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Evening Primrose and Rapeseed Yield Components and Grain Oil Concentrations Were Differentially Modulated by the N, P, and K Supplies in a Mediterranean Area

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Abstract: Evening Primrose (*Oenothera biennis* L.) is an industrial crop-producing seed with high oil concentration characterized by high gamma-linoleic acid. However, limited information is available on its response to the nutrient supply, especially P and K. The aim of this work was to compare the response of Evening Primrose to the application of N, P, and K alone or in combination in a P and K deficient soil in terms of grain yield, yield components, and oil composition in comparison to Rapeseed (*Brassica napus* L.). Evening Primrose yielded 54.4% less seed and 67.3% less oil than Rapeseed. Such differences were reduced when nutrients, especially N or P, were applied. N stimulated Evening Primrose more than Rapeseed. Application of K favored Evening Primrose oil yield when no N or P were added, and this especially occurred by an enhancement of the pod number. P favored yield per pod and oil yield in the Evening Primrose more than Rapeseed in almost all conditions. Fertilization scarcely affected lipid composition. In particular, an increase in the oleic acid concentration was found only when N + K or N + P + K were applied compared to the sole N or sole K applications. These results suggest that P and K differentially influenced yield components of both species and that Evening Primrose was less adapted than Rapeseed to a scarce nutrient supply.

Keywords: arid and semiarid; *Brassica napus*; fertilizer; Mediterranean; *Oenothera biennis*; nutrient response

1. Introduction

Evening Primrose (*Oenothera biennis* L., Family Onagraceae, Order Myrtales) is a facultative biennial species native to central and North America from arid and semiarid areas [1]. Evening Primrose is known for having plenty of uses, with the product of the high-oil seed characterized by high gamma-linoleic acid (GLA) and other important secondary compounds, especially ellagitannins and hydrolyzable tannins bearing the galloyl group residue [2–4]. In addition, its byproduct after the extraction of the seed oil also shows high potential for other uses [5,6].

Evening Primrose is primarily found in ruderal environments. Under cultivation, Evening Primrose is appreciated for both its seed oil, ability to grow as both a winter/spring or a spring/summer crop, and the brevity of its growth cycle. In temperate environments, Evening Primrose is mostly grown as a spring crop [7] and as a winter crop in arid and semiarid areas [8]. In particular, it has been shown that the reaction of Evening Primrose to low temperature can be critical for its growth and yield [9] and that temperatures higher than 26 °C before the onset of flowering may inhibit the reproduction and induce the biennial behavior of the species [10]. Evening Primrose is considered a promising industrial crop along with other species including *Borago officinalis*, *Echium plantagineum*, *Calendula officinalis*, and various species in the genus Brassica [11]. However, information on its agronomic traits, ability to withstand various stresses, and critical nutrient requirements are scarce. Such a lack of knowledge especially occurs for crops in Mediterranean areas, where stresses including drought were shown to reduce grain, oil yields, and quality to a variable extent [8,12–17], where the effect on oil content was similarly shown to be negligible in other species [18]. Most later reports have shown that a plant density higher than 100 plant m^{-2} for Evening Primrose is a prerequisite to achieving high yields when the water supply is adequate, but little information is available for water-limited conditions. Similarly, information on the nutrient needs of Evening Primrose is scarce. It was hypothesized that the N needs of the crops are minimal [2,19], but such information has relied on limited and contrasting reports. In pot conditions, Ghasemnezhad and Honermeier [20] showed that increasing N availability from 83 ppm to 167 ppm resulted in a doubling of the seed yield under adequate P and K application, whereas a further increase to 333 ppm N in the soil increased yield by only 11% to 17% depending on the sowing time. Later researchers found similar results in a similar experiment [21] irrespective of the harvest method. Under field conditions and high P supply, Şekeroğlu and Özgüven [14] found a minimal role of N application on the Evening Primrose yield with varying environmental traits and plant densities. In contrast, very limited information is available on the Evening Primrose response to the P and K application [22] and no information is available under field conditions. In contrast, other species with similar uses, e.g., rapeseed, showed a strong response to N and to a lesser extent, to P and K [23], the latter of which showed little effects on the seed oil composition. In addition, rapeseed P and K accumulation may also depend on the S availability [24].

