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[a] INRAE, INP Toulouse, AGIR, F-31326 Castanet Tolosan, France

[b] INRAE, ISPA, F-33882 Villenave d'Ornon, France

[c] Agriculture and Agri-Food Canada, Québec Research and Development Centre, Québec, G1V 2J3, Canada

[d] Agroscope, Plant Production Systems, 1260, Nyon, Switzerland

[e] BUAVMT, Faculty of Agriculture, RO-300645, Timișoara, Romania

* Corresponding author

Postal address : INRAE, AGIR, F-31326 Castanet Tolosan, France
E-mail address : Claire.Jouany@inrae.fr

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 + P_r) from the sum of oPion in the soil solution (Qw, mg P kg^{-1}) and diffusive oPion with time and C_P (P_r, 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, for a given crop or grassland sward, a single threshold value regardless of the soil type (Schulte and Herlihy, 2007; Jordan-Meille et al., 2012).

More recently, significant advances have been made with the development of innovative assessment tools. The approach of nutrition indices based on the nutrient concentration of shoot biomass allows grassland P nutrition status to be assessed during growth (Duru and Ducrocq, 1997). For P, this approach is more reliable than those based on a single critical concentration since it considers changes in nutrient concentration as a function of sward biomass accumulation and concentration of other nutrients (Duru and Ducrocq, 1997). The P nutrition index (PNI) is an effective tool for P fertilization management in grasslands since it assesses the sward P nutrition status well during growth and can verify and validate fertilization practices *a posteriori* (Thélier-Huché et al., 1999). The PNI is adequate for the interpreting of the effect of plant P nutrition status on plant growth in grasslands managed at different intensities (Liebisch et al., 2013); as well, PNI provides appropriate plant nutrients status evaluation at the interface between agricultural land and saline wetlands in protected saline habitats (Luna et al., 2019). At an ecosystem level, the N nutrition index (NNI) and PNI provide appropriate evaluation of the functional response of species and communities to fertility gradients induced by practices (Garnier et al., 2007; Lavorel et al., 2009).

At the same time, Morel (2002) developed a mechanistic model based on the assumption that the SPAP pool represents the sum of the amount of orthophosphate ions (oPion) in the soil solution (Qw) and the amount of soil P that can diffuse from the soil to the solution over time (P_r). This model assumes that (i) diffusion of oPion at the solid-to-solution interface of soils is quantitatively the dominant process in plant P nutrition and (ii) depletion of oPion concentration at the root surface, due to absorption, creates a gradient of oPion concentration between the root surface, the soil solution, and the soil solid phase. This gradient is the driving 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.

DÂMBOVICIOARA, ROMANIA (RO-DÂM). An experiment was established in 1963 on a permanent grassland in the Southern Carpathians Mountains in the Rucar-Bran-Dragoslavele corridor, Romania (Table 1). The grassland had a mixture of *F. rubra* and *A. capillaris*. The experiment consisted of two rates of P fertilizer [0 (P0) and 33 (P33) kg P ha^{-1}] applied in autumn each year as super phosphate on plots arranged in a randomized complete-block design with four replicates. N was applied as ammonium nitrate at 100 kg N ha^{-1} in early spring and 50 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 autumn to ensure that K did not limit plant growth (Ciubotariu et al., 2002).

More details on the five long-term experiments can be found in Bélanger et al. (2008) and Bélanger and Ziadi (2008) for CA-LEV, in Jeangros and Sinaj (2018) for CH-LES, in Stroia et al. (2007) for FR-ERC and FR-GRA, and in Ciubotariu et al. (2002) for RO-DÂM.

2.2. Yield and nutrition index determination

Dry matter (DM) yield and nutrient concentration were measured at the end of the first growth cycle (Table 2), a period of the year when growth is rarely limited by water. Forage production in this first growth cycle represented 40%-70% of the average total annual production, depending on the site and treatment. All plots were harvested again during the rest of the growing season, but no measurements were taken. DM yield was measured by cutting an area of at least 1m^2 to a height of 5-cm. A fresh sample of ca. 300-500 g was taken, dried at 55 °C for 48 h, and ground.

At CA-LEV, dried and ground (1 mm) forage samples (0.1 g) were wet-digested with 1.5 mL H_2SO_4-H_2SeO_3 and 2.0 mL H_2O_2 (Isaac and Johnson, 1976). N and P concentrations were measured by colorimetry using an automated continuous-flow injection analyzer (QuickChem 8000 FIA+ analyzer; Lachat Instruments, Loveland, Colorado, USA) with the salicylatenitroprusside 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).

