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1 **Plant and soil tests to optimize phosphorus fertilization management of**
2 **grasslands**

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25 **Highlights**

- 26 • 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 Developing more sustainable forage systems requires efficient decision support tools for
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
35 concentration of shoot biomass were developed to assess the P nutrition status of grasslands.
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 (≥ 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)
42 concentration (mg P L^{-1}), Olsen P (mg P kg^{-1}), and, a process-based assessment ($Q_w + P_r$) from
43 the sum of oPion in the soil solution (Q_w , mg P kg^{-1}) and diffusive oPion with time and C_P (P_r ,
44 mg P kg^{-1}). PNI was calculated as sward P concentration divided by the critical P
45 concentration.

46 . The cumulative effect of P fertilization resulted in a wide range of SPAP values. Overall, C_P
47 varied from $0.03\text{-}3.6 \text{ mg P L}^{-1}$, ($Q_w + P_r$) from $6\text{-}52 \text{ mg kg}^{-1}$, and Olsen P from $4\text{-}40 \text{ mg}$
48 kg^{-1} . The PNI varied from 48 %-94% in plots with no applied P, and from 83 %-121% in P-
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
53 stock basis for a target PNI value of 92% were similar for Olsen P ($12.9 \text{ kg P ha}^{-1}$) and ($Q_w +$
54 P_r) ($13.5 \text{ kg P ha}^{-1}$). 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

58

59 **1. Introduction**

60 Grasslands provide an essential source of cattle feed, and the development of more
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
63 by the amounts of nutrients supplied by soil reserves and/or organic or mineral fertilizers
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
73 assessing nutrient status and managing fertilizers have been developed. Nutrient concentration
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
78 (Güsewell, 2004). Soil P tests inform on soil plant available P (SPAP) and provide response
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,

84 for a given crop or grassland sward, a single threshold value regardless of the soil type (Schulte
85 and Herlihy, 2007; Jordan-Meille et al., 2012).

86 More recently, significant advances have been made with the development of innovative
87 assessment tools. The approach of nutrition indices based on the nutrient concentration of shoot
88 biomass allows grassland P nutrition status to be assessed during growth (Duru and Ducrocq,
89 1997). For P, this approach is more reliable than those based on a single critical concentration
90 since it considers changes in nutrient concentration as a function of sward biomass
91 accumulation and concentration of other nutrients (Duru and Ducrocq, 1997). The P nutrition
92 index (PNI) is an effective tool for P fertilization management in grasslands since it assesses
93 the sward P nutrition status well during growth and can verify and validate fertilization
94 practices *a posteriori* (Thélier-Huché et al., 1999). The PNI is adequate for the interpreting of
95 the effect of plant P nutrition status on plant growth in grasslands managed at different
96 intensities (Liebisch et al., 2013); as well, PNI provides appropriate plant nutrients status
97 evaluation at the interface between agricultural land and saline wetlands in protected saline
98 habitats (Luna et al., 2019). At an ecosystem level, the N nutrition index (NNI) and PNI
99 provide appropriate evaluation of the functional response of species and communities to
100 fertility gradients induced by practices (Garnier et al., 2007; Lavorel et al., 2009).

101 At the same time, Morel (2002) developed a mechanistic model based on the assumption
102 that the SPAP pool represents the sum of the amount of orthophosphate ions (oPion) in the soil
103 solution (Q_w) and the amount of soil P that can diffuse from the soil to the solution over time
104 (P_f). 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 P_r as a function of
110 the P_{ion} concentration in soil solution (C_P) and time (Morel, 2002). This model correctly
111 simulated the changes in SPAP in the 0-5 cm soil horizon over seven years from a long-term
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 ($Q_w + P_r$) 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*

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

130 LÉVIS, CANADA (CA-LEV). A grassland experiment, sown with timothy (*Phleum pratense*
131 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

133 (P15), 30 (P30), and 45 (P45) kg P ha⁻¹] assigned to main plots, and four application rates of N
134 fertilizer as calcium ammonium nitrate [0 (N0), 60 (N60), 120 (N120), and 180 (N180) kg N
135 ha⁻¹] assigned to subplots. Experimental treatments were replicated in four blocks. For this
136 study, plots that received the four P application rates and 120 kg N ha⁻¹ were selected within the
137 experimental setup. From 1999-2006, fertilizers were applied each year before the start of
138 growth in the first week of May. Potassium (K) as KCl was applied at 84 kg K ha⁻¹ as the same
139 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
144 (P0), 9 (P9), 17 (P17), and 26 (P26) kg P ha⁻¹] in plots arranged in a randomized complete-
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
147 different rates according to the P treatment: 0, 29, 58 and 116 kg K ha⁻¹ for treatments P0, P9,
148 P17, and P26, respectively. N was applied at a rate of 25 kg N ha⁻¹ as ammonium nitrate in all
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
155 with four replicates. N was applied as ammonium nitrate at rates of 100 kg N ha⁻¹ in February
156 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
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-Drăgoslavele
160 corridor, Romania (Table 1). The grassland had a mixture of *F. rubra* and *A. capillaris*. The
161 experiment consisted of two rates of P fertilizer [0 (P0) and 33 (P33) kg P ha⁻¹] applied in
162 autumn each year as super phosphate on plots arranged in a randomized complete-block design
163 with four replicates. N was applied as ammonium nitrate at 100 kg N ha⁻¹ in early spring and 50
164 kg N ha⁻¹ after the first cut. Potash salt was added at a rate of 108 kg K ha⁻¹ once a year in
165 autumn to ensure that K did not limit plant growth (Ciubotariu et al., 2002).

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

169 2.2. Yield and nutrition index determination

170 Dry matter (DM) yield and nutrient concentration were measured at the end of the first
171 growth cycle (Table 2), a period of the year when growth is rarely limited by water. Forage
172 production in this first growth cycle represented 40%-70% of the average total annual
173 production, depending on the site and treatment. All plots were harvested again during the rest
174 of the growing season, but no measurements were taken. DM yield was measured by cutting an
175 area of at least 1m² to a height of 5-cm. A fresh sample of ca. 300-500 g was taken, dried at 55
176 °C for 48 h, and ground.

177 At CA-LEV, dried and ground (1 mm) forage samples (0.1 g) were wet-digested with 1.5
178 mL H₂SO₄-H₂SeO₃ and 2.0 mL H₂O₂ (Isaac and Johnson, 1976). N and P concentrations were
179 measured by colorimetry using an automated continuous-flow injection analyzer (QuickChem
180 8000 FIA+ analyzer; Lachat Instruments, Loveland, Colorado, USA) with the salicylate-

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

183

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 H₂SO₄-H₂O₂ 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:

$$199 \quad \text{NNI} = N_{\text{measured}}/N_{\text{critical}} \times 100 \quad (1)$$

$$200 \quad N_{\text{critical}} = 48 (\text{shoot DM})^{-0.32} \quad (2)$$

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

206 $KNI = K_{\text{measured}}/K_{\text{critical}} \times 100;$ (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 $K_{\text{critical}} = 1.6 + 0.525 \times N_{\text{measured}}$ (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 the
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
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
230 ammonium acetate-EDTA mixture was used at the CH-LES site (Demaria et al., 2005), and
231 Mehlich-3 extractable P was used at the CA-LEV site (Tran and Simard, 1993).

232

233 *2.5 Soil plant available P*

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

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

252 that can diffuse (P_r , mg P kg⁻¹ soil) over time (t , minutes) and the soil-solution P
253 concentration (C_p , mg P L⁻¹ solution). This equation, the Freundlich kinetic equation, is as
254 follows:

$$255 \quad P_r = v \times C_p^w \times t^p \text{ with } P_r < P_{rLIMIT} \quad (7)$$

256 where v is the value of P_r at time $t = 1$ min and $C_p = 1$ mg P L⁻¹, w is the non-linear increase in
257 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
258 v , w and p are specific to soil at each site. The value of P_{rLIMIT} , which cannot be determined
259 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),
261 by combining studies of sorption-desorption in soil suspensions in which isotopic dilution
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
264 calculated by multiplying C_p by the solution-to-soil ratio (solution volume /soil mass).
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⁻¹
267 by using soil dry matter (Table 1).

