



Critical plant phosphorus for winter wheat assessed from long-term field experiments

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ABSTRACT

Optimizing the use of phosphorus (P) fertilizers is essential considering the environmental issues linked to over fertilization and the limited rock phosphate reserve. Diagnosing the crop P status by calculating a phosphorus nutrition index (PNI) based on the plant critical P concentration (P_c) could help determine the minimum amount of P fertilizer to ensure maximum crop yield. This study investigated (i) the effect of P fertilization on winter wheat (*Triticum hybernum* L.) shoot biomass and grain yield after several decades of different annual P fertilization rates, (ii) the stability over several site-years of the P_c expressed either as a function of shoot biomass or shoot nitrogen (N) concentration, and (iii) the possibility of using the PNI or the shoot N-to- P_c ratio as nutritional indicators to diagnose P deficiency and predict the expected response to P fertilization. Shoot biomass and its P and N concentrations were measured weekly at five site-years along with grain yield after several decades of P fertilization treatments that ranged between 0 and 5/3 of the theoretical P crop uptake. The P fertilization did not affect the grain yield, but it generally increased shoot biomass especially at the CD 27-37 developmental stages. The P_c expressed either as a function of shoot biomass or shoot N concentration differed among site-years and this was attributed to differences in crop N status. When N was not deficient, we developed a P_c dilution curve as a function of shoot biomass (SB) ($4.56 \times SB^{-0.279}$) along with a linear relationship ($P_c = 1.10 + 0.061 \times N$) and a power function ($P_c = 0.34 \times N^{0.632}$) between P_c and shoot N concentration. The N-to- P_c ratio was related to shoot biomass accumulation according to a power function, but the relationship also differed among site-years. The relative shoot biomass responded positively to the PNI up to different thresholds for limiting and non-limiting N conditions. The relative grain yield, however, was not related to the PNI. The PNI, based on the P_c dilution curve, has potential as a nutritional indicator to diagnose P deficiency and the expected response to P fertilization, but more research is needed to clarify the effect of N deficiencies on the P_c dilution curve.

1. Introduction

Phosphorus (P) is one of the major nutrients for plant growth, and P addition from fertilizers is often necessary to meet crop requirements and increase crop productivity (Marschner, 1995; Shen et al., 2011). However, the application of P fertilizers in excess of crop requirements is known to lead to eutrophication of aquatic systems (Sims et al., 1998; Sinaj et al., 2002). Furthermore, the limited rock phosphate reserve could eventually decrease the availability of P fertilizers (Gilbert, 2009). Consequently, improving P use efficiency in order to maintain high crop yields while limiting the P environmental impact is a critical issue.

Practically, this consists of applying the lowest P fertilizer rate that satisfies crop requirements.

Fertilization trials are crucial to establish this minimum P fertilizer rate to satisfy crop requirements. Crops, in most cases, respond to P fertilization only if soils have a low plant available P content (Valkama et al., 2009). For that reason, many short-term experiments have shown no effect of P fertilization on crop yield (McKenzie et al., 2003; Valkama et al., 2011). In some cases, P fertilization influenced crop yield only after several years of different P application rates (Gallet et al., 2003; Messiga et al., 2010). Therefore, long-term field experiments are more suitable to test the effect of P fertilization.

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Establishing a threshold soil P value indicating P limitation remains challenging since no chemical extraction accurately quantifies soil P availability (Demaria et al., 2005). Therefore, plant nutrient analysis can be used as an alternative to diagnose the nutrient status of crops and the need for P fertilization. A myriad of methods were developed to establish plant nutritional indicators, e.g. the critical value of nutrients (Bates, 1971), the Diagnosis and Recommendation Integrated System (DRIS) (Beaufils and Sumner, 1976), the vectorial method (Haase and Rose, 1995), the centered (Parent and Dafir, 1992) and isometric log ratios (Parent et al., 2009), and the boundary lines (Vizcayno-Soto and Côté, 2004). Another method consists in using nutrient dilution curves that have the advantage of taking into account the decrease of plant nutrient concentration (e.g. N and P) during plant growth (Williams, 1957). This method was first developed by using the allometric relationship between critical N concentration (N_c) and plant biomass accumulation as a power function, i.e. $N_c = a \times SB^{-b}$ where a and b are estimated coefficients and SB is the shoot biomass (Lemaire et al., 1984). This methodology was later extended to P by Salette and Huché (1991).

The critical concentration of N or P (P_c) is the lowest plant concentration needed to reach maximum crop growth and yield. Hence, the nutrient status can be diagnosed by comparing the concentration of a nutrient measured in the crop with the critical concentration predicted for the corresponding shoot biomass. This approach is particularly relevant for a crop species if the critical nutrient dilution curve is valid for different pedoclimatic conditions, a situation reported for the N_c dilution curve in several crop species (Greenwood et al., 1990), including potatoes (*Solanum tuberosum* L.; Giletto et al., 2020) and maize (*Zea mays* L.; Ziadi et al., 2008b). Regarding the P_c dilution curve, fewer crop species have been investigated (Cadot et al., 2018). For spring wheat, Bélanger et al. (2015a) found that the P_c dilution curve was affected by site conditions, whereas no effect was found when P_c was modeled using the shoot N concentration. Estimating P_c using shoot N concentration, instead of shoot biomass, has the advantage of avoiding shoot biomass measurements that are time consuming and not convenient for farmers (Bélanger et al., 2015a). Because N deficiency decreases the P_c dilution curve and affects the relationship between N and P_c , the N status of spring wheat should also be measured and considered (Ziadi et al., 2008a; Bélanger et al., 2015a). The use of P_c as a diagnostic tool, whether estimated via the dilution curve or the shoot N concentration, needs to be investigated under different pedoclimatic conditions in order to increase its reliability.

Our study investigated the relationships between P_c , shoot N concentration, and shoot biomass in winter wheat grown at four sites as part of long-term experiments with varying P fertilization rates applied annually over several decades. Our ultimate goal was to test the possibility to develop a general equation for P_c , either expressed as a function of shoot biomass or shoot N concentration that applies to a wide range of pedoclimatic conditions for the diagnosis of the P status of winter wheat. This study is the continuation of previous research on the P_c of three crop species as part of a crop rotation performed at a long-term field experiment in Changins (Switzerland) (Cadot et al., 2018). Because Cadot et al. (2018) observed only a marginal effect of P fertilization on grain yield in 2011, similar measurements were performed in 2018. In addition, we included measurements that were made at three other long-term field experiments in Switzerland. More specifically, our objectives were to determine: (i) the effect of P fertilization on shoot biomass and grain yield of winter wheat after several decades of different P fertilization rates; (ii) the stability over several sites and years of the P_c expressed either as a function of shoot biomass or shoot N concentration; and (iii) the possibility of using the phosphorus nutrition index (PNI) or the N-to- P_c ratio as nutritional indicators to diagnose P deficiency and the expected response to P fertilization.

Table 1
Site characteristics in the year of the measurements following decades of variable P fertilization at five site-years.

Site	Location	Altitude (m)	Soil type (FAO classification)	Establishment (year)	Plots (length × width, m)	Clay (g kg ⁻¹)	Sand (g kg ⁻¹)	pH (H ₂ O)	Year	Fertilization treatments	C _{org} (g kg ⁻¹)	N _{tot} (g kg ⁻¹)	P _{tot} (mg kg ⁻¹)	P _{NaHCO₃} (mg kg ⁻¹)
Changins	46°23'55.72"/06°14'24.72"	438	Gleyic Cambisol	1971	15.0 × 8.0	525	163	6.63	2011	0 P 3/3 P	28.2 A 29.9 A	3.0 A 3.0 AB	697 a B 879 b B	5 c D 15 b
	46°23'55.72"/06°14'24.72"													
Cadenazzo	46°9'37.66"/8°56'2.18"	197	Eutric Fluvisol	1989	8.0 × 4.5	91	362	5.80	2012	2/3 P 3/3 P	10.5 B 10.3 D	1.1 B 1.1 D	1107 b A 1194 ab A	18 18
Elighausen	47°36'35.06"/9°8'33.07"	440	Eutric Cambisol	1989	8.25 × 4.0	329	307	6.20	2018	2/3 P 3/3 P	18.5 A 18.0 C	2.3 A 2.4 C	793 B 803 B	15 ab 18 ab
Oensingen	47°17'2.95"/7°43'50.77"	422	Gleyic-calcaric Cambisol	1989	8.25 × 4.0	370	225	7.25	2018	2/3 P 3/3 P 5/3 P	21.4 C 21.7 BC	2.7 BC 2.8 AB	984 b B 1063 a AB	19 b 26 a BC

Different lowercase letters indicate significant differences ($p < 0.05$, ANOVA and Tukey test) between P fertilization treatments within the same site and uppercase letters indicate significant differences ($p < 0.05$, ANOVA and Tukey test) between sites for the same P fertilization treatment.

