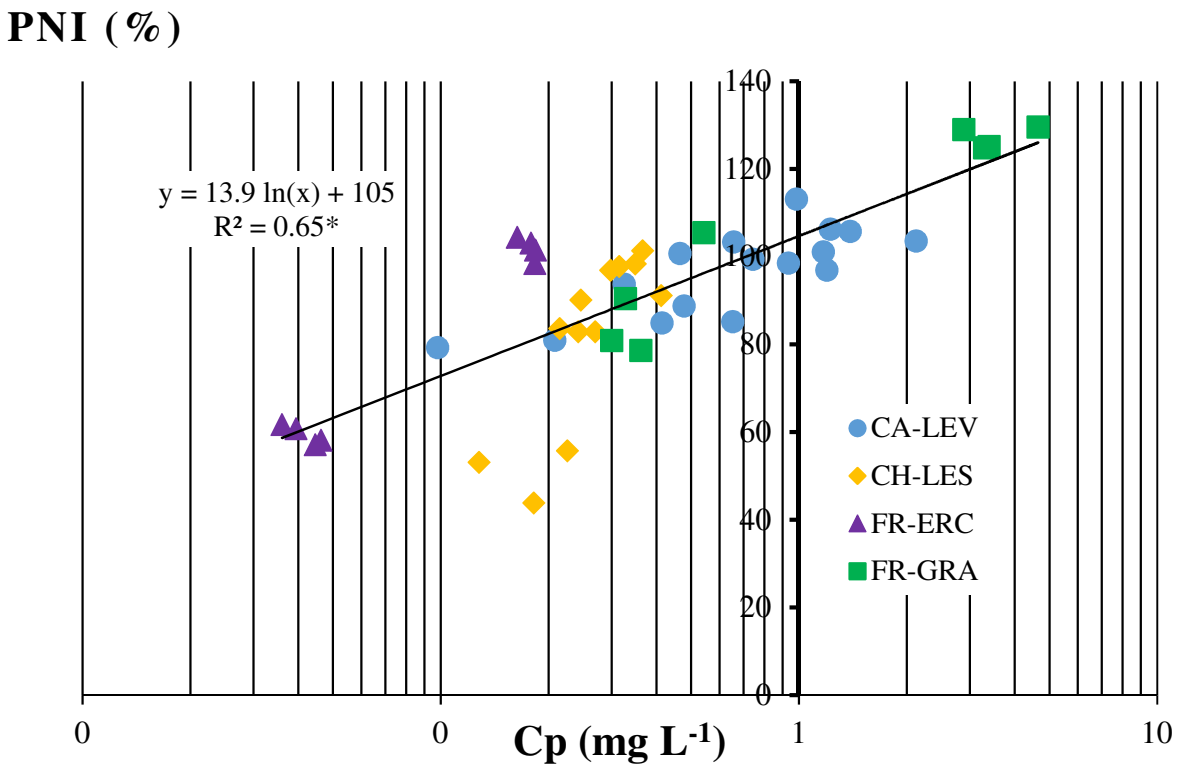
829 b


CA-LEV P0
p-value = 0.03
CA-LEV P1
p-value = 0.005
CA-LEV P2
p-value = 0.36
CH-LES P0
p-value = 0.0505
CH-LES P2
p-value = 0.186
CH-LES P1
p-value = 0.322
FR-ERC P0
p-value = 0.137
FR-GRA P0
p-value = 0.426
RO-DÂM P0
p-value = 0.602
RDMY (%)
PNI (%)