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

2.5 Soil plant available P

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

We also tested the functional- and process-based approach previously developed for soils with annual crops (Morel et al., 2014; Messiga et al., 2015) and perennial forages (Stroia et al., 2007; Messiga et al., 2012), and for rivers sediments (Némery et al., 2005). The model assumes that (i) roots absorb oPion only from the soil solution and (ii) this absorption generates a gradient of oPion concentration between the soil solid phase and solution that drives the oPion diffusion (Barbier et al., 1971; Barber, 1984). This method provides an experimental data-set 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 point of the linear-plateau curve, was 92%. Below this threshold, the increase in DM yield was proportional to that in PNI (Fig. 1). We observed a very significant positive association between the average values of PNI and RDMY for each combination site-treatment (p=9.88e-05, R^2=0.88), despites a moderate sample size (nine site-treatment combinations).

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

3.2 Soil plant available P

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

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

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

Data obtained from sorption-desorption and isotope dilution procedures showed that, for each site, the total amount of diffusive oPion in soils (Pr) increased as C_P and time increased (Fig. 3); the magnitude of the response differed among sites. For similar C_P ranges of less than 1 mg P L^{-1}, Pr measured in the FR-ERC soil was twice that measured in the CA-LEV soil, regardless of the time (t) (Fig. 3). The entire dataset served to estimate the parameters of Eq. (7) (Table 3). For each site, except RO-DÂM, the entire C_P dataset (Table 2; Table 4) and the parameter estimates of the Freundlich kinetic equation (Table 3) were used to calculate (Qw + Pr) values for increasingly long periods of diffusion. For each value of t, a general relationship was built between (Qw + Pr) and PNI (data not shown); the best fit was obtained for a diffusion time t of 60 minutes (Pr60min) (Fig. 2c).

Like C_P, the mean amount of diffusive P (Qw + Pr60min) varied at each site with treatments (Table 4). When several P fertilizer rates were applied (CH-LES and CA-LEV), the maximum (Qw + Pr60min) was measured for the highest P fertilizer rate. For each site, differences between control and fertilized plots were always significant.

For control treatments, mean (Qw + Pr60min) varied from 18.8 mg kg^{-1} at CH-LES to 22.3 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 fertilized treatments (Table 4).

3.3. Relationship between P nutrition index and soil plant available P

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

for Qw + Pr60min. We obtained a similar response pattern for the relationship between the 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). The threshold of 92% for PNI (Fig. 1b) served to calculate the critical SPAP values which value were 13.5 kg P ha^{-1} and 12.9 kg ha^{-1} for, respectively (Qw + Pr60min) and Olsen P.

4. Discussion

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

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

At the start of the experiments, the initial SPAP value exceeded the critical value of 25 mg Olsen P kg^{-1} at FR-GRA (Table 1) identified by Poulton et al. (1997). The experimental site of CA-LEV was chosen because of its expected positive response to P fertilization (Bélanger and Ziadi, 2008). Its soil (Mehlich-3 extraction; 0-15 cm) contained 54 kg available P ha^{-1} (Table 1) and was considered poor in P according to local recommendations (Conseil des Productions Végétales du Québec, 1996). During the experiment at FR-GRA, SPAP measured in the P0 treatment remained above the critical value despite large negative annual P budget (-35 kg ha^{-1}; Stroia, 2007). At CA-LEV, SPAP measured in the P0 and P15 treatments first decreased and then remained relatively stable over time, despite negative annual P budgets (-5 and -20 kg ha^{-1}, respectively) (Messiga et al., 2015). At FR-ERC and CH-LES, SPAP values laid below 25 mg Olsen P kg^{-1} at the start of the experiments, and they decreased over time as cumulative P budgets became more negative (Messiga et al., 2015).

For similar mean annual P outputs and budgets (Messiga et al., 2015), soils were less resilient at FR-ERC than at CA-LEV and FRA-GRA, where productivity was not affected after not applying P for nine and seventeen years, respectively. These results confirmed that the long-term impact of stopping P fertilization on grassland productivity varied greatly for European upland grasslands (Marriott et al., 2004) and that legacy P can contribute greatly to grassland nutrition (Sattari et al., 2012).

The significant relationship between relative DM yield and PNI demonstrated that PNI captures differences in DM yield between P fertilized and non-fertilized plots and that, under P limitation, the decrease in DM yield is directly proportional to the decrease in the grassland P nutrient status measured with PNI. The overall critical PNI value, corresponding to the inflection point of the linear-plateau curve, was 92%. This value was close to PNI = 100% which theoretically represents the boundary between P-limited and non-P-limited growth conditions according to Thélier-Huché et al. (1999). The difference between this threshold

(92%) and the statistical value (100%) given by Duru and Thélier-Huché (1997) and Thélier-Huché et al. (1999) is likely due to the smallest dataset that they used to determine the critical P-dilution curve or to establish the relationship between relative yield and PNI.