268 2.6. Data analysis

269 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
271 for PNI, C_p , ($Q_w + P_{r60min}$) and Olsen P. Statistical significance ($P < 0.05$) was tested using a
272 Fisher's exact test.

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

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

276 The maximum DM yield was the yield of P-fertilized plots at sites with only one P rate (FR-
277 ERC, FR-GRA, and RO-DAM) or the yield of plots that received the highest P rate at sites 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 × PNI if PNI < critical PNI (9)

281 Relative DM yield = plateau of relative DM yield if PNI ≥ 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
284 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
296 years of 61% at CH-LES, 66% at FR-ERC, and 48% at RO-DÂM. These values indicated that
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

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

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

309

310 *3.2 Soil plant available P*

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

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

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

322 Data obtained from sorption-desorption and isotope dilution procedures showed that, for
323 each site, the total amount of diffusive P in soils (P_r) 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} , P_r 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 ($Q_w +$
329 P_r) values for increasingly long periods of diffusion. For each value of t , a general relationship
330 was built between ($Q_w + P_r$) and PNI (data not shown); the best fit was obtained for a diffusion
331 time t of 60 minutes ($P_{r60\text{min}}$) (Fig. 2c).

332 Like C_P , the mean amount of diffusive P ($Q_w + P_{r60\text{min}}$) varied at each site with treatments
333 (Table 4). When several P fertilizer rates were applied (CH-LES and CA-LEV), the maximum
334 ($Q_w + P_{r60\text{min}}$) 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 ($Q_w + P_{r60\text{min}}$) varied from 18.8 mg kg^{-1} at CH-LES to 22.3
337 mg kg^{-1} at CA-LEV and, from 21.5 mg kg^{-1} for CH-LES (P1) to 84.3 mg kg^{-1} at FR-GRA for
338 fertilized treatments (Table 4).

339

340 *3.3. Relationship between P nutrition index and soil plant available P*

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

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

352 **4. Discussion**

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

354 A general relationship between relative DM yield and PNI was obtained by combining data
355 sets from five long-term experiments at contrasting sites with different response patterns to P
356 fertilization (Fig. 1 a). For CA-LEV and FR-GRA, no significant yield response to P
357 fertilization was observed throughout the experiments. At both sites, mean PNI values greater
358 than 80%, for control treatment plots, indicated that P did not limit growth (Duru and Ducrocq,
359 1997); consequently, mean relative DM yields remained close to 100% and did not vary. At
360 both sites, soil reserves provided enough P to meet grassland requirements and to maintain
361 optimal sward P nutrition for nine years at CA-LEV and seventeen years at FR-GRA. At CH-
362 LES, FR-ERC, and RO-DÂM, PNI values less than 80%, for control treatment plots, indicated
363 that growth was P-limited (Duru and Ducrocq, 1997). Soil reserves did not provide enough P to
364 meet grassland requirements for optimal growth and, consequently, a significant yield response
365 to P addition was observed. At CH-LES site, with four P rates tested, we observed a similar
366 response pattern to P; PNI increased in proportion with fertilizer rate between 0 and 26 kg P ha^{-1}
367 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
372 Olsen P kg⁻¹ at FR-GRA (Table 1) identified by Poulton et al. (1997). The experimental site of
373 CA-LEV was chosen because of its expected positive response to P fertilization (Bélanger and
374 Ziadi, 2008). Its soil (Mehlich-3 extraction; 0-15 cm) contained 54 kg available P ha⁻¹ (Table
375 1) and was considered poor in P according to local recommendations (Conseil des Productions
376 Végétales du Québec, 1996). During the experiment at FR-GRA, SPAP measured in the P0
377 treatment remained above the critical value despite large negative annual P budget (-35 kg ha⁻¹;
378 Stroia, 2007). At CA-LEV, SPAP measured in the P0 and P15 treatments first decreased and
379 then remained relatively stable over time, despite negative annual P budgets (-5 and -20 kg ha⁻¹,
380 respectively) (Messiga et al., 2015). At FR-ERC and CH-LES, SPAP values laid below 25 mg
381 Olsen P kg⁻¹ at the start of the experiments, and they decreased over time as cumulative P
382 budgets became more negative (Messiga et al., 2015).

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

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

396 (92%) and the statistical value (100%) given by Duru and Théliier-Huché (1997) and Théliier-
397 Huché et al. (1999) is likely due to the smallest dataset that they used to determine the critical
398 P-dilution curve or to establish the relationship between relative yield and PNI.

399 This study provides the first evidence that the relative DM yield of a P-limited sward, , is a
400 direct function of its P status assessed with PNI, within different site-treatments. These results
401 validate the PNI approach as an adequate tool for *a posteriori* assessment of grassland P
402 nutrition. They extend conclusions of studies conducted on grasslands at local scales (Duru et
403 al., 1993; Duru and Ducrocq, 1997; Duru and Théliier-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 Théliier 1997), we tested to what extent NNI and KNI, for CH-LES site, could explain relative
414 DM yield variability between years.

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

419 At site level, these results confirmed that for both sites and treatment optimal N and P
420 nutrition allowed optimal biomass production. On the other hand, negative relationships

421 between relative DMY and PNI at CA-LEV P0 and CA-LEV P1 (Fig. 1b) could result from P
422 luxury consumption and accumulation which increased as relative DM decreased.

423 For CH-LES, there was no significant relationship between NNI and PNI, relative DMY
424 and NNI and between relative DMY and KNI for any of the treatments. Including NNI or KNI
425 as co variable did not improve the model between relative DM and PNI for CH-LES P0 (Fig.
426 1b). Finally, when pooling the three CH-LES data sets, we got a significant relationship
427 between relative DMY and NNI ($R^2=0.44$; $p=0.018$); best fit was obtained with PNI as a single
428 variable ($R^2=0.65$; $p=0.0016$). This result demonstrated that, under P and N limited conditions,
429 inter annual relative DM variability was better explained by PNI than NNI.

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

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

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

445

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

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

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

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.

474 The assumption that maize obtains P from the plowed layer is reasonable since this layer
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
481 ($R^2 = 0.85$; $P < 0.05$; Fig. 2b) than the (Qw + Pr60min) stock ($R^2 = 0.70$; $P < 0.05$; Fig. 4a) or C_P
482 ($R^2 = 0.66$; $P < 0.05$; Fig. 4b) did. The NaHCO₃ extraction with the Olsen procedure
483 represented soil-type-specific effects on P_{ion} 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.

487 We hypothesize that the better fit observed for Olsen P is due to its ability to extract organic
488 P, which is partly mineralized when measuring phosphate ions. This is particularly likely to
489 occur when the test is performed for grassland soils rich in organic matter (Bowman and Cole,
490 1978). Tate et al. (1991) demonstrated that soil organic P pool in grassland soils can contribute
491 greatly to grassland P nutrition. In contrast, the (Qw + Pr) stock includes only the inorganic P
492 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).