[†] 0 P = no P fertilization; 2/3 P = 2/3 of the theoretical crop uptake (i.e. 27 kg P ha⁻¹) and so on.

2. Materials and methods

2.1. Field experiments

Four long-term field experiments were established in Switzerland to evaluate the effect of P fertilization on crop yield, P uptake, and soil P availability: Changins in 1971, and Cadenazzo, Ellighausen, and Oensingen in 1989 (Table 1). Phosphorus was applied as triple-superphosphate. The P fertilization treatments encompassed a gradient of P rates that ranged between 0 and 5/3 (or 6/3 at Changins) of the theoretical P crop uptake (Table 1). The long-term field experiments included four replicates of each P fertilization treatment (0 P, 2/3 P, 3/3 P, 5/3 P or 6/3 P) in a randomized complete block design. The theoretical crop P uptake was set at 27 kg P ha⁻¹ according to the official Swiss fertilization guidelines (Sinaj et al., 2017). Since the establishment of the long-term field experiments, winter wheat was grown in rotation with other crops at all sites (Gallet et al., 2003). At each site-year, various winter wheat cultivars (*cv Fiorina* at Cadenazzo, *cv Arina* at Changins, and *cv Nara* at Oensingen and Ellighausen) were sown in October of the year preceding sampling. Wheat row spacing was 18 cm at Ellighausen and Oensingen, and 15 cm at Cadenazzo and Changins. A mineral N fertilizer was applied three times during the growing season for a total of 140 kg N ha⁻¹ according to the official Swiss fertilization guidelines (Sinaj et al., 2017).

2.2. Plant sampling and analyses

After several decades of different P fertilization, the shoot biomass of winter wheat was sampled weekly from the end of March until the end of May at Changins in 2011 (Ch 2011) and 2018 (Ch 2018), at Cadenazzo in 2012 (Ca 2012), at Oensingen in 2018 (Oe 2018), and at Ellighausen in 2018 (El 2018). The sampling was done on seven consecutive weeks at Ca 2012, on two and then five consecutive weeks at Ch 2011, and on eight consecutive weeks at Ch 2018, Oe 2018, and El 2018. Developmental stages (CD) were determined according to Meier (2018) and shoot biomass was harvested using pruning shears at 2–3 cm above ground level on an area that ranged between 0.2 and 1 m² depending on site-year. Because the number of rows included within a square differed sometimes due to topographic variability, shoot biomass was first calculated on a linear meter basis (i.e. g of DM per row meter). We assumed that row spacing did not influence shoot growth during the sampling period. Therefore, shoot biomass (Mg DM ha⁻¹) was calculated using the same row spacing (i.e. 15 cm) at all site-years. Grain yield was measured by harvesting each plot on an area of 29.5 m² at Changins, 12 m² at Cadenazzo, and 8.88 m² at Oensingen and Ellighausen with a plot combine equipped with a scale. The entire amount of shoot biomass and grain sub-samples were oven dried at 55 °C for 72 h to determine the dry weight. The dry shoot biomass and grains were ground using a Retsch rotor mill. Residual crop humidity was evaluated at 105 °C. Total C and dry ashes were analyzed by calcination at 480 °C for 5 h. The Dumas method was used to measure the total N by an elemental analyzer (Thermo, flash 2000) (NF ISO 13878) (Masson et al., 2010). Total P was determined using a radial ICP-AES (Varian Vista RL Simultaneous or Varian 725ES Simultaneous) after calcination at 480 °C for 5 h and solubilization in hydrofluoric acid (Masson et al., 2010).

2.3. Soil sampling and analyses

After the grain harvest, at least three soil cores per plot were sampled to a depth of 20 cm. A composite sample was prepared for each plot by mixing the soil cores after removing plant residues. The composite soil samples were then air-dried and sieved with a 2-mm mesh size. Soil organic carbon (C_{Org}) was measured by sulfochromic oxidation (NF ISO 14235), whereas an elemental analyzer (Thermo, flash 2000) was used to measure total soil N (NF ISO 13878). To determine total P (P_{Total}), the molybdate colorimetric method (Murphy and Riley, 1962) was used

after an extraction of 0.25 g soil with 5 ml of hydrofluoric acid (40 %) and 1.5 ml of HClO₄ (65 %) (NFX 31-147). Available P (P_{NaHCO3}) was estimated following a sodium bicarbonate (NaHCO₃) extraction (Olsen, 1954). Soil texture and pH of each site were analyzed according to FAL et al. (2004).

2.4. Data analysis

Statistical analyses were performed using the R 3.01 software (Team, 2013). Tukey tests were performed to compare shoot biomass, grain yield, and N and P concentrations of shoots and grains between fertilization treatments within each site-year. For grain yield, site-years were also statistically compared with a Tukey test. The 6/3 P treatment at Changins was considered similar to the 5/3 P treatment at the other sites for the site-year comparison because there was no 5/3 P treatment at Changins.

Different methods have been proposed to identify data points that represent non-limiting conditions for shoot growth in experiments with several N or P fertilization rates (Greenwood et al., 1990; Justes et al., 1994). Because of the limited number of fertilization treatments and the high variability among replications within the fertilization treatments in our study, those methods could not be used satisfactorily. Data points that represent non-limiting conditions for shoot growth were identified on the basis of the whole growth cycle according to the two following criteria. Fertilization treatments were considered as P-limited when (i) relative shoot biomass averaged across all sampling dates was less than 0.80, a threshold used by Ziadi et al. (2008a) to identify the N-limited shoot biomass, and (ii) shoot biomass was decreased ($p < 0.05$) compared to the highest P fertilization rate (5/3 P or 6/3 P) on at least one sampling date. Among the non-limited P treatments, the lowest P fertilization rate was selected in order to estimate a P_c dilution curve at each site-year. Sampling dates were considered only when the shoot P concentration decreased concomitantly with an increase in shoot biomass. Sampling dates exhibiting an average shoot biomass less than 1 Mg DM ha⁻¹ were automatically excluded for estimating P_c dilution curves as already done in previous studies (Justes et al., 1994; Bélanger et al., 2015a; Cadot et al., 2018). At several site-years, P dilution started beyond this threshold of 1 Mg DM ha⁻¹ whereas at other site-years it started beyond 2 Mg DM ha⁻¹. Only the points linked to the P dilution were considered and, therefore, the number of observations used for the following analyses differed between site-years.

According to the dilution curve concept (Salette and Huché, 1991), the shoot P concentration was expressed as a power function of shoot biomass for each site-year. Because the P_c curve can be affected by the N status, the shoot N concentration was also expressed as a function of shoot biomass along with the critical N (N_c) dilution curve of Justes et al. (1994) for winter wheat (N_c = 53.5 × SB^{-0.44} with SB the shoot biomass) to assess the N nutrition status. To test the site-year effect, P_c dilution curves at the five site-years were linearized through a logarithmic (Ln) transformation of shoot P concentration and shoot biomass. Linear regressions were performed for each site-year and equations were compared for all combinations of two site-years. The comparison of the slopes was used to test if the P dilution rate differed across site-years. The *t* values were calculated as follows:

$$t = \frac{A1 - A2}{\sqrt{\sigma^2 \left(\frac{1}{\sum (x_{1i} - x_{1moy})^2} + \frac{1}{\sum (x_{2j} - x_{2moy})^2} \right)}} \quad (1)$$

with σ^2 the common variance, A the slopes, x the explanatory variable (i.e. Ln of shoot biomass), whereas 1 and 2 refer to the two site-years being compared. The *p* value was obtained by combining the *t* values and the number of degrees of freedom (d.f.) calculated as follows:

$$\text{d.f.} = n_1 + n_2 - 4 \quad (2)$$

When slopes differed significantly, the intercepts were not tested. If slopes did not differ, intercepts were compared to test the site-year effect

as follows:

$$t = \frac{B1 - B2}{\sqrt{\sigma^2 \left(\frac{1}{n_1} + \frac{x_{1moy}^2}{\sum (x_{1i} - x_{1moy})^2} + \frac{1}{n_2} + \frac{x_{2moy}^2}{\sum (x_{2i} - x_{2moy})^2} \right)}} \quad (3)$$

with B the intercepts. A difference between the two intercepts indicates a significant effect of site-year with similar P dilution rates for both site-years. In addition, confidence intervals (5%) for the slopes were determined for each site-year using *matplotlib* function (*graphics* package). The lack of overlapping of confidence intervals between two linear regressions indicates a significant site-year effect ($p < 0.05$) whether P dilution rates differ or not. A similar procedure was also carried out to compare linear relationships between (i) shoot P_c and N concentrations, (ii) the *Ln* transformed values of shoot P_c and N concentrations, and (iii) the *Ln* transformed values of N-to- P_c and shoot biomass; the last two being linearized power functions. Linear regressions of the site-years having similar slopes and intercepts were pooled to develop general models.