This study provides the first evidence that the relative DM yield of a P-limited sward, , is a direct function of its P status assessed with PNI, within different site-treatments. These results validate the PNI approach as an adequate tool for *a posteriori* assessment of grassland P nutrition. They extend conclusions of studies conducted on grasslands at local scales (Duru et al., 1993; Duru and Ducrocq, 1997; Duru and Thélier-Huché, 1997; Liebisch et al., 2013). Under P-limited nutrition status, relative DM yield was also shown to be a direct function of the PNI in canola (*Brassica napus* L.) (Cadot et al., 2018) and maize (*Zea Mays* L.) (Cadot et al., 2018; Gagnon et al., 2020).

While we observed a high positive association between average PNI and average relative DM measured within the nine site-treatment clusters (Fig. 1a), a similar relation was not observed for each site-treatment; relationships were positive for some site-treatments and negative for others (Fig. 1b). We considered that there were variables, other than PNI, that came into account to explain inter annual relative DM variability for a given site-treatment. Since interactions between N and P nutrition control forage response to fertilization (Duru and Thélier 1997), we tested to what extend NNI and KNI, for CH-LES site, could explain relative DM yield variability between years.

For each CA-LEV and FR-GRA treatments, there was no significant relationship between NNI and PNI, between relative DM and NNI; including NNI as a co variable did not improve none of the model obtained with PNI (Fig. 1b). No significant relationship was obtained either when testing the relationships for the three CA-LEV data sets pooled together.

At site level, these results confirmed that for both sites and treatment optimal N and P nutrition allowed optimal biomass production. On the other hand, negative relationships between relative DMY and PNI at CA-LEV P0 and CA-LEV P1 (Fig. 1b) could result from P luxury consumption and accumulation which increased as relative DM decreased.

For CH-LES, there was no significant relationship between NNI and PNI, relative DMY and NNI and between relative DMY and KNI for any of the treatments. Including NNI or KNI as co variable did not improve the model between relative DM and PNI for CH-LES P0 (Fig. 1b). Finally, when pooling the three CH-LES data sets, we got a significant relationship between relative DMY and NNI (R^2=0.44; p=0.018); best fit was obtained with PNI as a single variable (R^2=0.65; p=0.0016). This result demonstrated that, under P and N limited conditions, inter annual relative DM variability was better explained by PNI than NNI.

For FR-ERC, there was a significant relation between NNI and PNI (R^2=0.49; p=0.0353) which confirmed that, under P limitation, improving P nutrition has a positive and significant effect on herbage nutrient status as reported by Duru and Ducrocq (1997). Introduction of NNI as a co variable with PNI (Fig. 1b) improved the relationship (R^2=0.76; p=0.0135). Finally, best fit of the data was obtained with a simple linear model with NNI (R^2=0.75; p=0.0024. Under P limited and non N limited conditions, NNI explained better variations in relative DM than PNI; grassland response to P limitation varies according to NNI. For a given PNI, relative DM is higher, the higher NNI.

The results showed that P effect on relative DM was different according to the grassland nutritional status. At CH-LES site, with both N and P limitation, increasing PNI improved the sward efficiency for N conversion in biomass. P supply has a direct impact on forage growth. On the opposite, at FR-ERC site, where P was limiting growth but not N, increasing PNI improved sward N nutrition status and consequently relative DM.

These results confirmed at multi annual scale that interaction between N and P controls forage response to P fertilization (Duru and Ducrocq, 1997; Jouany et al (2011).

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

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

The stronger relationship observed between PNI and (Qw + Pr60min) compared to that with C_P alone (Fig. 2c) demonstrated that assessing SPAP as the amount of soil diffusive oPion, captured the specific effects of soil type on P-ion mobility better than C_P did. These results generalized results obtained for annual crops by Morel et al. (2000; 2021), to grassland soils. However, our study differed on two points. First, the time of diffusion (t) that minimized variance in the relationship between PNI and (Qw + Pr) (Fig. 2c) was 60 minutes (Pr60min), much shorter than 1360 minutes for annual crops (Morel et al., 2021). The critical (Qw + 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 applied) offer the opportunity to test grassland responses to P fertilization and set more reliable 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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Table 1.
Climate, soil, and management characteristics of the five experimental sites; NA = no data available

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.