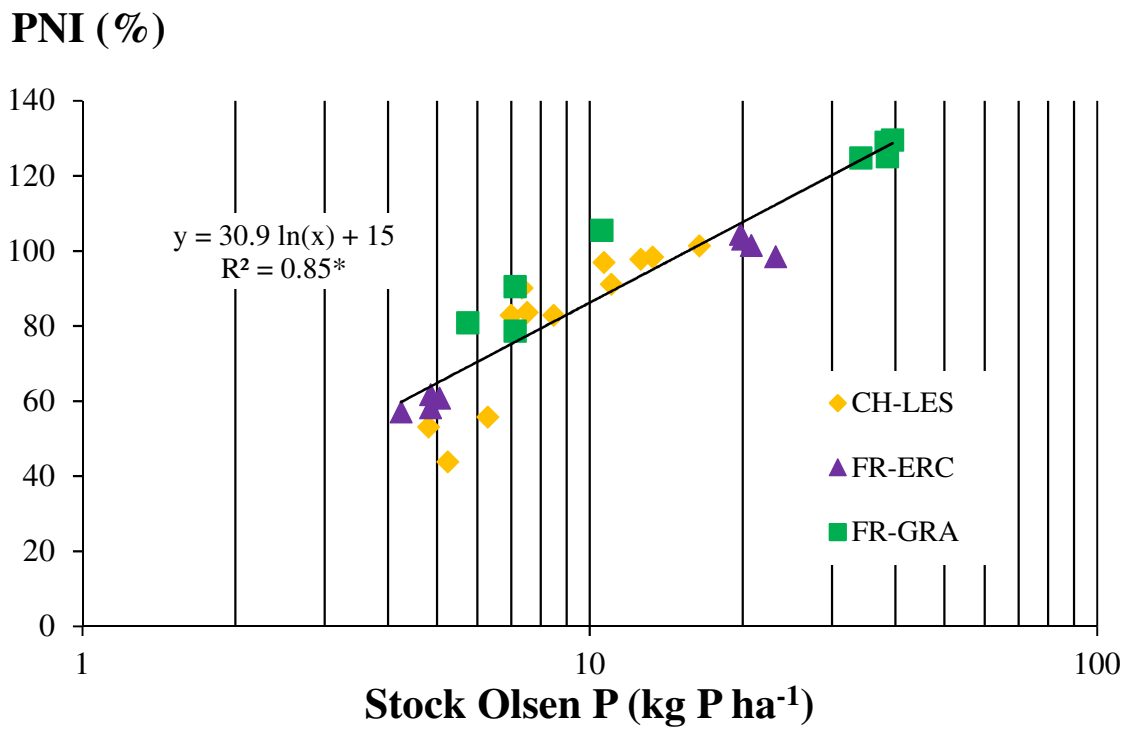
830

Fig. 2. Relationship between the phosphorus (P) nutrition index (PNI), measured at the plot scale, and soil plant-available P (SPAP); a) SPAP= $C_P$ at the CA-LEV, CH-LES, FR-ERC, and FR-GRA sites; b) SPAP=Olsen P at the CH-LES, FR-ERC, and FR-GRA sites; and c) SPAP=$Q_W$ + Pr60min at the CA-LEV, CH-LES, FR-ERC, and FR-GRA sites. The x-axis is on a log scale to visualize the experimental points better.

a



839 b
840


PNI (%)
140
120
100
80
60
40
20
0
y = 30.9 ln(x) + 15
R² = 0.85*
CH-LES
FR-ERC
FR-GRA
1
10
100
Stock Olsen P (kg P ha-1)