The PNI was calculated as follows:

$$PNI (\%) = (P_{\text{measured}} / P_c) \times 100 \quad (4)$$

with P_c estimated with three different equations: (i) dilution curve as a function of shoot biomass, (ii) linear regression as a function of shoot N concentration, and (iii) power function linking P_c and shoot N concentration. The relative shoot biomass at each sampling date and the relative grain yield were calculated by dividing the values from a given fertilization treatment by the highest value among the fertilization treatments. The relative shoot biomass and the relative grain yield were then related to the PNI calculated with P_c from the three equations. The Cate-Nelson procedure was carried out using the *cateNelson* function (*rcompanion* package) to test if critical values of the X variables (PNI) can separate the Y variables (shoot biomass or grain yield) in one group where X and Y are related and in another where Y does not respond to X (Cate et al., 1971). The best equation to estimate P_c for the PNI calculation used to develop the general relationship between the relative shoot biomass and PNI was selected according the highest R^2 and the

Table 2
Shoot biomass (Mg DM ha⁻¹) during wheat growth as affected by P fertilization at five site-years.

Site	Year	Fertilization ^a	Wheat development stages							
			23–29	26–31	27–32	27–32	29–35	30–37	34–45	45–53
Changins	2011	0 P	0.25 b	0.56 b	NA ^b	1.16 b	2.04 b	2.92 b	4.09 b	4.96 b
		3/3 P	0.45 a	0.90 a	NA	1.71 a	2.60 a	3.65 ab	5.11 a	6.37 a
		6/3 P	0.54 a	1.00 a	NA	1.83 a	2.94 a	4.07 a	5.81 a	6.9 a
	2018	0 P	0.59 b	0.69	0.88 b	1.24 b	2.06 b	3.62 b	4.86	7.20
		3/3 P	0.64 ab	0.78	1.07 ab	1.59 ab	2.87 ab	4.44 ab	6.09	8.61
		6/3 P	0.79 a	0.86	1.25 a	1.75 a	3.33 a	5.32 a	6.85	8.76
Cadenazzo	2012	0 P	NA	0.53 d	0.82 c	1.24 c	2.39 b	3.54 b	4.52	6.17
		2/3 P	NA	0.96 bc	1.51 ab	2.14 ab	3.36 ab	4.43 ab	5.62	8.62
		3/3 P	NA	1.17 ab	1.72 a	2.37 ab	3.66 a	4.72 a	6.03	8.71
	2018	5/3 P	NA	1.20 a	1.84 a	2.43 a	3.76 a	4.89 a	6.09	8.03
		0 P	0.95	1.23 b	1.50 b	1.85 b	2.96	4.44 b	6.46 b	8.83
		2/3 P	0.96	1.48 a	1.74 a	2.30 a	3.43	5.80 ab	8.04 ab	10.74
Ellighausen	2018	3/3 P	1.09	1.48 a	1.74 a	2.30 a	3.67	5.89 ab	8.67 ab	10.00
		5/3 P	1.03	1.36 ab	1.76 a	2.46 a	3.81	6.20 a	8.91 a	11.19
		0 P	1.12	1.52	1.74	2.43	3.48	5.76	6.31	9.15
	2018	2/3 P	1.17	1.72	2.02	2.94	4.50	6.96	8.67	11.48
		3/3 P	1.27	1.57	2.11	2.94	4.31	6.44	8.52	12.02
		5/3 P	1.20	1.52	2.20	3.04	4.89	7.37	9.24	12.00

Different letters indicate significant differences ($p < 0.05$, ANOVA and Tukey test) between P fertilization treatments within a sampling date and a site-year. The corresponding shoot P and N concentrations are presented in Supplementary Tables A1 and A2.

^a The P fertilization treatments ranged between 0 and 5/3 (or 6/3 at Changins) of the theoretical P crop uptake.

^b Data not available.

Table 3
Grain yield, and grain N and P concentrations as affected by P fertilization at five site-years.

Site	Year	Fertilization ^a	Grain yield (Mg ha ⁻¹)	Grain N concentration (g kg ⁻¹ DM)	Grain P concentration (g kg ⁻¹ DM)
Changins	2011	0 P	5.11 A	24 a B	4.2 AB
		3/3 P	5.54 A	22 b C	4.3 ABC
		6/3 P	5.34 A	22 b B	4.5 AB
	2018	0 P	3.30 B	21 a C	4.7 A
		3/3 P	3.39 B	20 b D	4.7 A
		6/3 P	3.40 B	19 b C	4.6 A
Cadenazzo	2012	0 P	3.55 B	20 C	3.8 ab B
		2/3 P	3.70 B	19 B	3.7 b B
		3/3 P	4.01 B	20 D	3.9 ab C
	2018	5/3 P	3.63 B	19 C	4.0 a B
		0 P	4.80 A	29 A	3.8 B
		2/3 P	5.09 A	28 A	4.2 A
Ellighausen	2018	3/3 P	5.11 A	29 B	4.4 AB
		5/3 P	5.08 A	28 A	4.4 AB
		0 P	3.71 B	30 A	4.0 B
	2018	2/3 P	4.30 AB	29 A	4.1 AB
		3/3 P	4.45 AB	31 A	4.0 BC
		5/3 P	4.80 A	29 A	4.2 AB

Different lowercase letters indicate significant differences ($p < 0.05$, ANOVA and Tukey test) between P fertilization treatments within a site-year and uppercase letters indicate significant differences between site-years for the same P fertilization treatment.

^a The P fertilization treatments ranged between 0 and 5/3 (or 6/3 at Changins) of the theoretical P crop uptake.

Table 4
 Estimated parameters of linear relationships between (i) Ln of shoot P_c and Ln of shoot biomass (SB), (ii) P_c and N, (iii) Ln of P_c and Ln of N, and (iiii) Ln of N-to-P_c and Ln of SB for each site-year with their respective R² as well as significance of slopes and intercepts. In addition, significant differences between site-years for either slopes or intercepts (i.e. only for similar slopes) are reported with different lowercase letters for Changins in 2011 (Ch 2011) and 2018 (Ch 2018), for Cadenazzo in 2012 (Ca 2012), Ellighausen in 2018 (El 2018), and Oensingen in 2018 (Oe 2018).

Site-year	n	Ln (P) ~ Ln (N)			Ln (P) ~ Ln (SB)			Ln (N-to-P) ~ Ln (SB)			
		Intercept	Slope	Adj R ²	Intercept	Slope	Adj R ²	Intercept	Slope	Adj R ²	
Ch 2011	21	1.53***	-0.456 (c)***	0.87	0.88***	0.083 (a)***	0.85	-1.33 (a)***	2.24 (c)***	-0.131 (a)***	0.31
Ch 2018	23	1.56 (a)***	-0.318 (b)***	0.67	1.66 (a)***	0.064 (b)***	0.82	-0.52 (c)*	2.34 (d)***	-0.281 (b)***	0.64
Ca 2012	18	1.10***	-0.138 (a)**	0.33	1.72***	0.032 (c)***	0.63	-0.06	2.80 (a)***	-0.386 (b)***	0.72
El 2018	20	1.58 (a)***	-0.328 (b)***	0.73	0.98 (b)**	0.064 (b)***	0.69	-1.30 (ab)***	2.54 (b)***	-0.130 (a)*	0.27
Oe 2018	22	1.46 (a)***	-0.239 (b)***	0.66	1.22 (b)***	0.057 (b)***	0.77	-1.00 (bc)***	2.53 (b)***	-0.134 (a)**	0.36

Regression coefficients are significant at $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***). Different lowercase letters indicate significant differences ($p < 0.05$, ANOVA and Tukey test) between site-years for either slopes or intercepts.

lowest percentage of observations that did not fall into the quadrants predicted by the Cate-Nelson procedure.

