

Plant and soil tests to optimize phosphorus fertilization management of grasslands

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Plant and soil tests to optimize phosphorus fertilization management of 1

grasslands 2

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25 Highlights

| 26 | ٠ | Positive relation between relative forage yield and the P nutrition index (PNI) |
|----|---|---|
| 27 | • | Critical PNI value of 92% separates P-limited and non-P limited grasslands |
| 28 | • | Stronger relationship of PNI with Olsen P than with other soil tests |
| 29 | • | Critical Olsen P stock for a target PNI of 92%: 12.9 kg P ha ⁻¹ |
| 30 | | |

31 Abstract

32

33 fertilization management. Soil phosphorus (P) tests have long been used as decision support 34 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. 35 36 The objectives of this study were to (i) evaluate the relationship between the phosphorus 37 nutrition index (PNI) and the yield response to P fertilization (ii) analyze relationships between 38 PNI and soil plant-available P (SPAP) indicators, and (iii) evaluate PNI assets for P diagnosis 39 in forage system. Five long-term (\geq 9 years) grassland P fertilization experiments under 40 different soil and climate environments in Canada, Switzerland, France and Romania were 41 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 42 the sum of oPion in the soil solution (Qw, mg P kg⁻¹) and diffusive oPion with time and $C_P(Pr,$ 43 mg P kg⁻¹). PNI was calculated as sward P concentration divided by the critical P 44 45 concentration. 46 . The cumulative effect of P fertilization resulted in a wide range of SPAP values. Overall, CP 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 47 kg⁻¹. The PNI varied from 48 %-94% in plots with no applied P, and from 83 %-121% in P-48 49 fertilized plots. A generally positive relationship between relative forage dry matter yield and 50 PNI was established, with a critical PNI value of 92% that distinguishes P-limited and non-P-51 limited grassland nutrition. Positive relationships between PNI and the three SPAP indicators 52 confirmed that the soil P status influenced the grassland P nutrition status. Critical values on a

Developing more sustainable forage systems requires efficient decision support tools for

53 stock basis for a target PNI value of 92% were similar for Olsen P (12.9 kg P ha⁻¹) and (Qw +

54 Pr60min) (13.5 kg P ha⁻¹). This study opens perspectives for P diagnosis improvement in forage

55 systems.

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

59 **1. Introduction**

Grasslands provide an essential source of cattle feed, and the development of more 60 61 sustainable agricultural systems tends to increase their contribution to livestock production 62 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 63 64 applied by farmers. The aim of phosphorus (P) fertilization is to meet crop requirements, which 65 are determined largely by the nitrogen (N) supply (Bélanger et al., 1989; Schellberg et al., 66 1999; Griffin et al., 2002; Valkama et al., 2016). Environmental and economic concerns require 67 developing more sustainable forage systems. Excess P threatens the integrity of terrestrial and 68 aqueous ecosystems (Sharpley and Menzel, 1987; Janssens et al., 1998. Ceulemans et al., 69 2011), while readily available and high-quality reserves of phosphate rocks will be exhausted in 70 the medium term (Cordel et al., 2011).

71 Developing more sustainable forage systems requires effective decision support tools for 72 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 73 74 ratios in plant tissues were developed as assessment tools for alfalfa (Medicago sativa L.; 75 Walworth et al., 1986) and perennial ryegrass (Lolium perenne L.) (Bailey et al., 1997; Bailey 76 et al., 2000). These ratios are also used for natural ecosystems to determine whether biomass 77 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 78 79 thresholds useful in decision support tools for fertilization management (Schulte and Herlihy, 80 2007; Reinjeveld et al., 2010). However, both plant and soil tests remain of limited general 81 value. Ratios that indicate of N or P limitation vary according to the type of ecosystems. For 82 instance, they are much lower in upland grasslands than in wetlands (Mamolos et al., 2005; 83 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 86 87 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, 88 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