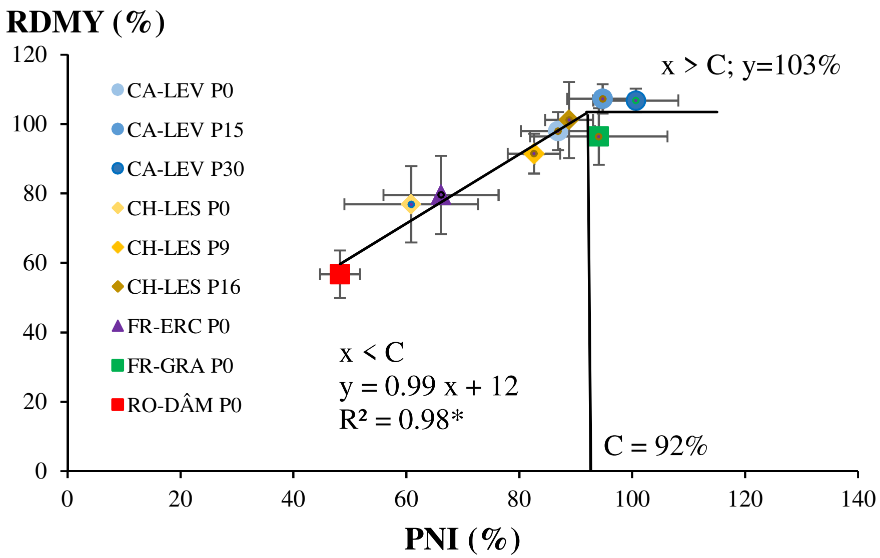
Site	Treatment	PNI[1] (%)		Cp mg P l^{-1}		Olsen-P mg P kg^{-1} soil		(Qw + Pr60min) mg P kg^{-1} soil	
CA-LEV	P0	83	a[2]	0.3	a	NA	NA	22.3	a
	P1	95	b	0.59	ab	NA	NA	31.5	ab
	P2	101	bc	1	bc	NA	NA	41.3	bc
	P3	106	c	1.37	c	NA	NA	49.2	c
CH-LES	P0	51	a	0.18	a	12.5	a	18.8	a
	P1	86	b	0.24	ab	16.7	a	21.5	ab
	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