3. Results

3.1. Soil characteristics

The sand-rich soil of Cadenazzo has the lowest organic C (C_{Org}) and total N (N_{Tot}) contents, and the highest total P (P_{Tot}) content (Table 1). At Ellighausen and Oensingen, the contents of sand, silt, and clay were roughly equivalent. The highest C_{Org}, N_{Tot}, and clay contents were observed at Changins. In the year of the measurements following decades of variable P fertilization, a strong gradient of the P_{NaHCO3} content was observed between P fertilization treatments at Changins, Ellighausen, and Oensingen, but there was no gradient at Cadenazzo.

3.2. Wheat shoot biomass and grain yield

Phosphorus fertilization increased wheat shoot biomass at development stages between CD 27-32 and CD 30-37 at all site-years, except at Oe 2018 (Table 2). At earlier stages, however, P fertilization increased wheat shoot biomass at only two site-years (Ch 2011 and Ch 2018) at stage CD 23-29 and at three site-years (Ch 2011, Ca 2012, and El 2018) at stage CD-26-31. At later stages, P fertilization increased wheat shoot biomass at two site-years (Ch 2011 and El 2018) at stage CD 34-45 and at one site-year (Ch 2011) at stage CD 45-53. The P fertilization did not affect grain yield at any of the site-years, whereas grain P concentration was affected only at Ca 2012 (Table 3). Phosphorus fertilization, however, decreased grain N concentration at Ch 2011 and Ch 2018, but there was no effect at the other site-years.

3.3. Critical phosphorus values

The P fertilization treatments used to determine the P_c values were 2/3 P at Ca 2012, 3/3 P at Ch 2011 and Ch 2018, 2/3 P at El 2018, and 2/3 P at Oe 2018. At Oe 2018, no significant effect of P fertilization was observed even though the shoot biomass with the 0 P treatment was lower than that with the 2/3 P treatment on all sampling dates with differences up to more than 2 Mg DM ha⁻¹ on the last sampling date. The relative shoot biomass averaged across all sampling dates was 0.79 for the 0 P treatment and 0.95 for the 2/3 P treatment. The lack of a statistical difference at that site-year was due to the greater coefficients of variation within fertilization treatments than at the other site-years. This is particularly true for the 0 P treatment with coefficients of variation of 70 % and 40 % on the last two sampling dates compared to a maximum coefficient of variation of 21 % at the other site-years.

The P_c values were expressed as a function of shoot biomass to establish a P_c dilution curve for each site-year. Following the Ln transformation of P_c values and shoot biomass, the statistical analysis of the P_c dilution curves showed that slopes (i.e. the dilution coefficient) differed between some site-years (Table 4), as it can also be seen by the lack of overlapping of the confidence intervals (Fig. 1A). Among site-years, the coefficients of determination (R²) ranged between 0.33 and 0.87 (Table 4). However, neither slopes nor intercepts differed between Ch 2018, El 2018, and Oe 2018.

Because the crop N status is known to affect the P_c values (Ziadi et al., 2008a; Bélanger et al., 2015a), the N nutrition status for the data points used to estimate the P_c values was analyzed according to Justes et al. (1994). Wheat was deficient in N at Ca 2012 with an average N nutrition index (NNI) of 82 %, at Ch 2011 with a NNI of 69 %, and at Ch 2018 with a NNI of 77 % (Fig. 2). Data points for the two site-years (El 2018 and Oe 2018) with similar slopes and intercepts, and without a N deficiency were then pooled to develop a general equation of the P_c dilution curve as follows:

$$P_c = 4.56 \times SB^{-0.279} \quad (n = 42, R^2 = 0.71, p < 0.001) \quad (5)$$

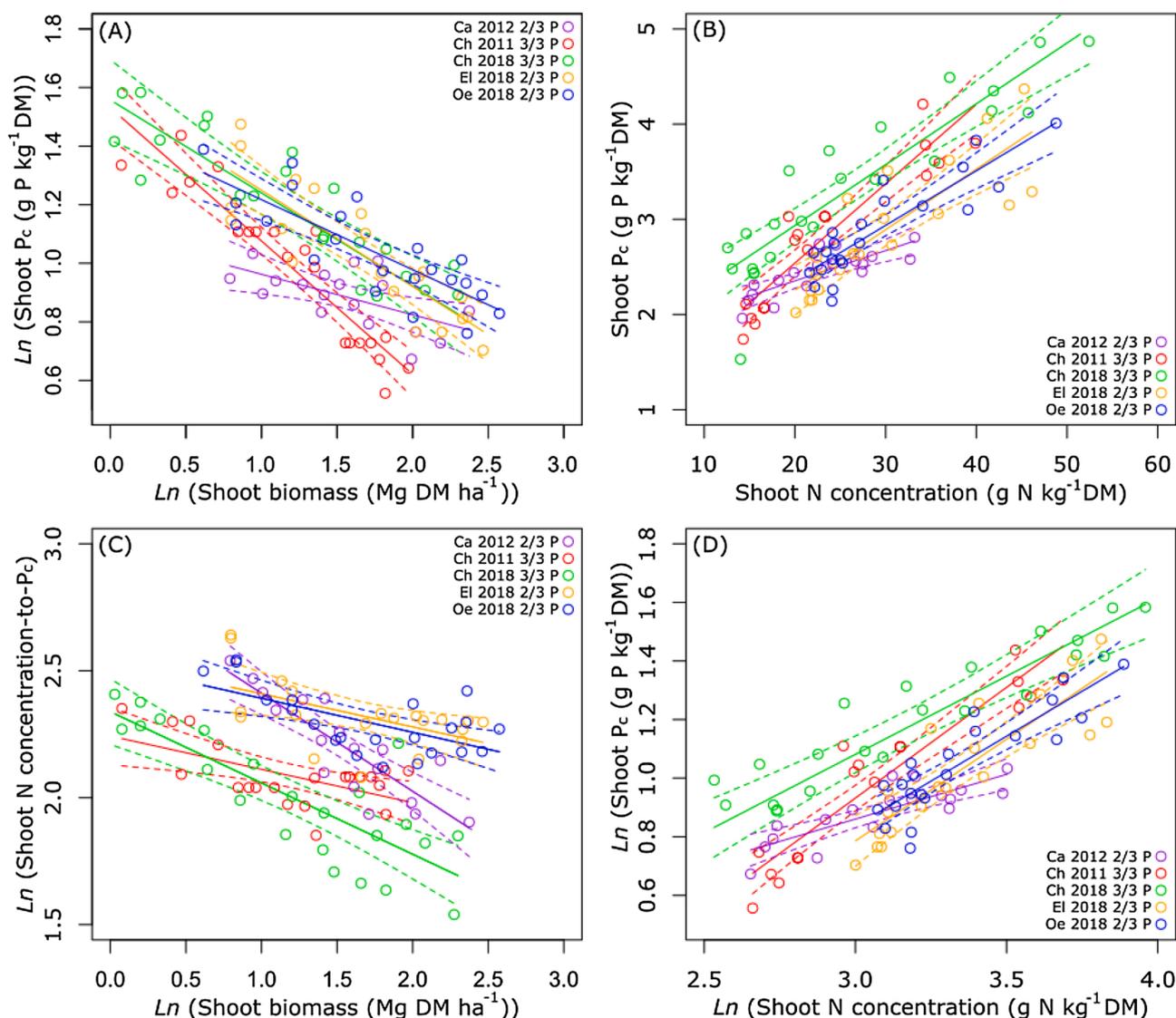


Fig. 1. Linear relationships between \ln of shoot P_c and \ln of shoot biomass (A), shoot P_c and shoot N concentration (B), \ln of N-to- P_c and \ln of shoot biomass (C) and \ln of P_c and \ln of shoot N concentration for each site-year (D). Solid lines represent the linear regressions whereas dashed lines indicate the confidence intervals.

with P_c in g kg^{-1} DM and SB the shoot biomass expressed in Mg DM ha^{-1} (Fig. 3).