109 model, based on a Freundlich kinetic equation, was developed to calculate Pr as a function of 110 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 111 112 grassland experiment with contrasting P fertilization regimes (Stroia et al., 2007). In the present 113 study, we examined the abilities of C_P (Morel et al., 2000), Olsen P (Olsen et al., 1954), and the 114 sum (Qw + Pr) to assess SPAP in grasslands. Both C_P and Olsen P are used around the world 115 for this purpose (Jordan-Meille et al., 2012; Ziadi et al., 2013). 116 The objectives of this study were to: (i) evaluate the relationship between PNI and the yield 117 response to P fertilization over a range of soil types and climate conditions; (ii) analyze 118 relationships between PNI and three SPAP indicators in order to compare their abilities to 119 assess the soil P status and (iii) evaluate PNI assets for P diagnosis in forage system. The study 120 relied on five long-term experiments that measured the response of forage yield to P

121 fertilization under contrasting environments representative of grassland ecosystems in Canada,

122 Switzerland, France and Romania. The four sites offered the opportunity to explore large

123 gradients in soil P fertility caused by cumulative effects of P fertilization over several years.

124 **2. Materials and Methods**

125 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

132 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.

140 LES VERRIÈRES, SWITZERLAND (CH-LES). A permanent grassland experiment was 141 established in 1993 on a Cambisol at Les Verrières, Switzerland (Table 1), with a mixture of 142 red fescue (Festuca rubra L.), common bent (Agrostis capillaris L.), and orchard grass 143 (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-144 145 block design with three replicates. P was applied each year in a single application as triple 146 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, 147 P17, and P26, respectively. N was applied at a rate of 25 kg N ha⁻¹ as ammonium nitrate in all 148 149 treatments once a year after the first cut.

150 ERCÉ (FR-ERC) AND GRAMOND (FR-GRA), FRANCE. An experiment was conducted at 151 Ercé and Gramond, France, on permanent multi-species grasslands (Table 1). The experiment 152 was established in 1999 at Ercé and in 1998 at Gramond. At both sites, the experiment 153 consisted of two rates of P fertilizer [0 (P0) and 50 (P50) kg P ha⁻¹] applied each year in 154 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 155 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 156 157 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 with four replicates. N was applied as ammonium nitrate at 100 kg N ha⁻¹ in early spring and 50 163 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 164 165 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 al. (2007) for FR-ERC and FR-GRA, and in Ciubotariu et al. (2002) for RO-DÂM. 168

169 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 1m² 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 H₂SO₄-H₂SeO₃ and 2.0 mL H₂O₂ (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).

| 184 | At CH-LES, concentrations of P and N in plant tissues were determined in samples that had |
|-----|---|
| 185 | been oven-dried (55°C for 72 h) and ground in a Retsch rotor. Total N was determined after |
| 186 | combustion using the Dumas method (Masson et al., 2010), while total P was determined by |
| 187 | radial ICP-AES (Varian Vista RL Simultaneous) after incineration (480°C for 5 h) and |
| 188 | solubilization in hydrofluoric acid (Masson et al., 2010). |
| 189 | |
| 190 | At FR-ERC, FR-GRA, and RO-DÂM, concentrations of P and N in plant tissues were |
| 191 | determined in dried and ground (0.5 mm) samples. Total P was determined after wet digestion |
| 192 | in H ₂ SO ₄ -H ₂ O ₂ with ceruleomolybdic blue colorimetry (Murphy and Riley, 1962). Total N |
| 193 | concentration was determined with a CN gas analyzer (LECO Corporation, St Joseph, |
| 194 | Michigan, USA). When present in samples, legumes were sorted by hand and excluded before |
| 195 | analysis, as recommended by Jouany et al. (2004). |
| 196 | The NNI, expressed as a percentage, was calculated as the sample N concentration |
| 197 | $(N_{measured}, mg g^{-1} DM)$ divided by the critical N concentration $(N_{critical}, mg g^{-1} DM)$ which was |
| 198 | estimated from the critical N-dilution curve (Lemaire and Gastal, 1997), as follows: |
| 199 | $NNI = N_{measured} / N_{critical} \times 100 $ (1) |
| 200 | $N_{\text{critical}} = 48 \text{ (shoot DM)}^{-0.32} $ (2) |
| 201 | with $N_{measured}$ and $N_{critical}$ expressed in mg g ⁻¹ DM and shoot DM in t ha ⁻¹ . |
| 202 | The PNI and KNI, expressed as a percentages, were calculated as the sample P ($P_{measured}$, mg |
| 203 | g^{-1} DM) or K (K _{measured} , mg g^{-1} DM) concentration divided by the critical P (P _{critical} , mg g^{-1} DM) |
| 204 | or K (P _{critical} , mg g ⁻¹ DM) concentration. |
| | |