b

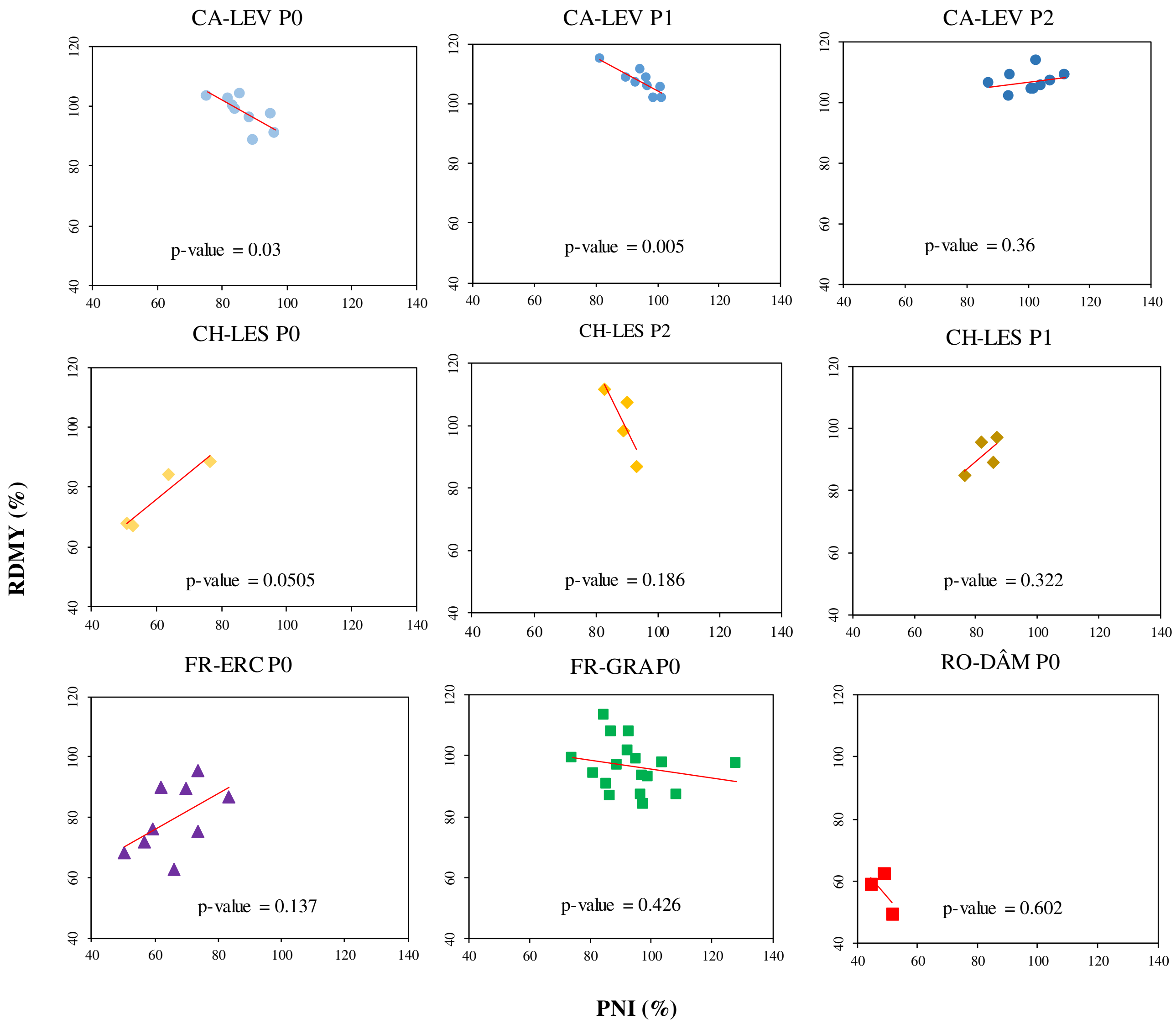