The aim of the present work was to study the relationship among N, P, and K supply on the yield and yield components of Evening Primrose compared to another well-known oilseed species, namely Rapeseed (*Brassica napus* L. cv. serw 1), both grown in a P and K deficient soil in two cropping seasons in a Mediterranean area. Rapeseed was also used as a reference in the studies by [11]. In particular, we hypothesized that under sufficient water availability, the application of P would stimulate plant yield through a direct effect on pod fertility, whereas the application of K would increase the ability of the crop to regulate the gas exchange and thus affect the plant's branching and pod number per unit area. We also hypothesize the effects of the P and K fertilization would be more pronounced under N fertilized conditions than the non-N fertilized treatments.

2. Materials and Methods

2.1. Field Experiment

A field experiment was conducted at the Agricultural Experimental Station (Faculty of Agriculture, Cairo University, Egypt (30°02′53.7″ N 31°12′36.2″) during the two growing seasons: 2017/18 and 2018/19. The climate of the area of the study is a subtropical desert (Bwh climate according to Köppen classification), with mild, wet winters and warm, dry summers. See Mostafa et al. [25] for weather and climatic data. The physical and chemical analyses of the soil were determined according to Jackson [26] and Cottenie [27]. The soil texture was a sandy loam, with 44.9% sand, 27.8% silt, 27.3% clay, and 0.89% organic matter. The results of soil chemical analysis were; pH = 8.13, E.C. = 0.88 dS m^{-1}, available phosphorus (Olsen) = 28.3 mg/kg, and potassium = 207 mg/kg. Field capacity (FC) and wilting point (WP) were determined according to Black [28]. Field capacity, permanent wilting point, available soil moisture (ASM), and soil bulk density (BD) were 34.50%, 16.01%, 18.49%, and 1.36 g L^{-1}, respectively. The plants were irrigated through lateral irrigation on average once every 10 days to reach field capacity.

Seeds of *Brassica napus L.* cv. serw 1 (hereafter referred to as "Rapeseed") and *Oenothera biennis* L., local genotype (hereafter referred to as "Evening Primrose") were obtained from the Agricultural Research Centre (Ministry of Agriculture, Egypt). Both species were sown on 15 November during both seasons, into 3 m × 3.5 m plots, in rows at a distance of 60 cm from one another and 20 cm between hills. The seedlings were thinned 30 and 45 days after sowing to leave two plants per hill to give 170 plants/plot.

The experimental layout was a split-plot (plant species in the main plots and the application of fertilization treatments were distributed randomly in the subplots) in three replicates.

Fertilization included the application of N, P, and K in a complete combination of the three fertilization factors (with and without, referred to as 'No' per each element). Thus, in total, 16 treatments were established, by the combination of the species (2 levels) each of which were fertilized with only N, K, P, with N + P, N + K, P + K, and N + P + K, in addition to the unfertilized control.

Plots fertilized with N and K received 142.9 kg N ha^{-1} as urea (46% N) and 171.4 kg K_2O ha^{-1} (i.e., 142.3 kg K ha^{-1}) as potassium sulfate (48% K_2O) per growing season. Both urea and potassium sulfate was applied as a dressing. The total amount of urea and potassium sulfate applied per growing season was split into four equal portions of each fertilizer at 45, 60, 90, and 120 days after sowing (DAS). Phosphorus was applied at a rate of 36.9 kg P_2O_5 ha^{-1} (i.e., 15.1 kg P ha^{-1}) as calcium superphosphate (P_2O_5, 15.5%) before sowing. The choice of the amount of fertilizer applied was done according to the recommendation for the area in similar species. The choice of the timing of the application of the N and K (i.e., split into 4 equal portions) was done to reduce the chance of loss of these nutrients by leaching or emission and thus ensure high availability to the fertilized crops compared to the unfertilized controls. Application of P was done on one single occasion (before sowing) to ensure that the element was well distributed along the profile explored by the roots (around 20 cm depth) since P has scarce movement into the soil and very scarce chance to be lost by leaching.