841

c

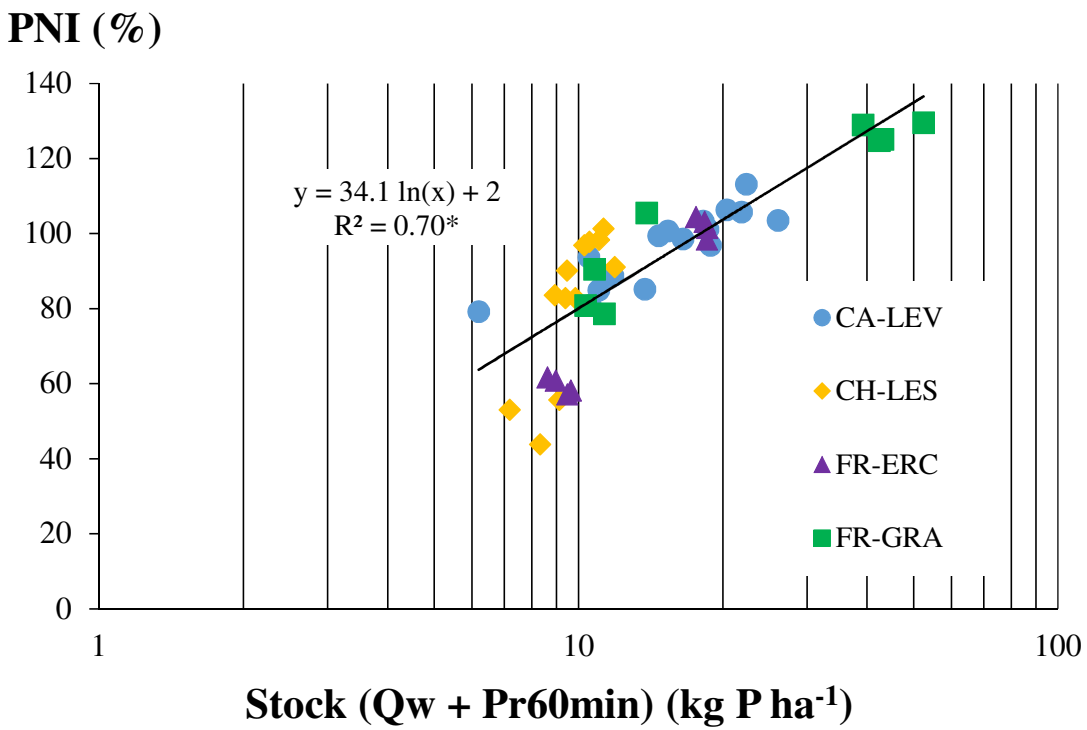
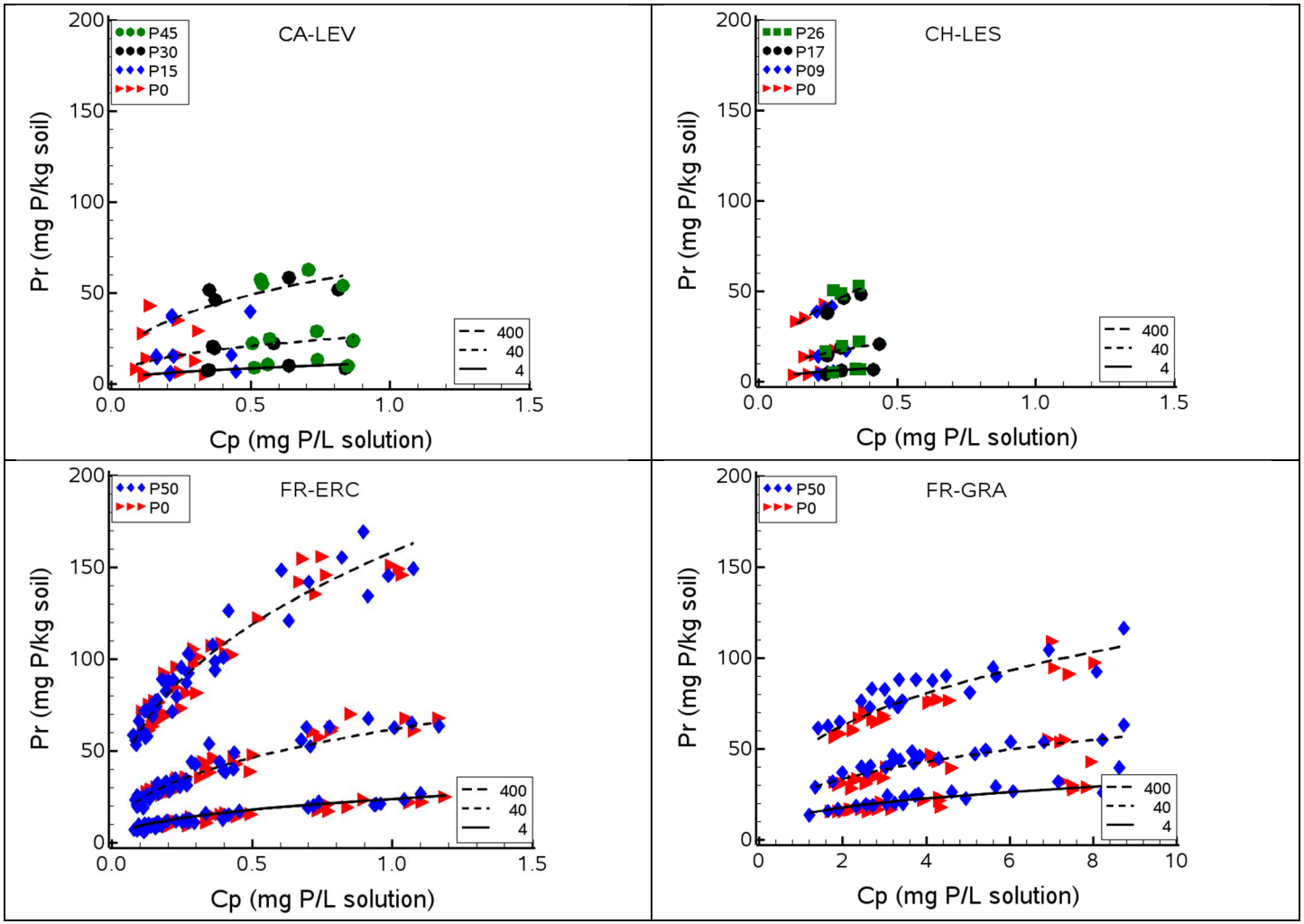