495 The comparison of response curves showed that, for both Olsen P and (Qw + Pr),
496 correlations were stronger when the PNI was related to SPAP measured in the 0-5 cm soil
497 horizon instead of the 0-10 cm horizon (data not shown). This result confirms that surface soil
498 horizons, where high nutrient concentrations (Kidd et al., 2017) foster intensive exploitation
499 and acquisition of resources by roots (Fort et al., 2016), contribute greatly to grassland P
500 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
509 permanent grasslands where soil analysis is difficult to perform. Taking a representative soil
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 the
521 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
550 analysis, Writing - review and editing. **Noura Ziadi**: Conceptualization, Investigation, Writing
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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**

558 The authors declare no conflict of interest of any kind that could have influenced the work
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560

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573 **References**

- 574 Afnor, 1994. *Qualité des sols*, vol. 1, Recueil de normes. Afnor, La Défense, Paris
- 575 Bailey, J., Beattie, J., Kilpatrick, D., 1997. The diagnosis and recommendation integrated
576 system (DRIS) for diagnosing the nutrient status of grassland swards: I. Model
577 establishment. *Plant Soil* 197, 127–135. <https://doi.org/10.1023/A:1004236521744>
- 578 Bailey, J.S., Dils, R.A., Foy, R.H., Patterson, D., 2000. The Diagnosis and Recommendation
579 Integrated System (DRIS) for diagnosing the nutrient status of grassland swards: III
580 Practical applications. *Plant Soil* 222, 255–262. <https://doi.org/10.1023/A:1004827206618>
- 581 Barber, S.A., 1984. *Soil nutrient bioavailability. A mechanistic approach*. John Wiley & Sons,
582 New York, 398p.
- 583 Barbier, G., Fardeau, J.-C., Marini, P., 1971. Sur la diffusivité des ions phosphates du sol. *Ann.*
584 *Agron.* 22, 309–342.
- 585 Bélanger, G., Richards, J.E., Walton, R.B., 1989. Effects of 25 years of N, P and K fertilization
586 on yield, persistence and nutritive value of a timothy sward. *Can. J. Plant Sci.* 69, 501–512.
587 <https://doi.org/10.4141/cjps89-061>
- 588 Bélanger, G., Tremblay, G. F., Mongrain, D., 2008. Yield and nutritive value of the spring
589 growth of an ageing timothy sward. *Can. J. Plant Sci.* 88, 457-464.
590 <https://doi.org/10.4141/cjps07154>
- 591 Bélanger, G., Ziadi, N., 2008. Phosphorus and nitrogen relationships during spring growth of
592 an aging timothy sward. *Agron. J.* 100, 1757-1762. <https://doi.org/10.2134/agronj2008.0132>
- 593 Bowman, R.A., 1989. A sequential extraction procedure with concentrated sulfuric acid and
594 dilute base for soil organic phosphorus. *Soil. Sci. Soc. Am. J.* 53, 362-366.
595 <https://doi.org/10.2136/sssaj1989.03615995005300020008x>

596 Cadot, S., Bélanger, G., Ziadi, N., Morel, C., Sinaj, S., 2018. Critical plant and soil phosphorus
597 for wheat, maize, and rapeseed after 44 years of P fertilization. *Nutr. Cycling Agroecosyst.*
598 112, 417–433. <https://doi.org/10.1007/s10705-018-9956-0>

599 Carrère, P., Farruggia, A., Dumont, B., Hulin, S., Theau, J.P., 2020. Valoriser les services
600 rendus par la prairie. Une voie pour assurer la durabilité des systèmes d'élevage herbagers ?
601 In *Les services écosystémiques dans les espaces agricoles. Paroles de chercheur(e)s*, pp.39-
602 50. https://doi.org/10.15454/nwq9-zk60_book_ch04

603 Ceulemans, T., Merckx, R., Hens, M., Honnay, O., 2011. A trait-based analysis of the role of
604 phosphorus vs. nitrogen enrichment in plant species loss across North-west European
605 grasslands. *J. Appl. Ecol.* 48, 1155–1163. <https://doi.org/10.1111/j.1365-2664.2011.02023.x>

606 Colwell, J.D., 1963. The estimation of phosphorus fertilizer requirements of wheat in southern
607 New South Wales by soil analysis. *Anim. Prod. Sci.* 3, 190-197.
608 <https://doi.org/10.1071/EA9630190>

609 Conseil des Productions Végétales du Québec. 1996. Fertilisation reference guide. (In French.)
610 Agdex 540. Gouvernement du Québec, Ministère de l'Agriculture, des Pêcheries et de
611 l'Alimentation, Québec, QC, Canada.

612 Cordell, D., White, S., 2011. Peak phosphorus: clarifying the key issues of a vigorous debate
613 about long-term phosphorus security. *Sustainability* 3, 2027–2049.

614 CRAA, 2016. Chambre Régionale d'Agriculture d'Auvergne [https://extranet-puy-de-](https://extranet-puy-de-dome.chambres-agriculture.fr/fileadmin/user_upload/National/FAL_commun/publications/Auvergne-Rhone-Alpes/Guide_regional_fertilisation_sept_2016.pdf)
615 [dome.chambres-](https://extranet-puy-de-dome.chambres-agriculture.fr/fileadmin/user_upload/National/FAL_commun/publications/Auvergne-Rhone-Alpes/Guide_regional_fertilisation_sept_2016.pdf)
616 [agriculture.fr/fileadmin/user_upload/National/FAL_commun/publications/Auvergne-](https://extranet-puy-de-dome.chambres-agriculture.fr/fileadmin/user_upload/National/FAL_commun/publications/Auvergne-Rhone-Alpes/Guide_regional_fertilisation_sept_2016.pdf)
617 [Rhone-Alpes/Guide_regional_fertilisation_sept_2016.pdf](https://extranet-puy-de-dome.chambres-agriculture.fr/fileadmin/user_upload/National/FAL_commun/publications/Auvergne-Rhone-Alpes/Guide_regional_fertilisation_sept_2016.pdf)

618 Craine, J.M., Morrow, C., Stock, W.D., 2008. Nutrient concentration ratios and co-limitation in
619 South African grasslands. *New Phytol.* 179, 829-836. [https://doi.org/10.1111/j.1469-](https://doi.org/10.1111/j.1469-8137.2008.02513.x)
620 [8137.2008.02513.x](https://doi.org/10.1111/j.1469-8137.2008.02513.x)

621 De Vries, D.M., De Boer, T.A., 1959. Methods used in botanical grassland research in the
622 Netherlands and their application. *Herb. Abstr.* 29, 1-7.

623 Demaria, P., Flisch, R., Frossard, E., Sinaj, S., 2005. Exchangeability of phosphate extracted by
624 four chemical methods. *J. Plant Nutr. Soil Sci.* 168, 89–93.
625 <https://doi.org/10.1002/jpln.200421463>

626 Duru, M., 1992. Diagnostic de la nutrition minérale de prairies permanentes au printemps. I.
627 Etablissement de références. *Agronomie* 12, 219-233. <https://hal.inrae.fr/hal-02714180>

628 Duru, M., Colomb, B., Cransac, Y., Fardeau, J.-C., Julien, J.-L., Rozière, M., 1993. Pédoclimat,
629 fertilisation et croissance des prairies permanentes au printemps I. Variabilité de la nutrition
630 minérale. *Fourrages* 133, 23-41. <https://hal.inrae.fr/hal-02713980>

631 Duru, M., Ducrocq, H., 1997. A nitrogen and phosphorus herbage nutrient index as a tool
632 assessing the effect of N and P supply on the dry matter yield of permanent pastures. *Nutr.*
633 *Cycling Agroecosyst.* 47, 59-69. <https://hal.inrae.fr/hal-02695147>

634 Duru, M., Thélier-Huché, L., 1997. N and P-K status of herbage: Use for diagnosis of
635 grasslands. In: G. Lemaire & I.G. Burns (eds.) *Diagnostic procedures for crop N*
636 *management and decision-making*. INRA, pp.125-138. <https://hal.inrae.fr/hal-02771864>

637 Duru, M., Jouany, C., Theau, J.-P., Granger, S., Cruz, P., 2015. A plant-functional-type
638 approach tailored for stakeholders involved in field studies to predict forage services and
639 plant biodiversity provided by grasslands. *Grass Forage Sci.* 70, 2-18.
640 <https://doi.org/10.1111/gfs.12129>

641 FAL, RAC, FAW. 2004 Méthodes de référence des stations fédérales de recherches
642 agronomiques. *Agroscope*, vol 2, Zürich-Reckenholz

643 Fort, F., Cruz, P., Lecloux, E., Bittencourt de Oliveira, L., Stroia, C., Theau, J.-P., Jouany, C.,
644 2016. Grassland root functional parameters vary according to a community-level resource