Both species were harvested on 20 May in both growing seasons at full fruits ripening by uprooting the plants from the soil by hand.

Plant morphological characteristics including plant height, the number of branches, and the number of pods/plant, seed yield, seed oil concentration, and oil yield were measured at plant harvest. In particular, a subplot area of 4 rows 1.5 m long within each plot was harvested to measure the above-mentioned characteristics. The row chosen was the middle one, avoiding the border rows to prevent a border effect. The 1.5-m lent was chosen in the middle of the 3.5 plot. Measures were conducted in a batch of all branches, irrespective of the branching order, to provide representative measurements per unit area (i.e., 2.4 m wide and 1.5 m long, for a total of 3.6 squared meters per subplot). Seeds harvested from a subplot were weighted and saved in bags in a 4 °C fridge until further chemical analyses, which were performed one week after the crop harvest.

2.2. Extraction of Seeds Oils and Gas Chromatography (GC) Analysis of Fatty Acid Methyl Esters (FAME)

Rapeseed and Evening Primrose seeds were crushed and grounded with a grinding mill (Petra electric, Burga, Germany). The oil was extracted from the ground material with n-hexane at 50–60 °C in a Soxhlet apparatus for 6 h following the AOCS method [29]. Total lipid concentration was determined as a percentage of the extracted total lipids to the sample weight (w/w). The oil obtained was stored at 4 °C for further investigation.

The fatty acid profile of total lipids extracted from Rapeseed and Evening Primrose was determined following the International Organization of Standards [30]. One drop of oil was dissolved in 1 mL of n-heptane, then 50 µL of 2 M sodium methanolate in methanol was added, and the closed tube was agitated vigorously for 1 min. The tube was centrifuged at 45,000× g, after the addition of 100 µL of water, for 10 min and the lower aqueous phase was removed. Fifty µL of 1 M HCl was added to the n-heptane phase, the two phases were mixed and the lower aqueous phase was discarded. About 20 mg of sodium hydrogen sulfate (monohydrate, extra pure, Merck, Darmstadt, Germany) was added, and after centrifugation at 45,000× *g* for 10 min, the top n-heptane phase was transferred into a vial and injected into a Varian 5890 gas chromatograph equipped with CP-Sil 88 capillary column, (100 m long, 0.25 mm ID, film thickness 0.2 lm). The temperature program was: from 155 °C to 220 °C (1.5 °C/min.), 10 min isotherm; injector 250 °C, detector 250 °C; carrier gas 1.07 mL/min hydrogen; split ratio 1:50; detector gas 30 mL/min hydrogen; 300 mL/min air and 30 mL/min nitrogen and manual injection less than 1 µL. The integration software computed the peak areas and percentages of FAME were obtained as weight percent by direct internal normalization. See [31] for an additional explanation of the normalization procedure.

2.3. Computations and Statistical Analyses

The analysis of variance was performed according to the statistical design by means of a general linear mixed model (Glimmix procedure in SAS/STAT 9.2 statistical package; SAS Institute Inc., Cary, NC, USA). This procedure can model non-normal data and correct for heteroscedasticity [32]. Restricted maximum likelihood was used to produce unbiased estimates of variance and covariance parameters. In particular, the plant species was the main factor and application of fertilization (as single factors) as sub-treatments. In total, 4 factors (plant species, application of N, P, and K) and their interactions were considered. The model used was similar to that shown by Saia et al. [33] (see the Supplementary Materials on Saia et al. [33] for both a description of the procedure and the SAS package model applied). In contrast to Saia et al. [33], year, rep (year), and rep (species) were added as random variables in the present experiment. A Kenward-Roger estimation of the denominator degrees of freedom of each error was used and least-square means (LSmeans, see below for a definition) of the treatment distributions were computed. Data were provided both as LSmeans and arithmetic means.

Restricted maximum likelihood (REML) was used to produce unbiased estimates of variance and covariance parameters. Differences among means were compared by applying t-grouping at the 5% probability level to the LSMEANS *p*-differences.