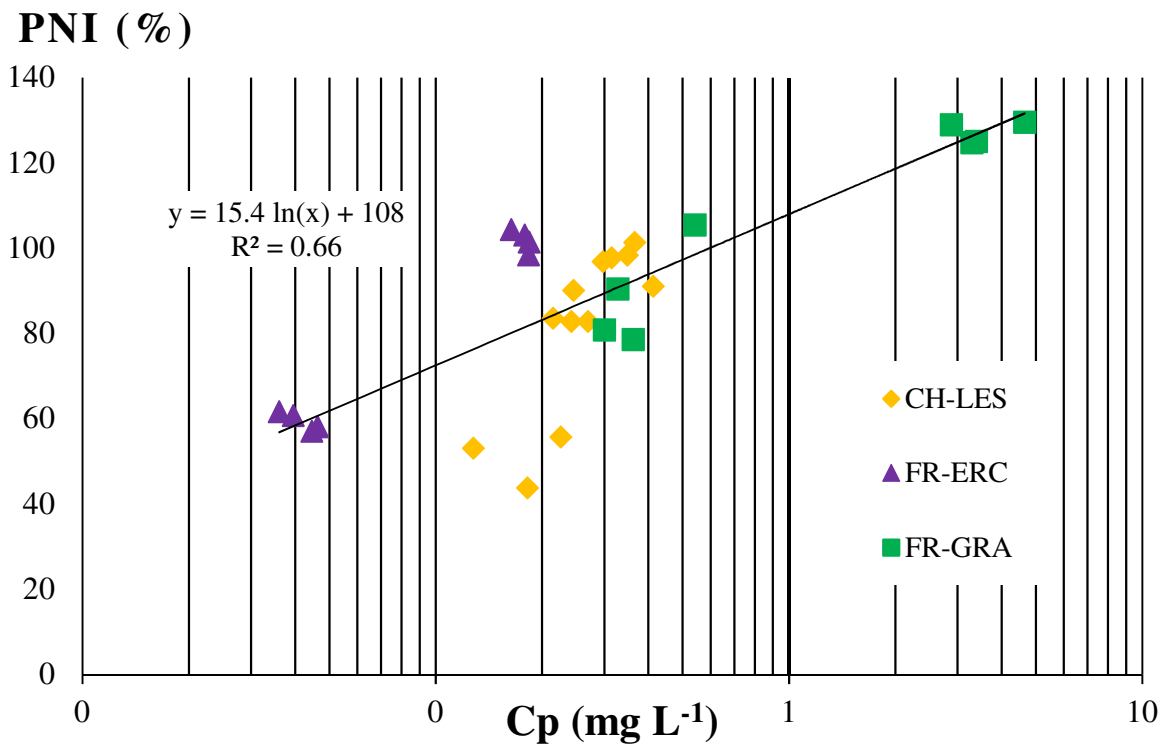

Fig. 3. Experimental (symbols) and regressed (lines) values of total amounts of diffusive orthophosphate P ions (Pr, mg P $kg^{-1}$ soil) as a function of the orthophosphate P ion concentration in solution ($C_P$, mg P $L^{-1}$ solution) and elapsed time (t, minutes) of isotope dilution at CA-LEV, CH-LES, FR-ERC, and FR-GRA sites. The regressed $P_r$ values were obtained using the Freundlich kinetic equation ($P_r = v \times C_P^w \times t^p$) (see Table 3 for values of parameters, v, w, and p)


CA-LEV
P45
P30
P15
P0
400
40
4
Pr (mg P/kg soil)
Cp (mg P/L solution)
CH-LES
P26
P17
P09
P0
FR-ERC
P50
FR-GRA