645 acquisition: conservation trade-off. *J. Veg. Sci.* 27, 749-758.
646 <https://doi.org/10.1111/jvs.12405>

647 Garnier, E., Lavorel, S., Ansquer, P., Castro, H., Cruz, P., Dolezal, J., Eriksson, O., Fortunel,
648 C., Freitas, H., Golodets, C., Grigulis, K., Jouany, C., Kazakou, E., Kigel, J., Kleyer, M.,
649 Lehsten, V., Leps, J., Meier, T., Pakeman, R., Papadimitriou, M., Papanastasis, V., Queded,
650 H., Quétier, F., Robson, M., Roumet, C., Rusch, G., Skarpe, C., Sternberg, M., Theau, J. P.,
651 Thébault, A., Vile, D., Zarovali, M., 2007. Assessing the effects of land-use change on plant
652 traits, communities and ecosystem functioning in grasslands : A standardized methodology
653 and lessons from an application to 11 European sites. *Ann. Bot.* 99, 967-985.
654 <https://doi.org/10.1093/aob/mcl215>

655 Gagnon, B., Ziadi, N., Bélanger, G., Parent, G., 2020. Validation and use of critical phosphorus
656 concentration in maize. *Eur. J. Agron.* 120. <https://doi.org/10.1016/j.eja.2020.126147>

657 Griffin, T., Gilbertson, E., Wiedenhoef, M., 2002. Yield response of long-term mixed grassland
658 swards and nutrient cycling under different nutrient sources and management regimes.
659 *Grass. Forage Sci.* 57, 268-278. <https://doi.org/10.1046/j.1365-2494.2002.00325.x>

660 Güsewell, S., 2004. N:P ratios in terrestrial plants: variation and functional significance. *New*
661 *Phytol.* 164, 243–266. <https://doi.org/10.1111/j.1469-8137.2004.01192.x>

662 Isaac, R.A., Johnson W.C., 1976. Determination of total nitrogen in plant tissue, using a block
663 digester. *J. Assoc. Off. Anal. Chem.* 59:98–100.

664 Janssens, F., Peeters, A., Tallowin, J.R.B., Bakker, J.P., Bekker, R.M., Fillat, F., Oomes,
665 M.J.M., 1998. Relationship between soil chemical factors and grassland diversity. *Plant Soil*
666 202, 69–78. <https://doi.org/10.1023/A:1004389614865>

667 Jeangros, B., Sinaj, S., 2018. Besoins en phosphore et en potassium d'une prairie de fauche du
668 Jura riche en fétuque rouge. *Recherche Agronomique Suisse* 9, 192-199.

669 Johnston, A.E., Poulton, P.R., 2019. Phosphorus in Agriculture: A Review of Results from 175
670 Years of Research at Rothamsted, UK. *J. Environ. Qual.* 48, 1133-1144.
671 <https://doi.org/10.2134/jeq2019.02.0078>

672 Jordan-Meille, L., Rubæk, G. H., Ehlert, P., Genot, V., Hofman, G., Goulding, K., Recknagel,
673 J., Provolo, G., Barraclough, P., 2012. An overview of fertilizer-P recommendations in
674 Europe: soil testing, calibration and fertilizer recommendations. *Soil Use Manage.* 28, 419–
675 435. <https://doi.org/10.1111/j.1475-2743.2012.00453.x>

676 Jouany, C., Cruz, P., Petibon, P., Duru, M., 2004. Diagnosing phosphorus status of natural
677 grassland in the presence of white clover. *Eur. J. Agron.* 21, 273-285.
678 <https://doi.org/10.1016/j.eja.2004.06.001>

679 Jouany, C., Cruz, P., Daufresne, T., Duru, M., 2011. Biological phosphorus cycling in
680 grasslands: interactions with nitrogen. In: Else Bünemann, Astrid Oberson, Emmanuel
681 Frossard, Phosphorus in action. *Biological Processes in Soil Phosphorus Cycling* (p. 275-
682 294.). Heidelberg, DEU : Springer - Verlag. 20 p. [https://doi.org/10.1007/978-3-642-
683 15271-9_11](https://doi.org/10.1007/978-3-642-15271-9_11)

684 Kidd, J., Manning, P., Simkin, J., Peacock, S., Stockdale, E. 2017. Impacts of 120 years of
685 fertilizer addition on a temperate grassland ecosystem. *PLoS ONE* 12, e0174632.
686 <https://doi.org/10.1371/journal.pone.0174632>

687 Lachat Instruments. 2005. Methods list for automated ion analyzers (flow injection analyses,
688 ion chromatography). Available at www.lachatinstruments.com/applications/Methods.asp
689 (accessed 9 Feb. 2007, 5 Oct.2007; verified 1 Oct. 2008). Lachat Instruments, Loveland,
690 CO.

691 Lavorel, S., Gachet, S., Sahl, A., Colace, M.-P., Gaucherand, S., Burylo, M., Bonet, R., 2009.
692 A plant functional traits data base for the Alps. In book: *Data mining for global trends in*

693 mountain biodiversity (pp.107-122) Publisher: Boca Raton: CRC Press Editors: EM Spehn,
694 C Körner. <https://doi.org/10.1201/9781420083705>

695 Lemaire, G., Gastal, F., 1997. N Uptake and Distribution in Plant Canopies. In: Lemaire G.
696 (eds) *Diagnosis of the Nitrogen Status in Crops*. Springer, Berlin, Heidelberg.

697 Li, H., Mollier, A., Ziadi, N., Shi, Y., Parent, L.-E., Morel, C. 2017. The long-term effects of
698 tillage practice and phosphorus fertilization on the distribution and morphology of corn
699 root. *Plant Soil* 412, 97-114. DOI 10.1007/s11104-016-2925-y

700 Liebisch, F., Bünemann, E., Huguenin-Elie, O., Jeangros, B., Frossard, E., Oberson, A., 2013.
701 Plant phosphorus nutrition indicators evaluated in agricultural grasslands managed at
702 different intensities. *Eur. J. Agron.* 44, 67– 77. <https://doi.org/10.1016/j.eja.2012.08.004>

703 Luna, E., Jouany, C., Castañeda del Alamo, C., 2019. Soil composition and plant nutrients at
704 the interface between crops and saline wetlands in arid environments in NE Spain. *Catena*,
705 173, 384-393. <https://doi.org/10.1016/j.catena.2018.10.032>

706 Marriott, C.A., Fothergill, M., Jeangros, B., Scotton, M., Louault, F., 2004. Long-term impacts
707 of extensification of grassland management on biodiversity and productivity in upland
708 areas. *Agronomie* 24, 447–462. <https://doi.org/10.1051/agro:2004041>

709 Masson P, Dalix T, Bussiere S (2010) Determination of Major and Trace Elements in Plant
710 Samples by Inductively Coupled Plasma-Mass Spectrometry. *Communications in Soil
711 Science and Plant Analysis* 41, 231-243 with <https://doi.org/...>

712 Messiga, A. J., Ziadi, N., Bélanger, G., Morel, C., 2012. Process-based mass-balance modeling
713 of soil phosphorus availability in a grassland fertilized with N and P. *Nutr. Cycling
714 Agroecosyst.* 92, 273–287. <https://doi.org/10.1007/s10705-012-9489-x>

715 Messiga, A. J., Ziadi, N., Bélanger, G., Morel, C. (2013). Soil nutrients and other major
716 properties in grassland fertilized with nitrogen and phosphorus. *Soil Sci. Soc. Am. J.*, 77
717 (2), 643-652. <https://doi.org/10.2136/sssaj2012.0178>

718 ...