The P_c values were also expressed as a function of shoot N concentration for each site-year using both a linear regression and a linearized power function following an \ln transformation. Among site-years, the coefficients of determination (R^2) of the linear regressions between P_c and shoot N concentration ranged from 0.63 to 0.85 whereas those of the linearized power functions ranged from 0.67 to 0.88 (Table 4). Intercept and slopes at all site-years were consistently significant using linear regressions and generally significant for linearized power functions. Slopes of linear regressions at Ch 2011 and Ca 2012 differed from those at the other three site-years (Ch 2018, El 2018, and Oe 2018), but no differences were observed among those three site-years as confirmed by the overlapping confidence intervals (Fig. 1B). However, the intercept differed between Ch 2018 and the two other site-years, indicating a site-year effect; this can also be seen by the non-overlapping confidence intervals of Ch 2018 with the other site-years except Ch 2011 (Fig. 1B).

A general equation was developed using only El 2018 (NNI = 112 %) and Oe 2018 (NNI = 114 %), two site-years with similar slopes and intercepts, and without N deficiency:

$$P_c = 1.10 + 0.061 \times N \quad (n = 42, R^2 = 0.73, p < 0.001) \quad (6)$$

with P_c and N in g kg^{-1} DM.

After \ln transformations, the differences in slopes and intercepts of the linearized power functions exhibited a similar trend than without the \ln transformations but differences between site-years were less pronounced (Table 4). A general power function was developed also using only El 2018 and Oe 2018:

$$P_c = 0.34 \times N^{0.632} \quad (n = 42, R^2 = 0.74, p < 0.001) \quad (7)$$

3.4. N-to- P_c ratio

The N-to- P_c ratio was also expressed as a function of shoot biomass for each site-year as was suggested by Greenwood et al. (2008). Among site-years, the coefficients of determination (R^2) of the linear functions between the N-to- P_c ratio and shoot biomass ranged from 0.27 to 0.72 (Table 4). The site-years of El 2018 and Oe 2018 had similar slopes and intercepts (Table 4), and the confidence intervals of their linear regressions overlapped (Fig. 1C). The site-year of Ch 2011 had a similar slope than El 2018 and Oe 2018, but a different intercept. There was no overlapping between the confidence intervals of their respective linear regressions, indicating a site-year effect. The slopes at Ch 2018 and Ca

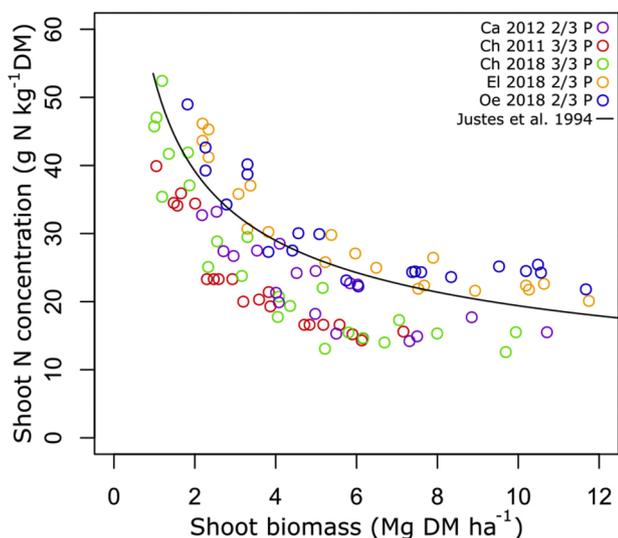


Fig. 2. Shoot N concentration as a function of wheat shoot biomass for treatments with the lowest P rate that achieved the maximum shoot biomass accumulation at Cadenazzo (Ca 2012, 2/3 P), Changins in 2011 (Ch 2011, 3/3 P) and 2018 (Ch 2018, 3/3 P), Ellighausen (El 2018, 2/3 P) and Oensingen (Oe 2018, 2/3 P) along with the critical N curve by Justes et al. (1994) ($N_c = 53.5 \times SB^{-0.44}$ with N_c the critical shoot N concentration and SB the shoot biomass).

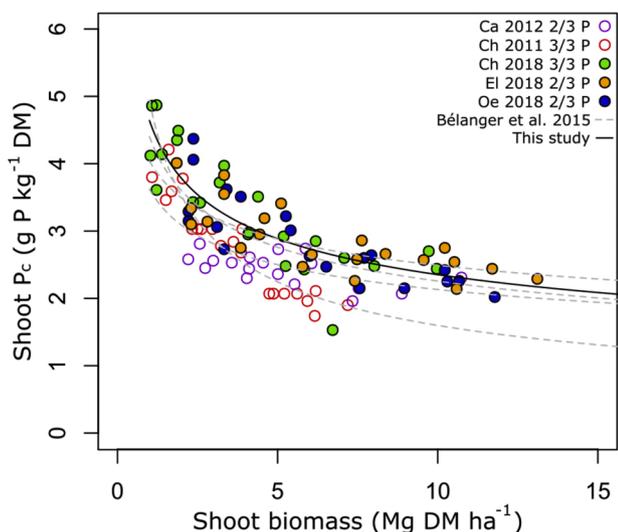


Fig. 3. Critical shoot P concentration (P_c) as a function of wheat shoot biomass for treatments with the lowest P rate that achieved the maximum shoot biomass accumulation at Cadenazzo (Ca 2012, 2/3 P), Changins in 2011 (Ch 2011, 3/3 P) and 2018 (Ch 2018, 3/3 P), Ellighausen (El 2018, 2/3 P) and Oensingen (Oe 2018 2/3, P). In addition, the P_c dilution curves by Bélanger et al. (2015a,b) and the general P_c dilution curve ($P_c = 4.56 \times SB^{-0.279}$ with SB the shoot biomass) developed in this study (using El 2018 and Oe 2018) are also included.

2012 were different from those of the other three sites-years (Table 4, Fig. 1). Our results show that the relationship between the N-to- P_c ratio and shoot biomass differed between site-years, except between those without N deficiency (i.e. El 2018 and Oe 2018). Data points of these two site-years were used to develop a general equation:

$$N\text{-to-}P_c = 13.02 \times SB^{-0.150} \quad (n = 42, R^2 = 0.39, p < 0.001) \quad (8)$$

with SB in Mg DM ha^{-1} .

As a whole, N-deficient site-years had lower N-to- P_c ratios than El 2018 and Oe 2018, the two site-years without N deficiency (Fig. 5).

3.5. Relationships between shoot biomass, grain yield, and PNI

The relationship between the relative shoot biomass and PNI was modeled using the Cate-Nelson procedure with P_c estimated with three equations. For site-years without a N deficiency (El 2018 and Oe 2018), results were similar for the three equations. The critical values for PNI calculated using the dilution curve as a function of shoot biomass, the linear regression as a function of shoot N concentration, and the power function of shoot N concentration were 101 %, 99 %, and 100 %, respectively. The percentages of observations that did not fall into the quadrants predicted by the model were 2.6 %, 5.3 %, and 5.3 % with R^2 values of 0.16, 0.15, and 0.15, respectively. For site-years with N deficiency (Ch 2011, Ch 2018, and Ca 2012), the critical values for PNI calculated using the dilution curve as a function of shoot biomass, the linear regression as a function of shoot N concentration, and the power function of shoot N concentration were 86 %, 113 %, and 114 %, respectively. The R^2 values were respectively 0.51, 0.40, and 0.39, while the percentages of observations that did not fall into the quadrants predicted by the model were 11.5 %, 17.3 %, and 19.0 %. The P_c estimated by the dilution curve as a function of shoot biomass was judged the most reliable equation for the calculation of PNI (Fig. 6).

For the relationship between relative grain yield and PNI, the critical values estimated by the Cate-Nelson procedure were not considered because the R^2 values were less than 0.01 and 0.03 for the site-years without and with N deficiency, respectively. The maximum values of grain yield were observed for the 3/3 P fertilization treatment, except at Oe 2018 and Ch 2018 (Table 1). Therefore, the relative grain yield generally decreased for the maximal values of PNI associated to the highest P fertilization rates (Fig. 6B). For the same P fertilization treatment, PNI values varied during the growing season and across site-years. Maximum relative shoot biomass was observed for PNI values ranging between 77 %–99 % for Ch 2011 and Ca 2012 (site-years with N deficiency), and between 93 %–118 % for the three other site-years.