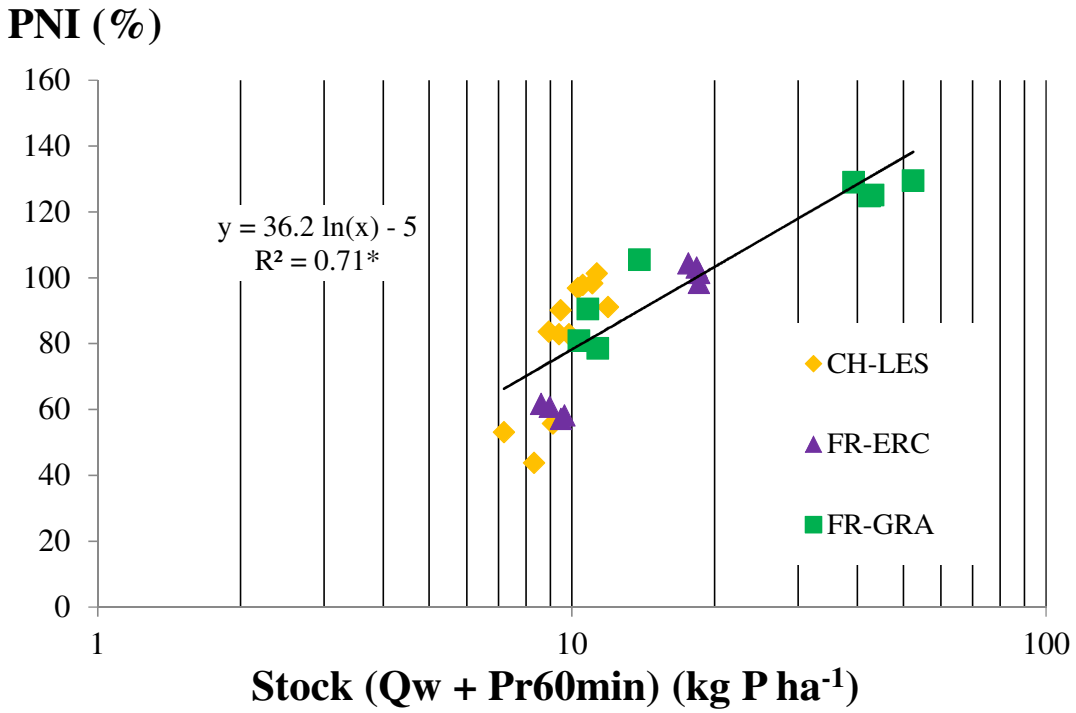
853

Fig. 4. Relationship between the phosphorus nutrition index (PNI), measured at the plot scale at CH-LES, FR-ERC, and FR-GRA sites, and soil plant-available P (SPAP); a) SPAP= $C_P$; b) SPAP=$Q_W$ + Pr60min. The x-axis is on a log scale to visualize the experimental points better.

a




b


PNI (%)
160
140
120
100
80
60
40
20
0
y = 36.2 ln(x) - 5
R² = 0.71*
CH-LES
FR-ERC
FR-GRA
1
10
100
Stock (Qw + Pr60min) (kg P ha-1)

863
864

865 # Supplementary materials

866

867 **Table S1** Mean (and standard error) of dry matter (DM; t $ha^{-1}$), nitrogen concentration (N; mg
868 $g^{-1}$), and phosphorus concentration (P; mg $g^{-1}$) measured at the first harvest at CA-LEV site
869 from 1999-2007 (n=4)