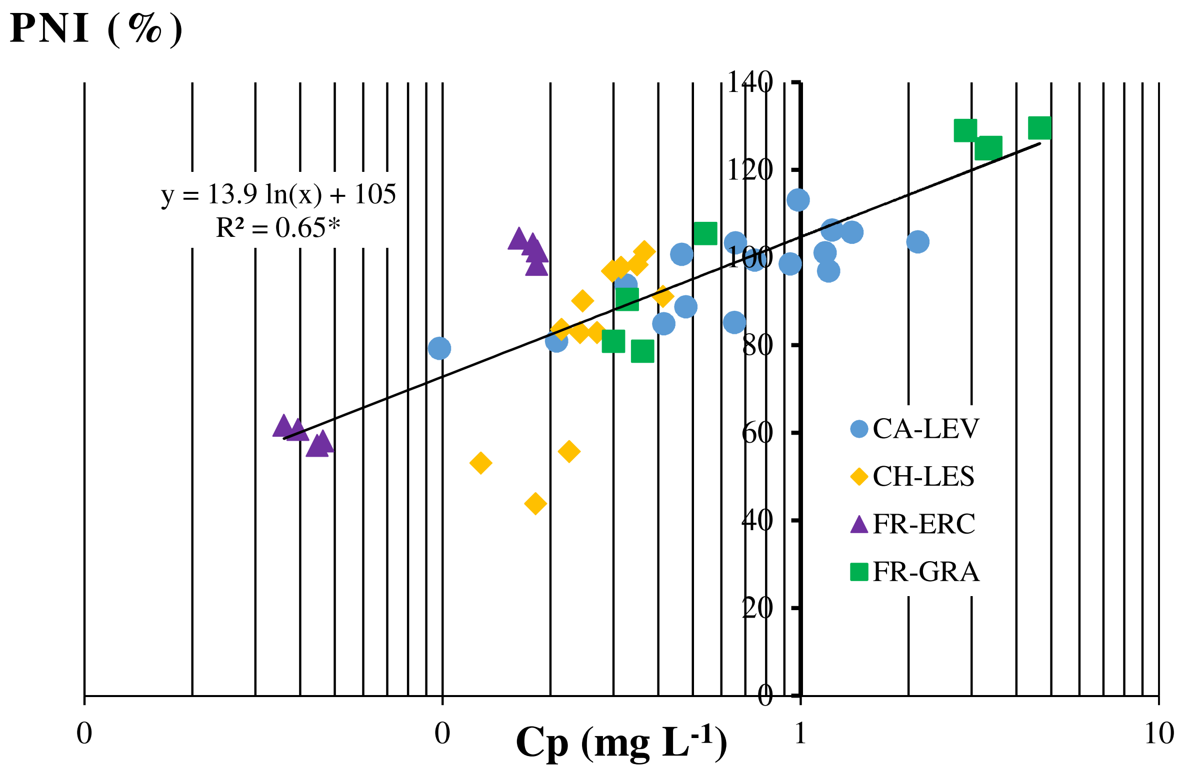
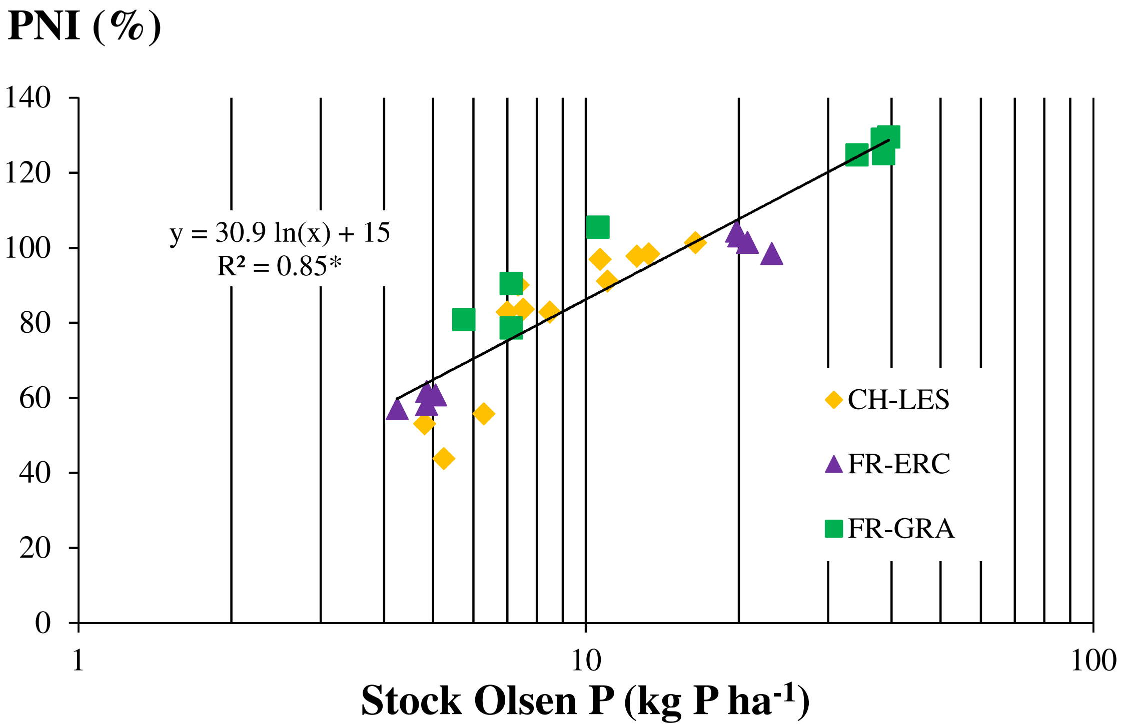