| 205 | $PNI = P_{measured} / P_{critical} \times 100; $ (3) |) |
|-----|--|---|
| 206 | $KNI = K_{measured} / K_{critical} \times 100; $ (4) |) |
| 207 | P _{critical} and K _{critical} were estimated from Duru and Ducrocq (1997), as follows: | |
| 208 | $P_{\text{critical}} = 1.50 + 0.065 \times N_{\text{measured}} $ (5) |) |
| 209 | $K_{critical} = 1.6 + 0.525 \times N_{measured}$ (6) |) |
| 210 | with $P_{measured}$ and $P_{critical}$ expressed in mg g ⁻¹ DM. | |
| 211 | 2.3. Grassland vegetation characterization | |
| 212 | At the four permanent multi-species grassland sites (CH-LES, FR-ERC, FR-GRA, and RO | - |
| 213 | DÂM), species composition was measured at the beginning of the experiments using | |
| 214 | exhaustive sorting of handfuls of vegetation, as described by De Vries and De Boer (1959). At | |
| 215 | least four species contributed 80% of community biomass (Table 1). Grasslands species | |
| 216 | compositions did not significantly change between control and fertilized plots for the whole | |
| 217 | period tested. | |
| 218 | | |
| 219 | 2.4. Soil physical and chemical properties | |
| 220 | Before the start of the experiment at each site, soil samples were taken air-dried, sieved (2 | |
| 221 | mm) and stored at ambient temperature before analysis (Table 1). Soils were analyzed using th | e |
| 222 | Swiss national reference methods for CH-LES (FAL et al., 2004) and the method | |
| 223 | recommended in the province of Quebec for CA-LEV, as reported by Bélanger and Ziadi | |
| 224 | (2008). For FR-ERC, FR-GRA and RO-DÂM sites, the soil samples were analyzed by the | |
| 225 | Laboratoire d'Analyses des Sols of the National Research Institute for Agriculture, Food and | |
| 226 | Environment (INRAE, 62000 Arras, France) using French standards (Afnor, 1994), as reported | ł |

227 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

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 sites, except CA-LEV and RO-DÂM (Table 2). Both tests are suitable for a wide range of soil 242 243 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).

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 asfollows:

255
$$P_r = v \times C_P^w \times t^p$$
 with $P_r < PrLIMIT$ (7)

where v is the value or Pr at time t = 1 min and $C_p = 1 \text{ mg P L}^{-1}$, w is the non-linear increase in Pr as a function of C_p , and p is the non-linear increase in Pr 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.

260 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 261 262 kinetics reached a steady-state for a few hours, as described by Stroia et al. (2007), Messiga et 263 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). 264 265 In order to better appreciate the amount of soil plant available P and compare the different 266 sites-treatments we expressed both Olsen P and the amount of diffusible P $(Q_w + P_r)$ as kg P ha⁻ 267 ¹by using soil dry matter (Table 1).

268 2.6. Data analysis

All statistics were performed with Statgraphics Centurion XV (version 15.2.06, StatPoint,

270 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:

| 275 | Relative DM yield = (DM yield in control plots / maximum DM yield) \times 100 | (8) |
|-----|---|-------------|
| 276 | The maximum DM yield was the yield of P-fertilized plots at sites with only one P rate (I | F R- |
| 277 | ERC, FR-GRA, and RO-DAM) or the yield of plots that received the highest P rate at sit | es with |
| 278 | several P rates (CA-LEV and CH-LES). To describe the relationship between the relative | DM |
| 279 | yield and PNI, a linear-plateau model was used, as follows: | |
| 280 | Relative DM yield = $a + b \times PNI$ if PNI < critical PNI | (9) |
| 281 | Relative DM yield = plateau of relative DM yield if PNI \geq critical PNI | (10) |
| 282 | where a and b are fitted parameters. | |
| 283 | 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
- 285 Generalized Reduced Gradient nonlinear solver.