719 Messiga, A. J., Ziadi, N., Jouany, C., Virkajarvi, P., Suomela, R., Sinaj, S., Bélanger, G.,

720 Stroia, C., Morel, C., 2015. Soil test phosphorus and cumulative phosphorus budgets in

721 fertilized grassland. *Ambio* 44, S252-S262. <https://doi.org/10.1007/s13280-015-0628-x>

722 Morel, C., 2002. Caractérisation de la phytodisponibilité du phosphore du sol par la

723 modélisation du transfert des ions phosphates entre le sol et la solution. Mémoire d'HDR,

724 INPL Nancy, 80 p.

725 Morel, C., Tunney, H., Plénet, D., Pellerin, S., 2000. Transfer of phosphate ion between soil

726 and solution. *Perspectives in soil testing. J. Environ. Qual.* 29, 50-59.

727 <https://doi.org/10.2134/jeq2000.00472425002900010007x>

728 Morel, C., Ziadi, N., Messiga, A., Bélanger, G., Denoroy, P., Jeangros, B., Jouany, C., Fardeau,

729 J.C., Mollier, A., Parent, L.-E., Proix, N., Rabeharisoa, L., Sinaj, S., 2014. Modeling of

730 phosphorus dynamics in contrasting agroecosystems using long-term field experiments.

731 *Can. J. Soil Sci.* 94, 377–387. <https://doi.org/10.1139/CJSS2013-024>

732 Morel, C., Plénet, D., Mollier, A. 2020. Calibration of maize phosphorus status by plant-

733 available soil P assessed by common and process-based approaches. Is it soil-specific or

734 not? *Eur. J. Agron.* 122, 126174. <https://doi.org/10.1016/j.eja.2020.126174>.

735 Murphy J., Riley J.B., 1962. A modified single solution method for the determination of

736 phosphate in natural waters. *Anal. Chim. Acta* 27, 31-36. <http://dx.doi.org/10.1016/S0003->

737 2670(00)88444-5

738 Némery, J., Garnier, J., Morel, C., 2005. Phosphorus budget in the Marne Watershed (France):

739 urban vs diffuse sources, dissolved vs particulate forms. *Biogeochemistry.* 72, 35-66.

740 <http://dx.doi.org/10.1007/s10533-004-0078-1>

741 Olsen. S.R., Cole, C.V., Watanabe, F.S., Dean, L.A., 1954. Estimation of available phosphorus

742 in soils by extraction with sodium bicarbonate. USDA Circ. 939. US Gov. Print. Office,

743 Washington, DC

744 Poulton, P.R., Tunney H., Johnston, A.E., 1997. Comparison of fertilizer phosphorus
745 recommendations in Ireland and England and Wales. In: Tunney H., Carton O.T., Brookes
746 P.C., Johnston A.E. (eds.): Phosphorus Loss from Soil to Water. CAB International,
747 Wallingford, 449–452.

748 Reinjeveld, J.A., Ehlert, P.A.I., Termorshuizen A.J., Oenema, O., 2010. Changes in the soil
749 phosphorus status of agricultural land in the Netherlands during the 20th century. *Soil Use*
750 *Manage.* 26, 399–411. <https://doi.org/10.1111/j.1475-2743.2010.00290.x>

751 Sattari, S.Z., Bouwman, A.F., Giller, K.E., van Ittersum M. K., 2012. Residual soil phosphorus
752 as the missing piece in the global phosphorus crisis puzzle. *Proc. Natl. Acad. Sci. U.S.A.*
753 109, 6348-6353. <https://doi: 10.1073/pnas.1113675109>

754 Schellberg, J., Moseler, B.M., Kuhbauch, W., Rademacher, I.F., 1999. Long-term effects of
755 fertilizer on soil nutrient concentration, yield, forage quality and floristic composition of a
756 hay meadow in the Eifel Mountains, Germany. *Grass Forage Sci.* 54, 195-207.
757 <https://doi.org/10.1046/j.1365-2494.1999.00166.x>

758 Schulte, R.P.O., Herlihy, M., 2007. Quantifying responses to phosphorus in Irish grasslands:
759 Interactions of soil and fertiliser with yield and P concentration. *Eur. J. Agron.* 26, 144–153.
760 <https://doi.org/10.1016/j.eja.2006.09.003>

761 Sharpley, A.N., Menzel, R.G., 1987. The impact of soil and fertilizer phosphorus on the
762 environment. *Adv. Agron.* 41, 297-324. [http://dx.doi.org/10.1016/S0065-2113\(08\)60807-X](http://dx.doi.org/10.1016/S0065-2113(08)60807-X)

763 Stroia, M. C., 2007. Etude de fonctionnement de l'écosystème prairial en conditions de
764 nutrition N et P sub-limitante ; application au diagnostic de nutrition (Thèse de doctorat,
765 Institut National Polytechnique (Toulouse), Toulouse, FRA, Universitatea de Stiinte
766 Agricole si Medicina Veterinaria, Timisoara, ROU). 255 p.
767 <http://prodinra.inra.fr/record/14785>

768 Stroia, C., Morel, C., Jouany, C., 2007. Dynamics of diffusive soil phosphorus in two grassland
769 experiments determined both in field and laboratory conditions. *Agric. Ecosyst. Environ.*
770 119, 60-74. <https://doi.org/10.1016/j.agee.2006.06.007>

771 Tate, K.R., Speir, T., Ross, D., Parfitt, R., Whale, K., Cowling, J., 1991. Temporal variations in
772 some plant and soil P pools in two pasture soils of widely different P fertility status. *Plant*
773 *Soil*. 132, 219-232. <https://www.jstor.org/stable/42936988>

774

775 Théliier-Huché, L., Farruggia, A., Castillon, P., 1999. L'analyse d'herbe: un outil pour le
776 pilotage de la fertilisation phosphatée et potassique des prairies naturelles et temporaires.
777 Institut de l'Élevage - ACTA, 31 p. hal-02842817

778 Tran, T.S., Simard, R.R., 1993. Mehlich-3 extractable elements. p. 43–50. In M.R. Carter (ed.)
779 *Soil sampling and methods of analysis*. Can. Soc. of Soil Sci., Lewis Publ., Boca Raton, FL.

780 Valkama, E., Virkajarvit, P., Uusitalo, R., Ylivainio, K., Turtola, E., 2016. Meta-analysis of
781 grass ley response to phosphorus fertilisation in Finland. *Grass Forage Sci.* 71, 36-53.
782 <https://doi.org/10.1111/gfs.12156>

783 Violleau, S., 2015. Témoignage d'un conseiller sur la mise en œuvre des outils de raisonnement
784 de la fertilisation des prairies auprès des éleveurs du Puy de Dôme. *Fourrages*, 224, 279-
785 286.

786 Walworth, J.L., Sumner, M.E., Isaac, R.A., Plank, C.O., 1986. Preliminary DRIS norms for
787 alphas in the southeastern United States and a comparison with midwestern norms.
788 *Agron. J.* 78, 1046–1052. <https://doi.org/10.2134/agronj1986.00021962007800060022x>

789 Zehetner, F., Wuenscher, R., Peticzka, R., Unterfrauner, H., 2018. Correlation of extractable
790 soil phosphorus (P) with plant P uptake: 14 extraction methods applied to 50 agricultural
791 soils from Central Europe. *Plant Soil Environ.* 64, 192–201.
792 <https://doi.org/10.17221/70/2018-PSE>

793 Ziadi, N., Whalen, J. K., Messiga, A. J., Morel, C., 2013. Assessment and modeling of soil
794 available phosphorus in sustainable cropping systems. In: Donald L. Sparks, dir., Advances
795 in agronomy (p. 85–126). Adv. Agron. 122, 85-126. [https://doi.org/10.1016/B978-0-12-](https://doi.org/10.1016/B978-0-12-417187-9.00002-4)
796 [417187-9.00002-4](https://doi.org/10.1016/B978-0-12-417187-9.00002-4)
797

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

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

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

801
802

803 Table 2.
 804 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
 805 model for CA-LEV (0-15 cm); NA = no data available.
 806

Analysis	Detail	Site and experiment period				
		CA-LEV 1999-2007	CH-LES 1991-2008	FR-ERC 1999-2007	FR-GRA 1998-2014	RO-DÂM 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 model parameterization	Sampling year	2007	2008	2007	2007	NA
	Soil horizon (cm)	0-15	0-5	0-5	0-5	NA

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Table 3.