CA-LEV P0
p-value = 0.03
CA-LEV P1
p-value = 0.005
CA-LEV P2
p-value = 0.36
CH-LES P0
p-value = 0.0505
CH-LES P2
p-value = 0.186
CH-LES P1
p-value = 0.322
FR-ERC P0
p-value = 0.137
FR-GRA P0
p-value = 0.426
RO-DÂM P0
p-value = 0.602
RDMY (%)
PNI (%)
40
60
80
100
120
140

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



b


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

c

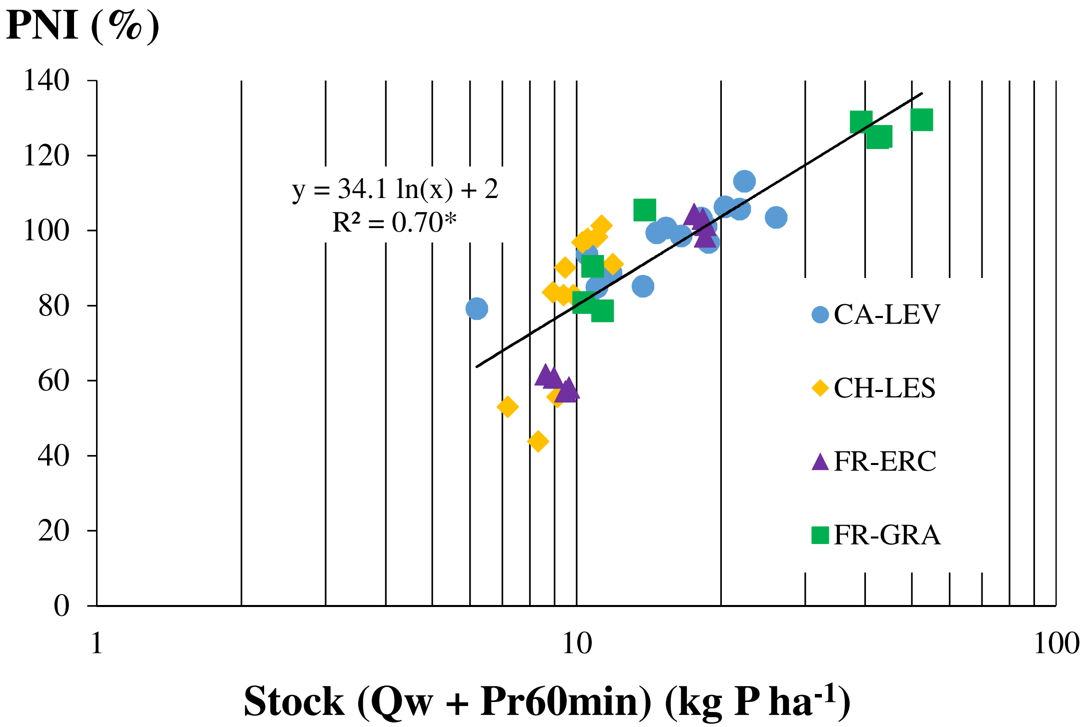
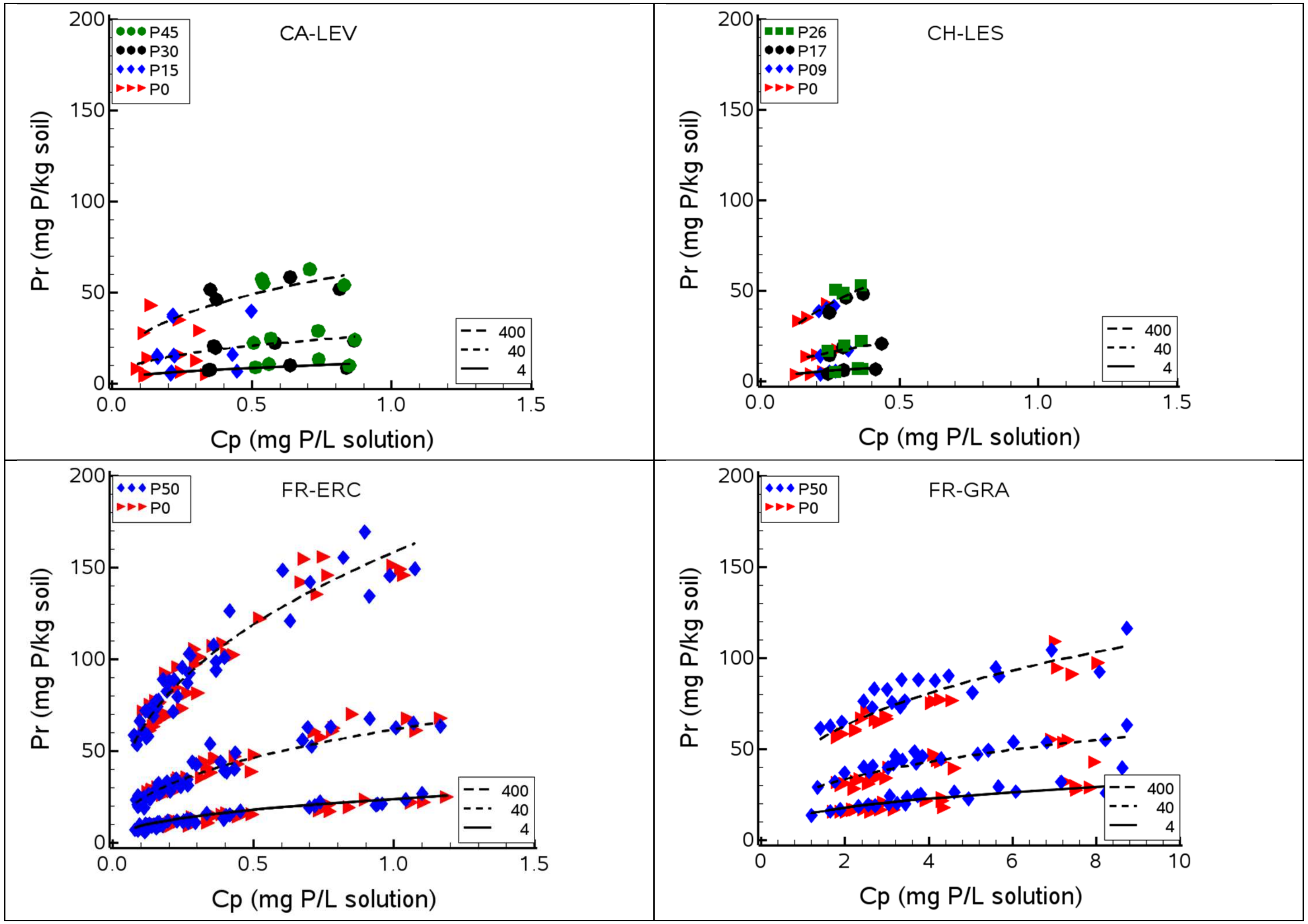