286 **3. Results**

287 3.1. Forage yield response to the grassland P nutrition status

288 DM yield response to P fertilization varied greatly among sites. P fertilization increased DM 289 yield significantly in 2 out of 4 years at CH-LES, 1 out of 3 years at RO-DÂM, and 3 out of 9 290 years at FR-ERC, but had no effect at CH-LEV or FR-GRA. On control plots (P0), the mean 291 PNI among years was 87% at CA-LEV and 94% at FR-GRA; these values were representative 292 of adequate P nutrition for growth. Consequently, mean relative DM yields among years were 293 high, with values of 98% at CA-LEV and 96% at FR-GRA, and remained constant even when 294 the PNI increased due to P fertilization (Fig. 1a). For the three sites with a positive response to 295 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 296 297 grassland growth was P-limited (relative DM yields of 77%, 80%, and 57%, respectively) (Fig. 298 1a). The linear plateau model satisfactorily described the relationship between relative DM 299 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=9.88e05, R²=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.

309

310 *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).

| 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

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 R²=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.

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⁻ 367 ¹, 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 Olsen P kg⁻¹ at FR-GRA (Table 1) identified by Poulton et al. (1997). The experimental site of 372 373 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 374 375 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 PO 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 then remained relatively stable over time, despite negative annual P budgets (-5 and -20 kg ha⁻¹, 379 380 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 381 382 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 ThélierHuché 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.

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.

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

419 At site level, these results commined 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 (R²=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).

Positive relationships between PNI and each SPAP indicator (Fig. 2) indicates that the plant 447 448 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 together, the critical C_P value was 0.26 mg P L⁻¹ (Fig. 2a), but it varied among sites, from a 453 454 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 contents of Fe and Al oxide, and hydroxide (Stroia et al., 2007), had the lowest critical CP 458 459 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 C_P alone (Fig. 2c) demonstrated that assessing SPAP as the amount of soil diffusive oPion, 464 465 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. 466 467 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 + Pr60min) value for grasslands was 13.5 kg P ha⁻¹ (Fig. 2c), twice as large as that for maize (7.9 470

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

The assumption that maize obtains P from the plowed layer is reasonable since this layer 474 475 contains 80% of maize root biomass (Li et al., 2017) and the soil horizons below it contribute 476 little to crop P nutrition. This assumption is more questionable for permanent grasslands, in 477 which soil horizon below 5 cm may contribute greatly to sward P nutrition. This is more likely 478 to occur in non-fertilized grasslands with a soil with a low P status (Fort et al., 2016). 479 SPAP tests could be compared only for three sites where the three indicators were measured 480 (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 (Ow + Pr60min) stock ($R^2 = 0.70; P < 0.05; Fig. 4a$) or C_P 481 $(R^2 = 0.66; P < 0.05; Fig. 4b)$ did. The NaHCO₃ extraction with the Olsen procedure 482 483 represented soil-type-specific effects on oPion mobility better than C_P (Fig. 2a) or (Qw + 484 Pr60min) (Fig. 2c) did. These results for grasslands, however, differ from those for maize of 485 Morel et al. (2000), who reported that (Qw + Pr) stock explained the yield response better than 486 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.

493 Expressed on a stock basis, the Olsen P critical value was 12.9 kg P ha⁻¹ (Fig. 2b) which
494 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.