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

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 .

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813

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 (%)		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

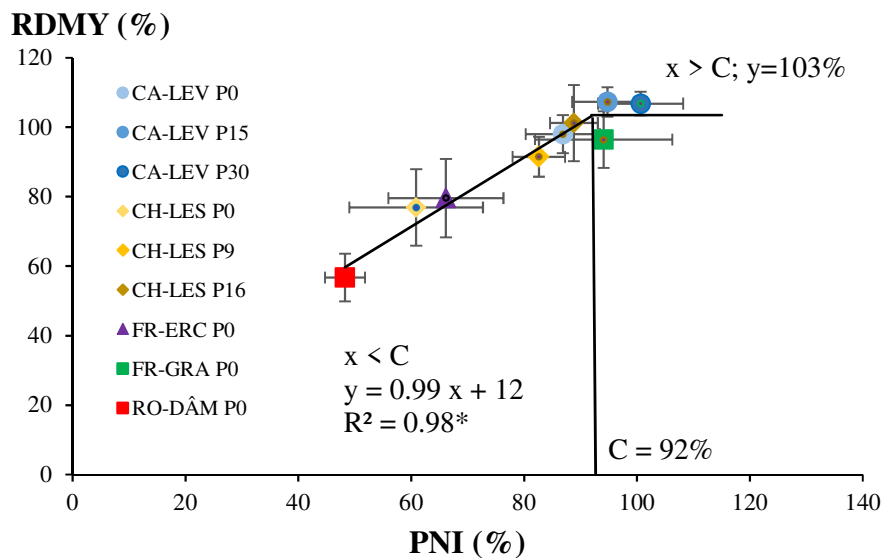
¹ Measured on the soil sampling date (Table 2)

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

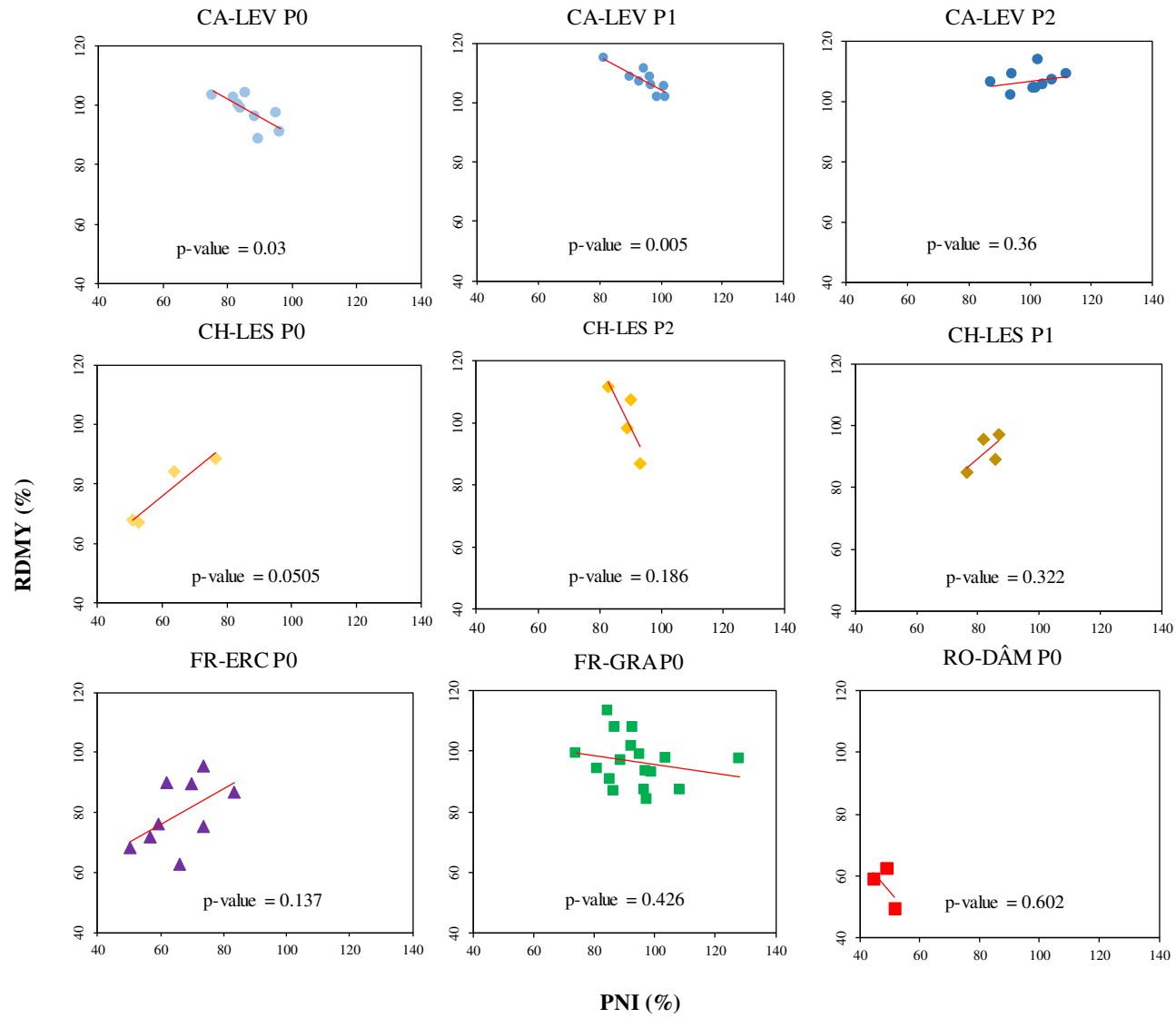
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820 Fig. 1. Relationship between relative dry matter yield (RDMY) and Phosphorus nutrition index (PNI) a) the average RDMY as a function of the
 821 average PNI, with averages computed over all years for each site-treatment combination; C represents 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 a
 826

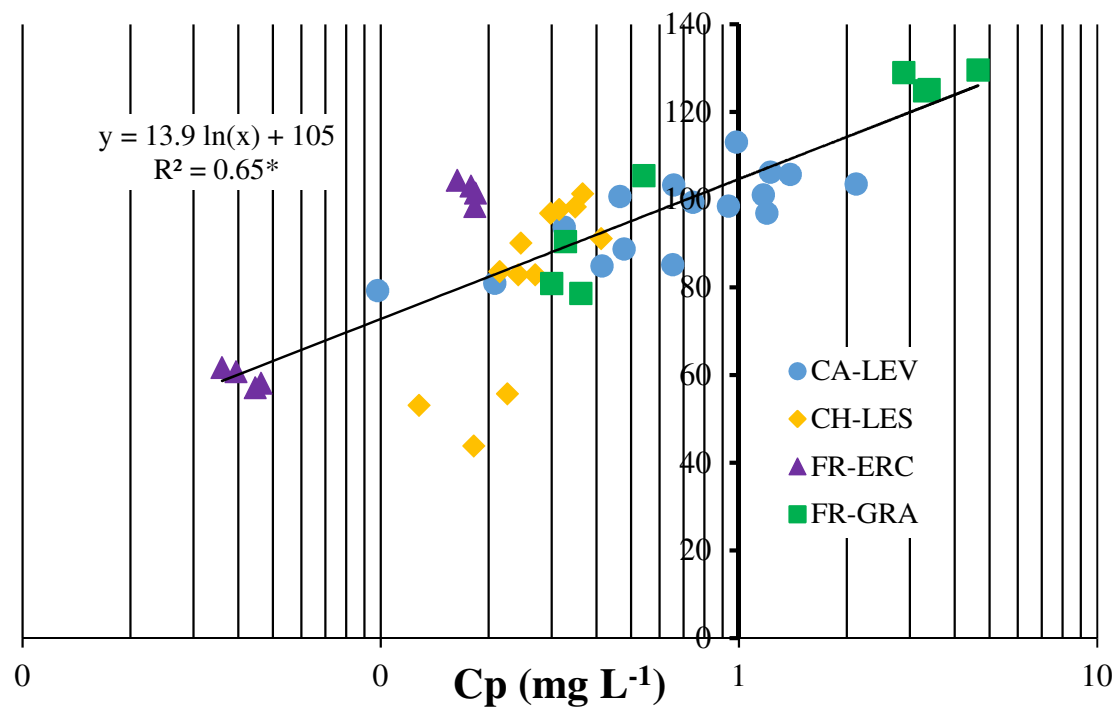


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831 Fig. 2. Relationship between the phosphorus (P) nutrition index (PNI), measured at the plot scale, and soil plant-available P (SPAP); a) SPAP=
832 C_p at the CA-LEV, CH-LES, FR-ERC, and FR-GRA sites; b) SPAP=Olsen P at the CH-LES, FR-ERC, and FR-GRA sites; and c) SPAP= $Q_w +$
833 Pr_{60min} 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.
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835
836 a