4. Discussion

4.1. Shoot biomass and grain yield

Differences in shoot biomass and grain yield were greater among site-years than among the P fertilization treatments, a result that has been frequently reported (Morel et al., 1992; Gallet et al., 2003). Site-year differences can be attributed to a combination of climatic conditions and soil characteristics such as soil texture and organic C content (Table 1). The P_c values were therefore tested under a significant range of soil and climatic conditions that resulted in a range of shoot biomass and grain yield (Tables 2 and 3). The fact that the highest and the lowest grain yields were both measured at Changins in 2011 and 2018, respectively (Table 3), suggests that the grain yield was more affected by climatic conditions than by the range of soil conditions across sites. The greater grain yield at Changins in 2011 could be partly explained by a greater cumulated precipitation from flowering to harvest than in 2018 (140 vs. 95 mm, météo Suisse), while the lower shoot biomass at Changins in 2011 could be attributed to less cumulated precipitation from tillering to flowering (29 vs. 60 mm).

Phosphorus fertilization significantly affected wheat shoot biomass at 22 of the 36 sampling dates, particularly for stages CD 27–37, whereas grain yield was not affected at any of the site-years (Tables 2 and 3). In a study conducted at eight site-years, Bélanger et al. (2015a) reported that the spring wheat shoot biomass was significantly affected by P fertilization at 7 of the 40 sampling dates, but no effect was observed on grain yield at any of the site-years. The response of the shoot biomass to P fertilization, however, was greater in our study than in the study of Bélanger et al. (2015a). This result can be explained by the fact that the P fertilization treatments were applied only once in the study of Bélanger et al. (2015a), while in our long-term experiments the P fertilization treatments were applied annually over several decades. Our results

confirm that the response to P fertilization is often greater for shoot growth than for grain yield (Otto and Kilian, 2001). In spring wheat grown in Canada, the reduction in grain yield (12–25 %) due to a P deficiency was less than the reduction in shoot biomass earlier in the season (25–50 %) (Grant et al., 2001). In canola, Bélanger et al. (2015b) concluded that a reduction in early crop growth of more than 30 % was required to affect grain yield.

Different responses to P fertilization in long-term experiments have been reported and they might be due to soil types (Gallet et al., 2003; McKenzie et al., 2003) or to the duration of the experiments (Messiga et al., 2010). In field experiments longer than 20 years, P fertilization systematically increased wheat grain yield in a Japanese Andosol whereas no effect was observed in a Chinese loess soil (Takahashi and Anwar, 2007; Ming-De et al., 2007). In a loamy black earth soil in the Czech Republic, P fertilization did not affect the wheat grain yield the first decade whereas the effect was systematically significant during the fifth decade (Kunzová and Hejman, 2009). Even though our study was conducted at four sites with contrasted pedoclimatic conditions, the lack of a grain yield response to P fertilization was observed at all sites after three decades of variable P fertilization. This result highlights the capacity of several soils to provide sufficient P for grain yields. As the soil P_{NaHCO_3} values of the 0 P treatment for each site-year (Table 1) were in the range of the critical values reported for wheat (from 4.9 to 24 mg P kg⁻¹, Jackson et al., 1991; Bollons and Barraclough, 1999; Colomb et al., 2007; Tang et al., 2009; Wang et al., 2016), we can expect the effect of P fertilization to become significant in the future. Because the critical values of soil P_{NaHCO_3} differ across soil conditions, shoot P_c for diagnostic purposes offer a promising alternative for improving P fertilization practices.

4.2. Critical phosphorus values

Site differences for the P_c dilution curves were also reported by Bélanger et al. (2015a) for spring wheat grown at site-years in Canada, Finland, and China. The highest and the lowest P_c dilution curves in our study were in the range of those reported in the study of Bélanger et al. (2015a; Fig. 3) in which the site-years with the lowest P_c dilution curve tended also to be those with the lowest shoot N concentrations. Differences in the relationship between P_c and shoot biomass among some of the site-years can be attributed to the crop N status since it can influence the P_c dilution curve. Nitrogen was deficient at three site-years with NNI values ranging from 69 to 82 %, while the NNI was greater than 100 % at the other two site-years. It is therefore very likely that, in both studies, the difference in P_c dilution curves at some site-years can be ascribed to N deficiency. The P_c dilution curve developed in our study may be generalizable for winter wheat as well as for spring wheat across site-years providing that N is not limiting. However, critical N dilution curves of spring wheat and winter wheat likely differ as previously reported (Ziadi et al., 2010).

We also tested if P_c can be predicted independently of site-years using the stoichiometric relationship with shoot N concentration. This relationship was first proposed by Duru and Ducrocq (1997) for grasslands and later investigated for timothy grass (*Phleum pratense* L.) (Bélanger and Richards, 1999; Bélanger and Ziadi, 2008), grasslands under Swiss conditions (Liebisch et al., 2013), maize (Ziadi et al., 2007), and spring wheat (Ziadi et al., 2008a). In all those previous studies, linear regressions were used. A power function might be more appropriate than the linear regression to describe the relationship between shoot P and N concentrations because both shoot P and N concentrations decrease with increasing shoot biomass according to a power function. For that reason, we tested both the linear regression and the power function.

The linear relationship and the linearized power function were also found to vary with site-years, likely due to the wheat N status (Figs. 1B, D and 2). Indeed, N deficiency and N luxury consumption are known to affect the relationship between P_c and shoot N concentration (Ziadi

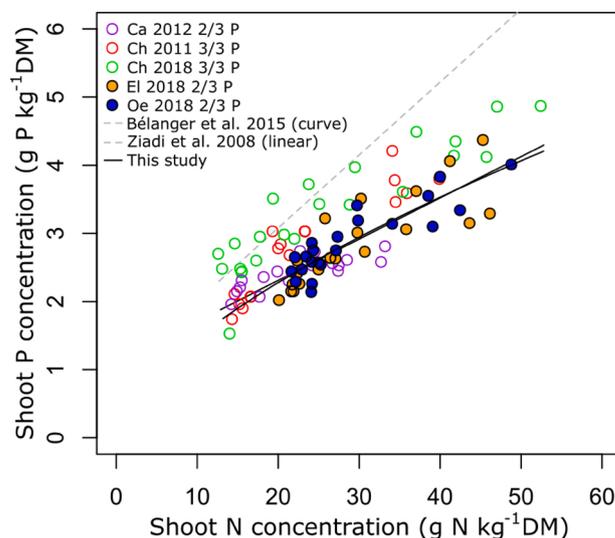


Fig. 4. Critical shoot P concentration (P_c) as a function of shoot N concentration along with the linear ($P_c = 1.10 + 0.061 \times N$, $R^2 = 0.73$) and the power ($P_c = 0.34 \times N^{0.632}$, $R^2 = 0.74$) regressions for the data points from Ellighausen (El 2018, 2/3 P) and Oensingen (Oe 2018 2/3 P). The linear relationship reported by Ziadi et al. 2008 ($P_c = 0.94 + 0.107 \times N$) for spring wheat is also shown.

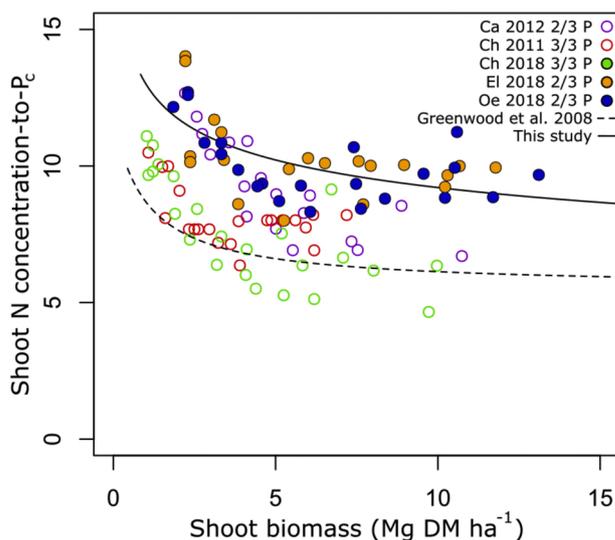


Fig. 5. Shoot N concentration-to-critical shoot P concentration (P_c) ratio as a function of wheat shoot biomass at Ca 2012, Ch 2011, Ch 2018, El 2018, and Oe 2018 along with the critical curve by Greenwood et al. (2008) and the shoot N concentration-to- P_c curve developed in our study using El 2018 and Oe 2018.

et al., 2008a; Bélanger et al., 2015a). Furthermore, the slope of the relationship between N and P concentrations can interact with the developmental stage (Hamner et al., 2017). In our study, the N deficiency resulted in higher P_c for a given shoot N concentration at Ch 2011 and Ch 2018 compared to the other site-years (Fig. 1B and D). Bélanger et al. (2015a) also observed a site-year effect for the linear relationship between P_c and shoot biomass but site-years did not differ for their relationship between P_c and shoot N concentration although a N deficiency may have occurred at some site-years.