501 4.3. Strengths and limits of nutrient index approach for Phosphorus diagnosis in agro 502 ecosystems

503 Our results confirmed that PNI was a reliable indicator of the level of satisfaction of plant's P 504 needs from soil. Over large soil P gradients, a significant relationship existed between 505 grassland P nutrition status, evaluated with PNI, and SPAP, assessed according to conventional 506 soil tests. Situations exist in agro systems where nutrition index approach could be used in 507 association with soil analysis, or be an alternative to, when soil analysis is difficult to perform. 508 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 509 510 sample is tough because of nutrients stratification and non-uniform vertical repartition of 511 nutrients (; this issue specially applies to P that is not mobile in soils (Messiga et al, 2013). 512 Moreover, little soil references are available for permanent grasslands, compare to cropping 513 systems, and thresholds responses based on soil analysis are rarely available. Likewise, 514 nutrition indices could be implemented for P diagnosis in cropping system where non-uniform 515 nutrients repartition makes soil sampling more tedious (no tillage, Conservation agriculture, 516 precision farming) and where available soil thresholds and references are unsuitable.. 517 In other situations, PNI allows specifying, or even correct, soil diagnosis as it is the case for 518 CA-LEV site in this study. Although initial soil P status was diagnosed as limiting for growth, 519 based on soil P test, our study demonstrated that forage P nutrition status was adequate on

520 control treatment plots and its conclusion questioned soil based diagnosis, or at least the521 method used.

522 On an other hand, this approach presents some limits since the 'critical' P concentrations (Eq.

523 4) that serves for PNI calculations (Eq. 3) were more an approximation of critical P

524 concentration than critical concentration *sensu stricto* as defined in Justes et al. (1994). As a

525 consequence, P critical value (Eq. 4) was higher and PNI lower than expected. This bias results

526 in underestimation of forage P status diagnosis and, as a consequence, a risk of excess P supply

527 in subsequent fertilization recommendations.

528 In order to improve the reliability of the nutrient index approach for P diagnosis, a more precise

529 critical curve is necessary. In that purpose, the interaction between N and P must be

530 investigated on P deficient grasslands, by combining different P and N doses in order to

531 identify precisely the true critical P concentration.

532

533 5. Conclusions

534 This study of five long-term grassland experiments conducted on contrasting soil types and 535 climates demonstrated that a direct and general relationship exists between PNI and the forage 536 yield of the first growth cycle. Forage yield increased linearly as sward P nutrition status, 537 assessed by the PNI, increased up to nearly 100%. This critical threshold differentiated P-538 limited growth from that with adequate P nutrition. Significant positive relationships between 539 PNI and three SPAP indicators confirmed that the soil P status influences sward P nutrition 540 status. The Olsen P extraction procedure provided the best fit with PNI, with a critical value of 541 12.9 kg ha⁻¹.

542 The study confirmed the potential of using plant analysis and nutrient indices for P diagnosis in 543 forage systems and making fertilizer P recommendations. Our study highlights the utility of 544 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.

547 Author contribution section

- 548 Claire Jouany: Conceptualization, Investigation, Formal analysis, Writing original draft,
- 549 Writing review and editing. Christian Morel: Conceptualization, Investigation, Formal
- analysis, Writing review and editing. Noura Ziadi: Conceptualization, Investigation, Writing
- 551 review and editing. Gilles Bélanger: Conceptualization, Investigation, Writing review and
- 552 editing. Sokrat Sinaj: Investigation, Writing review and editing. Ciprian Stroia:
- 553 Investigation, Writing review and editing. Pablo Cruz: Investigation, Writing Review and
- 554 Editing. Jean-Pierre Theau: Investigation, Writing review and editing. Michel Duru:
- 555 Writing review and editing.
- 556

557 **Declaration of competing interest**

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

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Table 1. 798

Climate, soil, and management characteristics of the five experimental sites; NA = no data available 799

| 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 |

803 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.

806

| | | 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 |

808

809 Table 3.

Mean (and standard error) estimates of parameters v, w and p of the Freundlich kinetic equation $(P_r = v \times C_p^w \times t^p)$. 810

811

| 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.