870

| Year | Treatment | DMY | | N | | P | |
|---|---|---|---|---|---|---|---|
| | | t $ha^{-1}$ | | mg $g^{-1}$ | | mg $g^{-1}$ | |
| 1999 | P0 | 0.8 | (0.2) | 23.6 | (1.4) | 2.3 | (0.1) |
| | P15 | 9.1 | (0.6) | 21.4 | (0.5) | 2.4 | (0.1) |
| | P30 | 8.4 | (0.6) | 22.0 | (0.7) | 2.6 | (0.1) |
| | P45 | 7.9 | (0.7) | 22.1 | (0.8) | 2.5 | (0.1) |
| 2000 | P0 | 7.3 | (0.4) | 19.9 | (0.8) | 2.5 | (0.1) |
| | P15 | 8.2 | (0.7) | 19.1 | (1.1) | 2.5 | (0.1) |
| | P30 | 7.8 | (0.2) | 19.1 | (0.8) | 2.6 | (0.1) |
| | P45 | 7.6 | (0.5) | 19.0 | (0.3) | 2.6 | (0.0) |
| 2001 | P0 | 7.1 | (0.3) | 18.0 | (0.8) | 2.2 | (0.0) |
| | P15 | 7.5 | (0.5) | 17.7 | (1.0) | 2.4 | (0.1) |
| | P30 | 7.5 | (0.4) | 18.1 | (0.4) | 2.5 | (0.0) |
| | P45 | 6.9 | (0.3) | 18.8 | (0.8) | 2.7 | (0.1) |
| 2002 | P0 | 5.9 | (0.4) | 23.7 | (0.7) | 2.9 | (0.1) |
| | P15 | 6.4 | (0.3) | 25.0 | (1.3) | 3.2 | (0.1) |
| | P30 | 6.5 | (0.2) | 21.3 | (0.8) | 3.1 | (0.1) |
| | P45 | 6.0 | (0.1) | 23.4 | (0.9) | 3.2 | (0.1) |
| 2003 | P0 | 3.2 | (0.2) | 23.3 | (1.2) | 2.9 | (0.1) |
| | P15 | 3.6 | (0.1) | 23.8 | (0.7) | 3.1 | (0.1) |
| | P30 | 3.8 | (0.1) | 24.4 | (0.9) | 3.5 | (0.1) |
| | P45 | 3.5 | (0.2) | 24.2 | (1.1) | 3.5 | (0.1) |
| 2004 | P0 | 4.0 | (0.1) | 22.0 | (1.4) | 2.5 | (0.1) |
| | P15 | 4.1 | (0.2) | 24.5 | (0.9) | 3.0 | (0.1) |
| | P30 | 4.1 | (0.3) | 23.4 | (1.4) | 3.2 | (0.1) |
| | P45 | 3.9 | (0.2) | 24.9 | (0.8) | 3.4 | (0.1) |
| 2005 | P0 | 4.5 | (0.1) | 23.4 | (0.8) | 2.5 | (0.1) |
| | P15 | 4.6 | (0.1) | 23.8 | (0.9) | 3.0 | (0.1) |
| | P30 | 4.7 | (0.2) | 21.4 | (0.8) | 3.0 | (0.1) |
| | P45 | 4.5 | (0.1) | 22.2 | (0.8) | 3.1 | (0.1) |
| 2006 | P0 | 3.5 | (0.4) | 24.1 | (0.7) | 2.8 | (0.1) |
| | P15 | 4.3 | (0.5) | 23.2 | (1.6) | 2.9 | (0.1) |
| | P30 | 4.5 | (0.2) | 22.7 | (0.4) | 3.1 | (0.0) |
| | P45 | 4.0 | (0.3) | 23.3 | (0.4) | 3.2 | (0.1) |
| 2007 | P0 | 5.0 | (0.3) | 21.0 | (0.8) | 2.4 | (0.0) |
| | P15 | 5.5 | (0.1) | 20.7 | (0.8) | 2.7 | (0.1) |
| | P30 | 5.1 | (0.1) | 20.5 | (0.3) | 2.9 | (0.0) |
| | P45 | 4.9 | (0.3) | 19.8 | (0.4) | 3.0 | (0.1) |

871

872 **Table S2** Mean (and standard error) of dry matter (DM; t $ha^{-1}$), nitrogen concentration (N; mg
873 $g^{-1}$), and phosphorus concentration (P; mg $g^{-1}$) measured at the first harvest at CH-LES site in
874 1997, 2001, 2005 and 2008 (n=3)
875

| Year | Treatment | DMY | | N | | P | |
|---|---|---|---|---|---|---|---|
| | | t $ha^{-1}$ | | mg $g^{-1}$ | | mg $g^{-1}$ | |
| 1997 | P0 | 3.7 | (0.3) | 13.8 | (0.3) | 1.8 | (0.1) |
| | P9 | 4.0 | (0.1) | 13.0 | (0.6) | 2.0 | (0.1) |
| | P17 | 3.6 | (0.4) | 13.8 | (0.7) | 2.2 | (0.1) |
| | P26 | 4.2 | (0.2) | 14.2 | (0.9) | 2.3 | (0.1) |
| 2001 | P0 | 3.1 | (0.3) | 14.8 | (0.2) | 1.6 | (0.1) |
| | P9 | 3.5 | (0.3) | 14.3 | (0.5) | 2.0 | (0.1) |
| | P17 | 3.6 | (0.4) | 14.5 | (0.9) | 2.2 | (0.1) |
| | P26 | 3.7 | (0.1) | 15.1 | (0.6) | 2.4 | (0.0) |
| 2005 | P0 | 2.4 | (0.2) | 15.4 | (0.2) | 1.3 | (0.1) |
| | P9 | 3.0 | (0.2) | 16.2 | (0.3) | 2.0 | (0.1) |
| | P17 | 4.0 | (0.3) | 16.1 | (0.6) | 2.1 | (0.1) |
| | P26 | 3.5 | (0.4) | 15.5 | (0.9) | 2.2 | (0.1) |
| 2008 | P0 | 2.6 | (0.1) | 14.4 | (0.1) | 1.2 | (0.1) |
| | P9 | 3.3 | (0.1) | 16.9 | (0.4) | 2.2 | (0.1) |
| | P17 | 4.0 | (0.4) | 18.1 | (0.4) | 2.4 | (0.1) |
| | P26 | 3.8 | (0.2) | 14.4 | (0.1) | 1.2 | (0.1) |