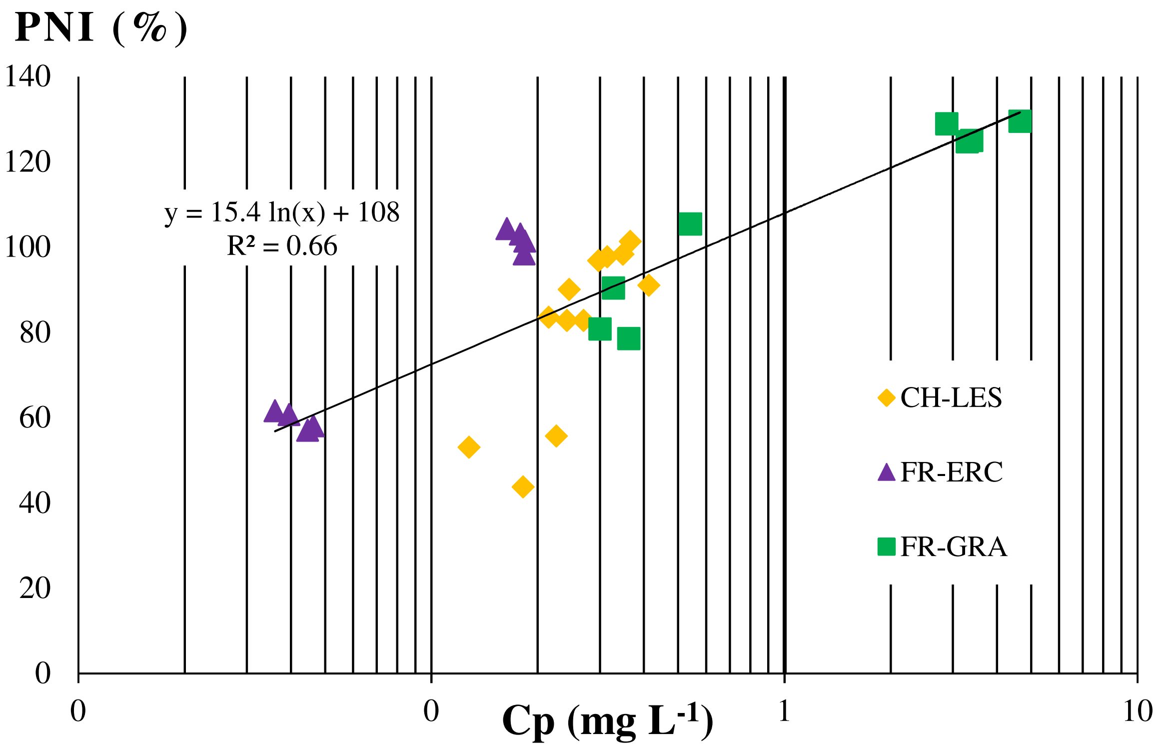
Fig. 3. Experimental (symbols) and regressed (lines) values of total amounts of diffusive orthophosphate P ions (Pr, mg P kg^{-1} soil) as a function of the orthophosphate P ion concentration in solution (C_P, mg P L^{-1} solution) and elapsed time (t, minutes) of isotope dilution at CA-LEV, CH-LES, 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
P0
FR-GRA

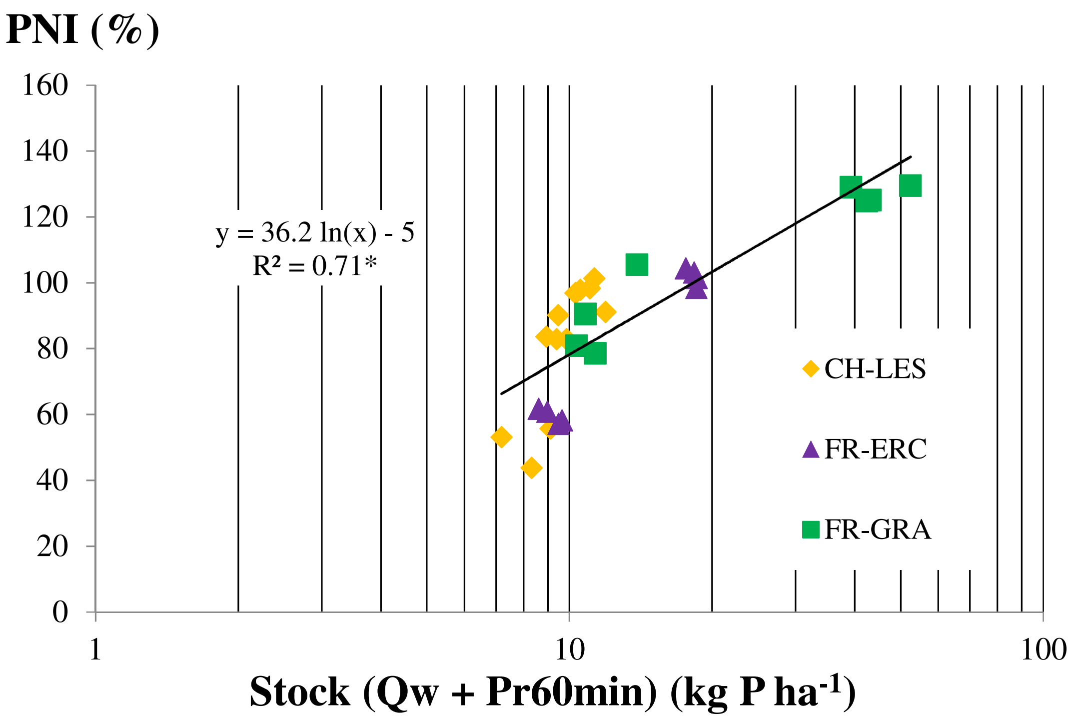
853

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
1
10
100
y = 36.2 ln(x) - 5
R² = 0.71*
CH-LES
FR-ERC
FR-GRA
Stock (Qw + Pr60min) (kg P ha-1)

Supplementary materials

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

Year	Treatment	DMY t ha^{-1}		N mg g^{-1}		P 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)

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

Year	Treatment	DMY t ha^{-1}		N mg g^{-1}		P 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)

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

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)

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

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)

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

Year	Treatment	DMY t ha^{-1}		N mg g^{-1}		P 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)