814 Table 4.

815 Mean Phosphorus nutrition Index (PNI), soil solution oPions concentration (Cp), Olsen extracted Phosphorus (Olsen-P) and diffusive P (Qw +

816 Pr60min); NA = no data available.

817

| Site | Treatment | PNI ¹ | | Ср | | Olsen-P | | (Qw + Pr60min) | |
|--------|-----------|------------------|-------|--------------|----|--------------------|----|----------------------------|----|
| | | (%) | | mg P 1^{1} | | $mg P kg^{-1} sol$ | 1 | mg P kg ⁻¹ soil | |
| CA-LEV | P0 | 83 | a^2 | 0.3 | а | NA | NA | 22.3 | а |
| | 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 | с | NA | NA | 49.2 | с |
| CH-LES | P0 | 51 | а | 0.18 | а | 12.5 | а | 18.8 | a |
| | P1 | 86 | b | 0.24 | ab | 16.7 | а | 21.5 | ab |
| | P2 | 90 | bc | 0.32 | b | 23 | b | 24.1 | b |
| | P3 | 99 | c | 0.34 | b | 32.3 | с | 25 | b |
| FR-ERC | P0 | 59 | а | 0.042 | а | 10.3 | а | 19.8 | a |
| | P1 | 102 | b | 0.18 | b | 45.3 | b | 39.4 | b |
| FR-GRA | P0 | 89 | а | 0.38 | а | 14.5 | а | 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)

- 820 Fig. 1. Relationship between relative dry matter yield (RDMY) and Phosphorus nutrition index (PNI) a) the average RDMY as a function of the
- 821 average PNI, with averages computed over all years for each site-treatment combination; C represents the critical PNI (%) b) RDMY
- 822 measurements as a function of PNI measurements, for each site-treatment combination. The line corresponds to the linear regression of PNI on 823 RDMY, and *p* is the Fisher p-value.
- 824
- 825
- 826

a

RDMY (%)







PNI (%)

831 Fig. 2. Relationship between the phosphorus (P) nutrition index (PNI), measured at the plot scale, and soil plant-available P (SPAP); a) SPAP=

- 832 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 +
- 833 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.
- 834
- 835

837 838

836

a

PNI (%)









847

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 × C_P^w ×t^p) (see Table 3 for values of parameters, v, w, and p)





854 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 855 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.



865 Supplementary materials

866

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)

870

| Year | Treatment | DMY | | Ν | Ν | | Р | |
|------|-----------|-------|-------|--------------------|-------|------|-------|--|
| | | t ha⁻ | 1 | mg g ^{_1} | 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) | |

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)

874

| Year | Treatment | DMY | Ν | Р |
|------|-----------|--------------------|--------------------|--------------------|
| | | t ha ⁻¹ | mg g ⁻¹ | mg g ⁻¹ |
| 1997 | PO | 3.7 (0. | 3) 13.8 (0.3 | 3) 1.8 (0.1) |
| | P9 | 4.0 (0. | 1) 13.0 (0.6 | 6) 2.0 (0.1) |
| | P17 | 3.6 (0. | 4) 13.8 (0.7 | 7) 2.2 (0.1) |
| | P26 | 4.2 (0. | 2) 14.2 (0.9 | 9) 2.3 (0.1) |
| 2001 | P0 | 3.1 (0. | 3) 14.8 (0.2 | 2) 1.6 (0.1) |
| | P9 | 3.5 (0. | 3) 14.3 (0.5 | 5) 2.0 (0.1) |
| | P17 | 3.6 (0. | 4) 14.5 (0.9 | 9) 2.2 (0.1) |
| | P26 | 3.7 (0. | 1) 15.1 (0.6 | 6) 2.4 (0.0) |
| 2005 | P0 | 2.4 (0. | 2) 15.4 (0.2 | 2) 1.3 (0.1) |
| | P9 | 3.0 (0. | 2) 16.2 (0.3 | 3) 2.0 (0.1) |
| | P17 | 4.0 (0. | 3) 16.1 (0.6 | 6) 2.1 (0.1) |
| | P26 | 3.5 (0. | 4) 15.5 (0.9 | 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) 879

| Year | Treatment | DMY | | Ν | | Р | |
|------|-----------|--------------------|-------|--------------------|-------|------|-------|
| | | t ha ⁻¹ | | mg g ⁻¹ | l | 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 | N | | Р | |
|------|-----------|-------------------|--------------------|------|--------------------|-----|-------|--|
| | | t ha ⁻ | t ha ⁻¹ | | mg g ⁻¹ | | -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 | | Ν | | Р | |
|------|-----------|---------------------------|-------|--------------------|-------|--------------------|-------|
| | | | | 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) |