General equations (Eqs. 6 and 7) were developed using only El 2018 (NNI = 112 %) and Oe 2018 (NNI = 114 %), two site-years with similar slopes and intercepts, and without any N deficiency (Table 4). The close results from the linear relationship and the power function (Fig. 4) suggest that although the use of a power function makes more sense from a theoretical perspective, linear regressions can be reasonably used

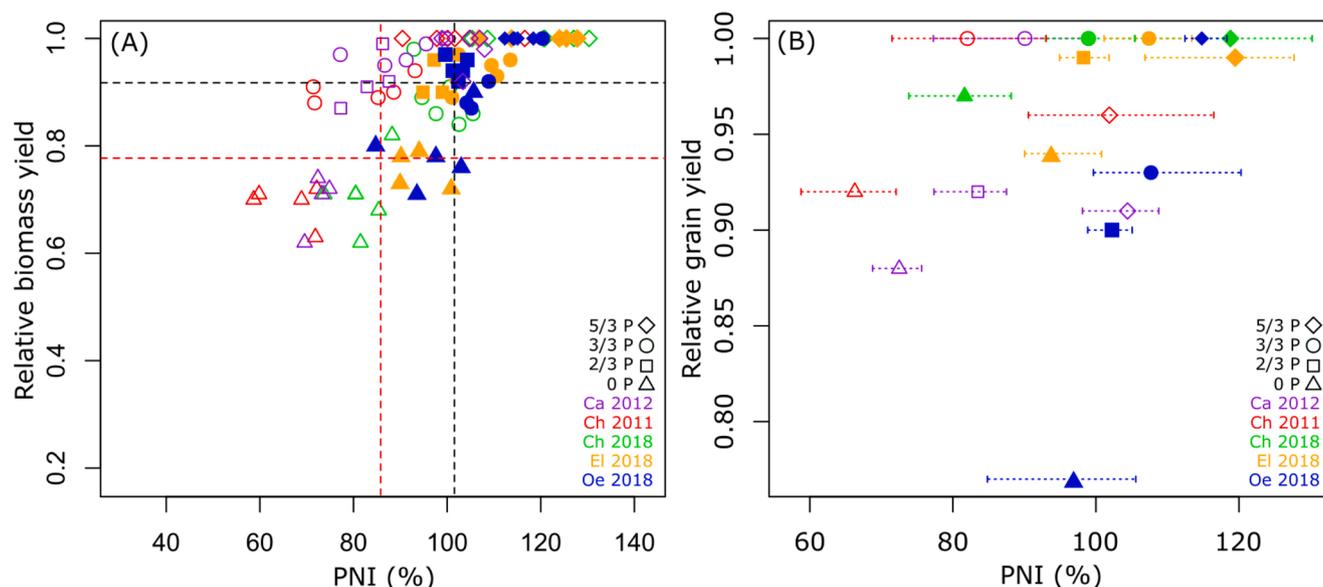


Fig. 6. (A) Relative wheat shoot biomass as a function of the P nutrition index ($PNI = P_{\text{measured}} / P_c \times 100$) for all fertilization treatments and sampling dates at Cadenazzo in 2012 (Ca 2012), Changins in 2011 and 2018 (Ch 2011 and Ch 2018), Ellighausen in 2018 (El 2018), and Oensingen in 2018 (Oe 2018); only data with shoot biomass greater than 1 Mg DM ha^{-1} and for which the P dilution had started are included. Black and red dashed lines indicate the critical PNI thresholds set by the Cate-Nelson procedure for, respectively, site-years without a N deficiency (El 2018, and Oe 2018, full points) and site-years with a N deficiency (Ca 2012, Ch 2011 and Ch 2018, empty points). Horizontal dashed line indicates the thresholds of relative shoot biomass set by the Cate-Nelson procedure for the site-years with and without a N deficiency. (B) Relative grain yield as a function of the PNI with mean and maximum range of PNI values.

to estimate P_c . Compared to linear relationships previously observed for spring wheat (Ziadi et al., 2008a; Bélanger et al., 2015a), our relationships were different with greater N concentration for a given P_c (Fig. 4), likely due to the higher N concentration of winter wheat compared to spring wheat (Ziadi et al., 2010).

The equations (Eqs. 6 and 7) developed in our study may be used to diagnose the P status of winter wheat only for site-years without either N deficiency or strong luxury consumption in accordance with the N_c developed by Justes et al. (1994). However, this assumption needs additional validation because only two site-years of this study were undoubtedly not N deficient. Overall, our data confirmed the strong relationship between P_c and shoot N concentration initially reported for spring wheat (Ziadi et al., 2008a; Bélanger et al., 2015a), maize (Ziadi et al., 2007), timothy grass (Bélanger and Richards, 1999; Bélanger and Ziadi, 2008), and multi-species swards (Duru and Ducrocq, 1997). Our results also confirmed that the relationship between P_c and shoot N concentration for achieving maximum shoot growth varies with the level of N nutrition. Estimating P_c using the shoot N concentration has the advantage of avoiding the time-consuming shoot biomass measurements (Bélanger et al., 2015a). However, this advantage would be lost if a NNI estimation is required for determining P_c with the relationship between P_c and shoot N concentration.

4.3. Shoot N-to- P_c ratio

The shoot N-to- P_c ratio decreased with increasing shoot biomass at all site-years. This decrease, previously reported by Greenwood et al. (2008) for several crop species and by Bélanger et al. (2017) for grasslands, is due to the faster decrease in shoot N concentration than in shoot P concentration as shoot biomass increases. The relative decrease in nutrient concentration with increasing shoot biomass is quantified by the dilution coefficient. For P, our dilution coefficient across the two site-years with no evident N deficiency was 0.30, while the dilution coefficient for N was 0.45, very close to 0.44 reported by Justes et al. (1994). The linearized relationship between the shoot N-to- P_c ratio and shoot biomass differed across site-years (Fig. 1C, Table 4). The relationships at Ch 2011 and Ch 2018 were particularly different from

those at the other three site-years. The low shoot N concentrations at Ch 2011 and Ch 2018 most likely decreased the shoot N-to- P_c ratio. At Ca 2012, the other site-year with a N deficiency, both N and P concentrations were lower than those at El 2018 and Oe 2018 (Tables A.1 and A.2), resulting in a different relationship even though there was no site-year effect (i.e. overlapping of the confidence intervals, Fig. 1C). Our results indicate that a N deficiency at different site-years affect the relationship between the shoot N-to- P_c ratio and shoot biomass.

Our relationship between the shoot N-to- P_c ratio and shoot biomass, based on two site-years with no evident N deficiency, differed from that previously published by Greenwood et al. (2008). For a given shoot biomass, the estimated shoot N-to- P_c ratio in our study was greater than that predicted by the equation of Greenwood et al. (2008). The luxury N consumption at El 2018 and Oe 2018, indicated by NNI values greater than 100 %, might explain the greater shoot N-to- P_c ratio than that predicted by Greenwood et al. (2008). In forage grasses (Bélanger et al., 2017), the estimated shoot N-to- P_c ratio also tended to be greater than that predicted by the equation of Greenwood et al. (2008). Bélanger et al. (2017) also explained the particularly high values of the shoot N-to- P_c ratio at one site by NNI values greater than 100 %. In both our study and that of Bélanger et al. (2017), there were no treatments with varying rates of N fertilization. Further studies with both rates of N and P fertilization are required to determine the optimal relationship between shoot N-to- P_c ratio and shoot biomass. Our results, in accordance with Greenwood et al. (2008) and Bélanger et al. (2017), strongly suggest that the level of shoot biomass should be taken into account when interpreting shoot N-to- P_c ratio for diagnostic purposes.