A correlation among all traits was performed by means of the CORR procedure in SAS/STAT 9.2. Since most of the variables related to the FAME composition and were highly correlated to each other, no multivariate analysis was performed.

3. Results

3.1. Seed Yield, Yield Components, and Seed Oil Concentration on Yield

As expected, all productive traits strongly varied by the plant species and N availability. Seed yield, yield per pod, and oil concentration were the only productive traits with Sp × N × P × K showing a $p < 0.05$ (Tables 1 and 2, and Supplementary Materials Table S1).

On average, Rapeseed produced 119% more grain than Evening Primrose and such an increase was more evident when no N was applied (from +122% to +179%) than under N fertilization (from +82% to +124%). Application of fertilizer increased on average the yield by the species to a dramatically lower extent compared to the species choice, with N, P, and K fertilization increasing grain yield by 18.5%, 13.2%, and 6.4% on average between species. When no N was applied, K fertilization increased grain yield more than P, whereas under N fertilization the role of both P and K fertilization in grain yield was scarce (Figure 1).

Under N and K fertilization, the addition of P consisted in an increase of the grain yield in the Evening Primrose, but not in the Rapeseed. Results for the plant height were similar to those of the grain yield but less pronounced.

Application of P and K differentially affected the yield per pod when varying the N availability and plant species (Figure 2).

In the non-N fertilized Evening Primrose, K increased the yield per pod by 8.8%, whereas P did not affect it. In the N fertilized Evening Primrose, a similar increase in the yield per pod (+9.8%) was found when both P and K were applied, but not when applied singularly. In contrast to the Evening Primrose, yield per pod in the non-N fertilized Rapeseed increased by 18.0% when P was applied alone and by 16.9% when both P + K was applied, whereas when K but not P was applied, it increased by only 6.1%. In the N fertilized plot, application of P alone slightly increased the grain per pod (+6.0%), whereas application of K alone did not, and the application of both P and K decreased grain per pod by 7.9%.