876

877 **Table S3** Mean (and standard error) of dry matter (DM; t $ha^{-1}$), nitrogen concentration (N; mg
878 $g^{-1}$), and phosphorus concentration (P; mg $g^{-1}$) measured at the first harvest at FR-ERC site
879 from 1999-2018 (n=4)
880

| Year | Treatment | DMY | | N | | P | |
|---|---|---|---|---|---|---|---|
| | | t $ha^{-1}$ | | mg $g^{-1}$ | | mg $g^{-1}$ | |
| 1999 | P0 | 3.2 | (0.2) | 28.9 | (0.3) | 2.4 | (0.2) |
| | P50 | 3.6 | (0.2) | 26.2 | (0.6) | 2.8 | (0.2) |
| 2000 | P0 | 2.8 | (0.2) | 37.9 | (1.9) | 2.9 | (0.3) |
| | P50 | 3.0 | (0.2) | 37.6 | (2.3) | 4.8 | (0.1) |
| 2001 | P0 | 4.0 | (0.3) | 33.0 | (1.1) | 3.0 | (0.1) |
| | P50 | 4.6 | (0.4) | 25.7 | (1.0) | 3.7 | (0.1) |
| 2002 | P0 | 3.0 | (0.2) | 23.9 | (1.2) | 2.2 | (0.0) |
| | P50 | 4.0 | (0.4) | 23.7 | (1.2) | 3.6 | (0.1) |
| 2003 | P0 | 6.3 | (0.5) | 18.3 | (0.7) | 1.8 | (0.1) |
| | P50 | 10.0 | (1.0) | 13.9 | (0.7) | 2.7 | (0.0) |
| 2004 | P0 | 3.6 | (0.5) | 28.0 | (1.0) | 2.1 | (0.1) |
| | P50 | 4.4 | (0.6) | 27.7 | (0.5) | 4.4 | (0.1) |
| 2005 | P0 | 2.3 | (0.2) | 28.6 | (0.6) | 1.9 | (0.1) |
| | P50 | 3.2 | (0.5) | 28.6 | (1.5) | 2.9 | (0.1) |
| 2006 | P0 | 4.7 | (0.4) | 17.9 | (0.8) | 1.3 | (0.1) |
| | P50 | 6.9 | (0.4) | 17.5 | (1.4) | 2.8 | (0.3) |
| 2007 | P0 | 3.6 | (0.5) | 25.8 | (1.1) | 1.9 | (0.1) |
| | P50 | 4.8 | (0.1) | 24.4 | (0.5) | 3.1 | (0.0) |

881

882 **Table S4** Mean (and standard error) of dry matter (DM; t $ha^{-1}$), nitrogen concentration (N; mg
883 $g^{-1}$), and phosphorus concentration (P; mg $g^{-1}$) measured at the first harvest at FR-GRA site
884 from 1998-2014 (n=4)
885