4.4. PNI and N-to- P_c as diagnostic tools

The Cate-Nelson procedures identified two different PNI thresholds for site-years with and without N deficiency beyond which a lack of positive response of relative shoot biomass was expected (Fig. 6A). The values of the PNI for the site-years with a clear N deficiency might have been underestimated (except at Ch 2018) because of an overestimation of the P_c values which were estimated from the equation based on data from sites with no N deficiency. The PNI thresholds separated the 0 P

treatments that were P limited from the other P treatments. This result shows that N deficiency lowers P_c without affecting the positive effect of P fertilization on wheat biomass production, suggesting that the efficiency of P fertilizer was not affected by N limiting conditions. Our results for winter wheat confirm those of [Ziadi et al. \(2008a\)](#) found on spring wheat in that separate P_c curves are needed for N-deficient and N-sufficient conditions. A similar conclusion was drawn for forage grasses ([Bélanger et al., 2017](#)).

The lowest relative grain yields were associated with the lowest PNI values at each site-year. We could not, however, establish a relationship between the relative grain yield and PNI. As previously discussed, the shoot biomass is more sensitive to P deficiency than grain yield. Therefore, maintaining the PNI above the critical threshold could be used as safeguard to avoid a decrease of grain yield due to P limitation.

No relationship between the N-to- P_c ratio and relative grain yield or relative shoot biomass was found. The P fertilization decreased the N-to- P_c ratio as already observed in a long-term field trial ([Takahashi and Anwar, 2007](#)). These authors reported that the wheat N-to-P ratio decreased with P applications and increased with N applications. However, the N-to-P ratio is more sensitive to P than to N fertilization ([Güsewell and Koerselman, 2002](#); [Sadras, 2006](#); [Yan et al., 2015](#)). In a meta-analysis by [Sadras \(2006\)](#), maximum grain yields of cereals were predicted for a mean N-to-P ratio in shoots of 5.6, which is far below the values observed in our study. Because the N-to-P ratio declines during crop growth and development as seen in our study on winter wheat and as reported by [Greenwood et al. \(2008\)](#) for spring wheat, defining one critical value of the N-to-P ratio is risky except if it is associated with a level of shoot biomass or a given stage of development. The variability of the N-to-P ratio due to the interaction between biomass accumulation, fertilization, and site-years conditions makes it difficult to develop a general relationship to predict grain yield. Environmental conditions influence the N-to-P ratio. In European non-forested wetlands, the N-to-P varied more across sites than across different plant species grown at the same site ([Güsewell and Koerselman, 2002](#)). For winter wheat shoot biomass of 5 and 10 Mg ha⁻¹, we assume that N-to- P_c ratios of 10 and 9, respectively, consist in reasonable trade-off to identify P limitation ([Fig. 5](#)). The N-to-P ratios below these values suggest a P sufficiency but does not exclude P limitation, in particular for N-deficient winter wheat. The N-to-P ratios above these values clearly indicates a P deficiency.

4.5. Perspectives and limitations

The identification of data points that represent non-limiting conditions for shoot growth in experiments with several N or P fertilization rates is an important issue and several methods have been used ([Greenwood et al., 1990](#); [Justes et al., 1994](#)). The method proposed by [Justes et al. \(1994\)](#), based on a statistical analysis of both shoot biomass and N concentration, requires more than four fertilizer rates along with a positive response to the fertilization and, therefore, could not be used in our study. The method proposed by [Greenwood et al. \(1990\)](#) identifies critical data points by comparing P fertilization treatments for shoot biomass on each sampling date. In our study, the P_c data points identified through this last method were associated to different fertilization treatments depending on the sampling date (not shown). To avoid this problem, we chose to consider the entire sampling period by considering as P-limited the P fertilization treatments when the shoot biomass decreased compared to the highest P fertilization rate on at least one sampling date. As a result, the P_c curves developed by considering the entire sampling period had greater coefficients of determination than those using P_c data points for each sampling date. Overall, the methods proposed by [Justes et al. \(1994\)](#) and [Greenwood et al. \(1990\)](#) as well as that used in our study are sensitive to the magnitude of the variability of shoot biomass among replications within fertilization treatments. For instance, in this study no effect of P fertilization on shoot biomass was observed at Oe 2018 due to the high variance coefficient compared to

the other sites, whereas a relative shoot biomass as low as 80 % was observed with the 0 P treatment. Hence, the identification of P_c data points is particularly influenced by the heterogeneity across replications when the number of replications is low, as frequently observed in field experiments testing P fertilization ([Johnstone and Sinclair, 1991](#)). Recently, a Bayesian statistical approach was proposed to overcome this problem by estimating the most probable coefficients of the critical dilution curve, while including in the model the uncertainty of the threshold separating the limiting and the non-limiting fertilizer rates ([Makowski et al., 2020](#)). This approach should be considered in future research on critical nutrient concentrations.

Luxury P consumption might have occurred for some fertilization treatments, including those that were identified for determining the P_c values. There is, therefore, the risk of a slight overestimation of P_c with our general P_c dilution curve that might eventually result to an over-fertilization. This risk of overestimating P_c was reported on spring wheat ([Bélanger et al., 2015a](#)), canola ([Bélanger et al., 2015b](#)), and maize ([Gagnon et al., 2020](#)). The small number of P fertilizer rates in our study limited our ability to determine precisely the P_c values. Our results strongly suggest that P fertilizer experiments with a large gradient of P limiting conditions and more than three rates are required to determine the P_c values and the corresponding critical curves and relationships.

Our results confirm the effect of an N deficiency on the P_c curve expressed either as a function of shoot biomass or shoot N concentration. Consequently, the P_c dilution curve and the relationship between P_c and shoot N concentration determined in our study apply only for conditions when N is not limiting shoot growth. This limits their usefulness for predicting P_c and estimating the level of P nutrition. In a study with varying rates of both fertilizer P and N applied to forage grasses, [Bélanger and Ziadi \(2008\)](#) proposed different P_c curves for different N rates and they concluded that the degree of N deficiency should be considered when establishing the critical P concentration. The interaction between P and N, and its effect on the determination of the P_c curves should therefore be investigated in experiments that include a wide range of N and P deficiencies.

Different wheat cultivars were used at the four sites used in this study and we hypothesized that cultivars did not differ in their P_c dilution curve as was observed for potato cultivars ([Soratto et al., 2020](#)). This assumption, however, was not formally tested in our study. To our knowledge, there are no reports of cultivar differences in the critical N and P concentration curves of winter wheat.

The study was conducted under rain-fed conditions at all sites. The level of water stress was not measured, but we can assume that it varied among the five site-years. Water deficit was shown to affect the critical N concentration curve in potatoes ([Bélanger et al., 2011](#)). Soil water restriction is known to affect indirectly soil N availability, N uptake, and crop N status ([Kunrath et al., 2020](#)). It is likely that the soil water restriction will also affect the P_c dilution curves. Studies of the interaction between water stresses and P nutrition with the objective of defining P_c curves are required.

5. Conclusion

Phosphorus fertilization ranging from no P addition up to twice the theoretical crop uptake did not affect the grain yield of winter wheat after three decades of annual applications. This result confirms that current soil P availability can be sufficient to ensure near maximum yield after several decades without any fertilizer P application. Shoot biomass during the growth cycle, however, was generally influenced by P fertilization, particularly at development stages from CD 27 to 37. The critical P concentration, either expressed as a function of shoot biomass or shoot N concentration, and the N-to- P_c ratio, expressed as a function of shoot biomass, differed among site-years and this difference was attributed to varying crop N status. The relative shoot biomass responded positively to PNI but the critical PNI differed in relation to the degree of N deficiency. The relative grain yield, however, was not related

to the PNI because of the limited range of PNI. The PNI based on a P_c dilution curve has the potential to diagnose P deficiency and the response to P fertilization. More research, however, is needed to clarify the effect of N deficiencies on the P_c dilution curve and to improve the precision of the reference curve with multi-site experiments including at least five fertilizer P rates using a novel statistical approach.

Author contribution

S.S. planned and designed the study. S.E., M.F. and J.H. collected the data set. M.F. analyzed the data and performed statistical analyses, figures and tables. M.F., G.B. and S.S. wrote the first version of the draft. All authors provided critical feedback on the manuscript and gave final approval for publication.

Declaration of Competing Interest

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

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Appendix B. Supplementary data

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