PNI (%)

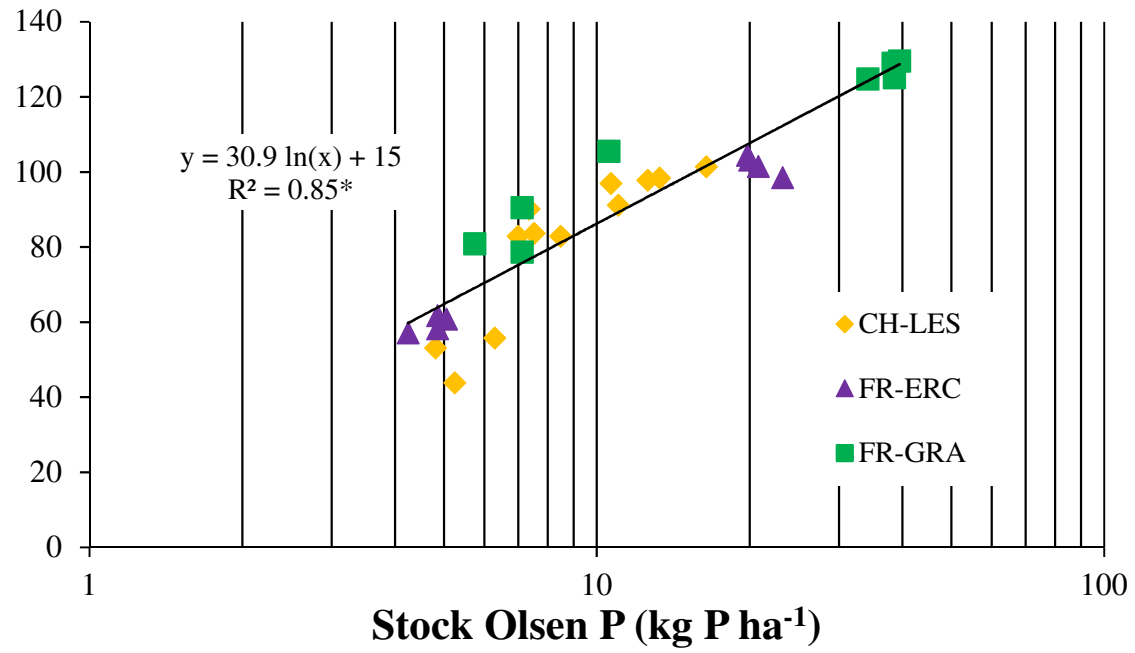


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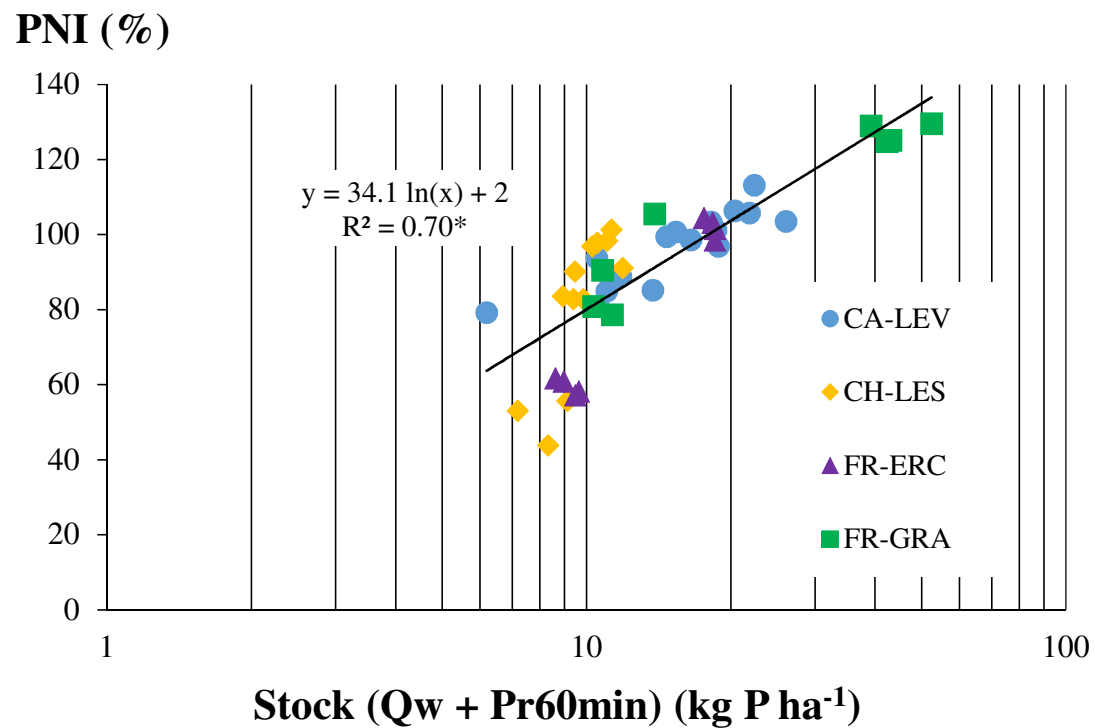
b

PNI (%)