| Year | Treatment | DMY | | N | | P | |
|---|---|---|---|---|---|---|---|
| | | t $ha^{-1}$ | | mg $g^{-1}$ | | mg $g^{-1}$ | |
| 1998 | P0 | 4.3 | (0.1) | 32.5 | (0.2) | 4.5 | (0.1) |
| | P50 | 4.4 | (0.3) | 31.8 | (0.4) | 4.7 | (0.1) |
| 1999 | P0 | 4.8 | (0.8) | 21.5 | (0.4) | 2.4 | (0.1) |
| | P50 | 4.2 | (0.3) | 19.7 | (0.6) | 2.7 | (0.1) |
| 2000 | P0 | 3.8 | (0.1) | 26.6 | (0.7) | 3.3 | (0.2) |
| | P50 | 4.6 | (0.3) | 27.3 | (2.4) | 4.2 | (0.3) |
| 2001 | P0 | 5.9 | (0.8) | 19.5 | (2.3) | 2.6 | (0.2) |
| | P50 | 6.0 | (0.3) | 18.8 | (1.4) | 3.3 | (0.1) |
| 2002 | P0 | 3.7 | (0.6) | 26.3 | (2.1) | 3.2 | (0.2) |
| | P50 | 4.0 | (0.3) | 23.5 | (0.8) | 3.8 | (0.1) |
| 2003 | P0 | 6.0 | (1.0) | 20.4 | (1.7) | 2.1 | (0.2) |
| | P50 | 6.0 | (0.7) | 20.1 | (0.7) | 2.4 | (0.2) |
| 2004 | P0 | 3.9 | (0.4) | 29.2 | (1.8) | 3.7 | (0.4) |
| | P50 | 4.5 | (0.9) | 23.4 | (0.5) | 4.3 | (0.2) |
| 2005 | P0 | 5.7 | (1.1) | 19.0 | (1.2) | 2.8 | (0.1) |
| | P50 | 5.8 | (0.5) | 19.5 | (1.8) | 3.5 | (0.4) |
| 2006 | P0 | 4.6 | (0.6) | 24.3 | (1.6) | 2.9 | (0.2) |
| | P50 | 4.3 | (0.6) | 20.7 | (1.1) | 3.9 | (0.2) |
| 2007 | P0 | 3.6 | (0.5) | 31.0 | (2.9) | 3.1 | (0.4) |
| | P50 | 3.5 | (0.5) | 30.9 | (2.1) | 4.3 | (0.1) |
| 2008 | P0 | 2.8 | (0.4) | 30.6 | (2.0) | 3.4 | (0.3) |
| | P50 | 3.0 | (0.3) | 30.9 | (1.1) | 3.8 | (0.4) |
| 2009 | P0 | 5.8 | (0.3) | 24.4 | (1.1) | 4.4 | (0.2) |
| | P50 | 6.1 | (0.6) | 18.5 | (1.9) | 4.7 | (0.4) |
| 2010 | P0 | 6.4 | (0.5) | 20.5 | (1.0) | 3.3 | (0.2) |
| | P50 | 7.3 | (0.2) | 17.7 | (0.9) | 4.1 | (0.2) |
| 2011 | P0 | 5.2 | (0.3) | 26.0 | (0.7) | 2.7 | (0.1) |
| | P50 | 5.7 | (0.4) | 21.3 | (0.8) | 3.3 | (0.2) |
| 2012 | P0 | 8.2 | (1.0) | 16.6 | (0.6) | 2.3 | (0.1) |
| | P50 | 7.6 | (0.7) | 15.5 | (0.7) | 3.0 | (0.3) |
| 2013 | P0 | 5.1 | (0.2) | 19.1 | (1.9) | 2.7 | (0.2) |
| | P50 | 5.8 | (0.1) | 18.8 | (1.3) | 2.9 | (0.2) |
| 2014 | P0 | 4.4 | (0.4) | 23.5 | (0.9) | 2.7 | (0.1) |
| | P50 | 4.6 | (0.3) | 22.2 | (0.9) | 3.7 | (0.1) |

886

887 **Table S5** Mean (and standard error) of dry matter (DM; t $ha^{-1}$), nitrogen concentration (N; mg
888 $g^{-1}$), and phosphorus concentration (P; mg $g^{-1}$) measured at the first harvest at RO-DÂM site
889 from 2002-2004 (n=4)
890

| Year | Treatment | DMY | | N | | P | |
|---|---|---|---|---|---|---|---|
| | | t $ha^{-1}$ | | mg $g^{-1}$ | | mg $g^{-1}$ | |
| 2002 | P0 | 3.5 | (0.7) | 31.6 | (1.9) | 1.7 | (0.1) |
| | P33 | 5.7 | (0.8) | 26.0 | (2.0) | 2.7 | (0.1) |
| 2003 | P0 | 1.4 | (0.3) | 33.5 | (1.1) | 1.9 | (0.3) |
| | P33 | 2.9 | (0.5) | 34.1 | (2.6) | 3.8 | (0.2) |
| 2004 | P0 | 2.0 | (0.3) | 34.3 | (0.6) | 1.7 | (0.1) |
| | P33 | 3.5 | (0.5) | 32.8 | (2.2) | 3.8 | (0.1) |

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