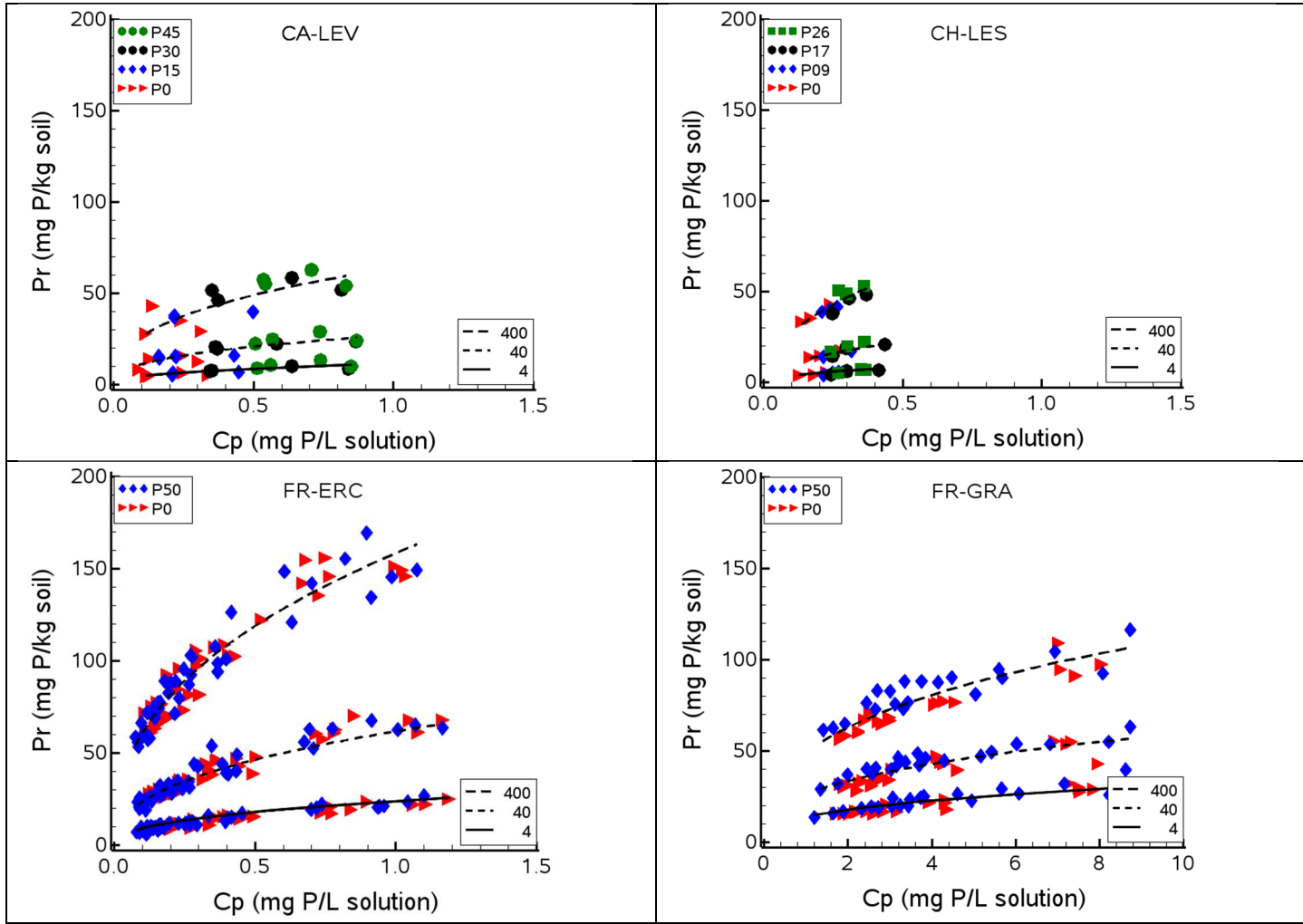
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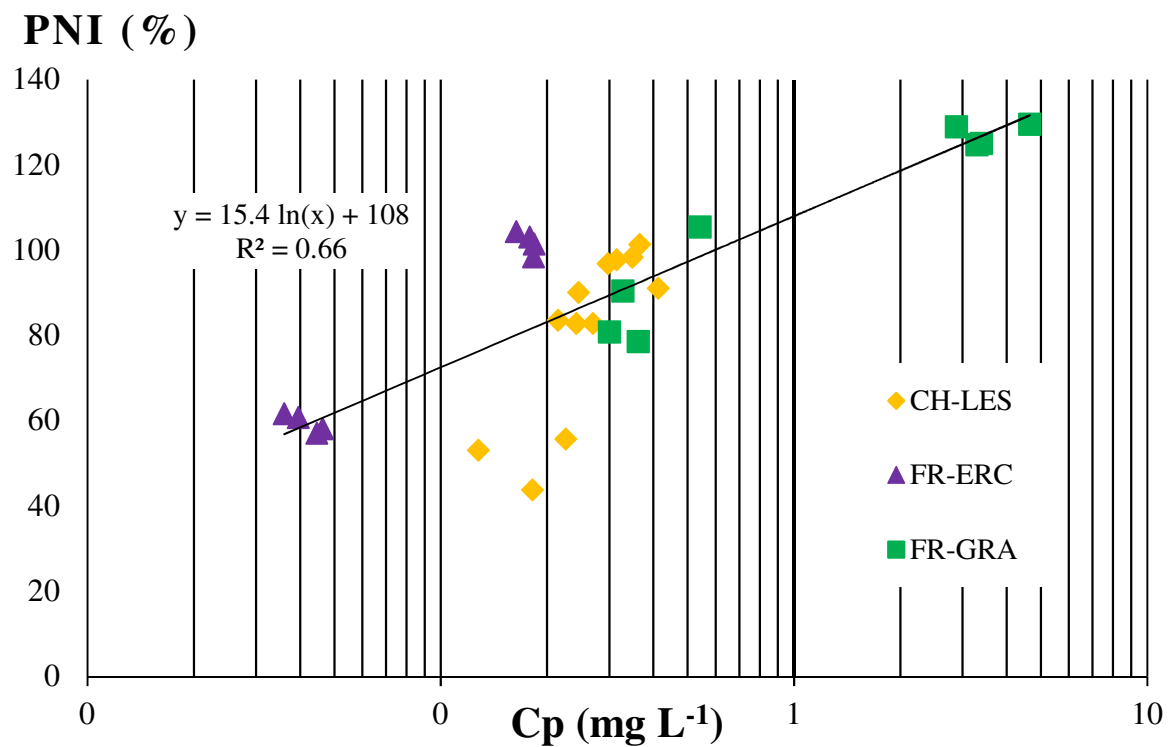
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Fig. 3. Experimental (symbols) and regressed (lines) values of total amounts of diffusive orthophosphate P ions (P_r , mg P kg⁻¹ soil) as a function of the orthophosphate P ion concentration in solution (C_p , mg P L⁻¹ solution) and elapsed time (t , 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)



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= $Q_w + Pr_{60min}$. The x-axis is on a log scale to visualize the experimental points better.
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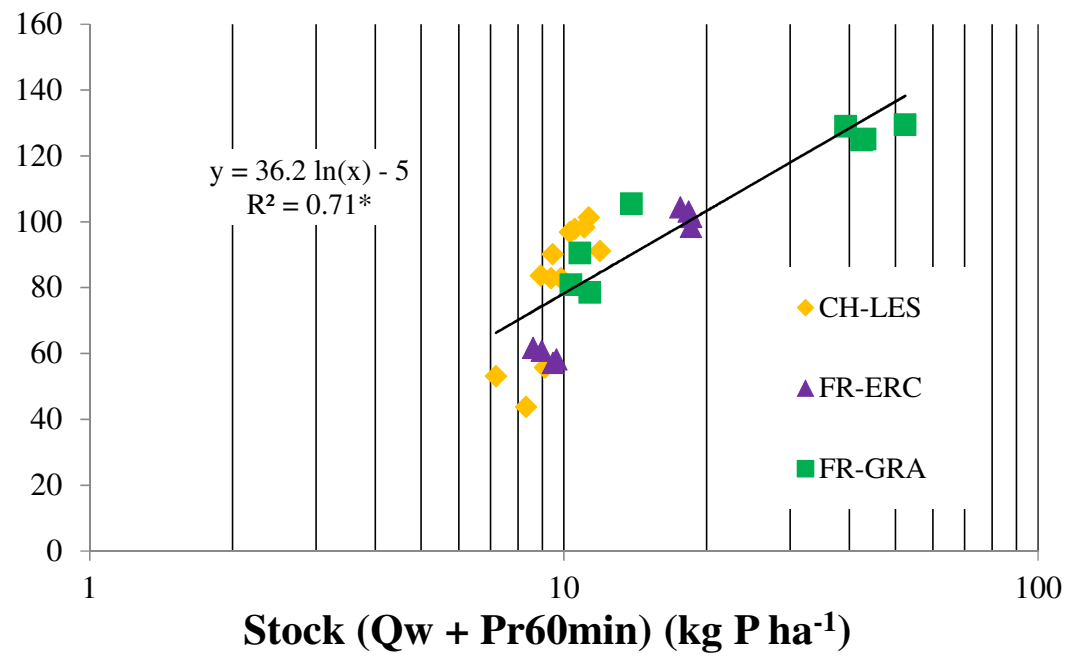
a



859
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861
862

b

PNI (%)



863
864

865 **Supplementary materials**

866

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

870

Year	Treatment	DMY 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)

871

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

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

876

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

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

881

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

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

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

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

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