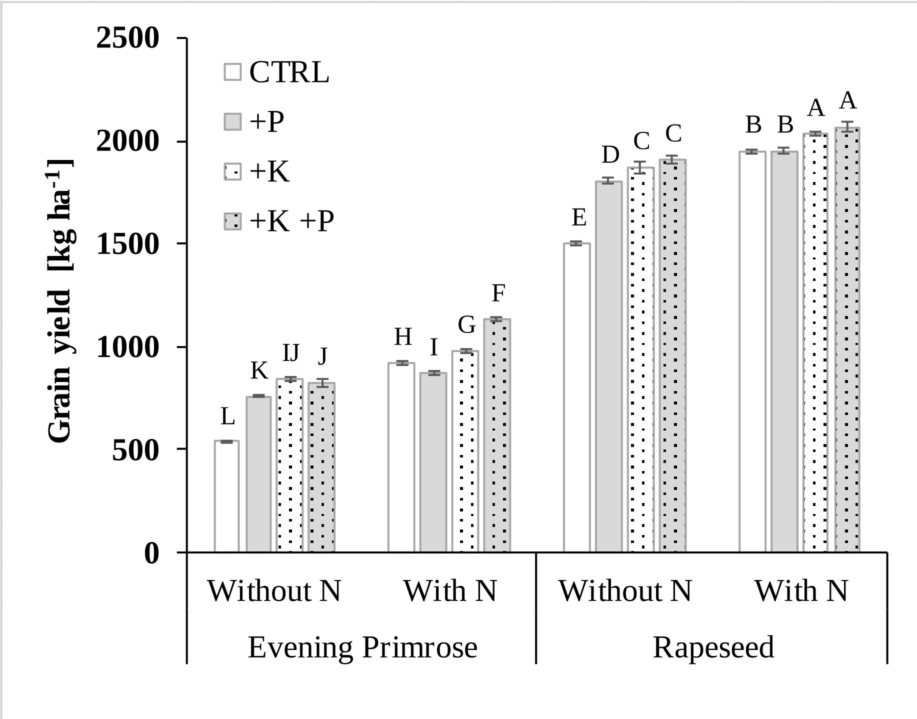

Figure 1. Least squares means (LSmeans) and standard error estimate of the LSmeans of the grain yield of Oenothera biennis L. local genotype (Evening Primrose) and Brassica napus L cv. serw 1 (Rapeseed) grown in 2 growing seasons in a P and K deficient soil and fertilized with 142.9 kg N ha^{-1}, 171.4 kg K_2O ha^{-1}, or 36.9 kg P_2O_5 ha^{-1}. Means with a letter in common cannot be considered different according to a conservative Tukey-grouping applied to the p differences of the LSmeans. n = 6. Please note that error bars are standard error estimates of the LSmeans and not the computed standard error, which can be found in Supplementary Materials Table S1. CTRL indicates a lack of P and K applications.

Evening Primrose and Rapeseed showed, on average, 4.4 and 5.1 branches per plant, respectively. The role of the fertilizers in affecting the number of branches per plant was similar between species (Figure 3). In particular, K mostly increased the branch number irrespective of the other fertilizers, whereas the role of P was evident only when both N and K were supplied.

The role of the treatments on the pod number per unit area (Figure 4) was similar to those on the branch numbers but less pronounced: Rapeseed produced 59.4% more pods than Evening Primrose and differences between species were higher and more variable under non N fertilization (with Rapeseed producing +39 to +90% pods per unit area than Evening Primrose) than under N fertilized plots, where Rapeseed produced +58% to +68% more pods than Evening Primrose.

Under non-N fertilization, K increased the number of pods in Evening Primrose by 14.7%, but it did not have this effect in Rapeseed. The results showed a decreasing number of pods per branch when N or P were applied, but not with K application. The number of pods per branch reduced by 21.3% with the application of N, 13.5% with the application of P, with no differences between species or with K application.

Table 1. F statistics and p values of the general linear mixed model applied to the yield, yield components, and plant height of Evening Primerose and Rapeseed as affected by ferlizer application. Factors were the plant species (Sp) and each of the three main nutrients added as a fertilizer (N, P, K). Plant species were Evening Primrose or Rapeseed and each fertilizer factor had two treatments: either added or not. Fertilizers were nested into Sp. When the *p*-values were lower than 0.05, *F* and *p* are shown in bold.

Effect	Grain Yield		Yield per Pod		Number of Branches		Number of Pods		Pods per Branch		Plant Height		Oil Concentration in the Seed		Total Oil Yield	
	F	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Sp	**9001**	**<0.0001**	**1302**	**<0.0001**	**10.9**	**0.0297**	**1339**	**<0.0001**	**26.2**	**0.0069**	**785**	**<0.0001**	**32,020**	**<0.0001**	**12,781**	**<0.0001**
N	**1178**	**<0.0001**	**41.4**	**<0.0001**	**326**	**<0.0001**	**696**	**<0.0001**	**58**	**<0.0001**	**759**	**<0.0001**	**218**	**<0.0001**	**1069**	**<0.0001**
Sp × N	0.21	0.6507	**128**	**<0.0001**	2.22	0.1400	**67**	**<0.0001**	0.11	0.7432	**61.0**	**<0.0001**	**9.7**	**0.0026**	**25.1**	**<0.0001**
P	**636**	**<0.0001**	3.80	0.0549	**102**	**<0.0001**	**174**	**<0.0001**	**21.4**	**<0.0001**	**216**	**<0.0001**	**127**	**<0.0001**	**579**	**<0.0001**
Sp × P	0.19	0.6613	**22.3**	**<0.0001**	2.22	0.1400	**6.3**	**0.0144**	1.18	0.2810	**9.1**	**0.0035**	**4.9**	**0.0307**	**13.0**	**0.0006**
N × P	**37.0**	**<0.0001**	**10.6**	**0.0017**	**9.9**	**0.0023**	0.20	0.6596	0.25	0.6163	**6.5**	**0.0130**	0.60	0.4393	**33**	**<0.0001**
Sp × N × P	**16.7**	**0.0001**	**6.6**	**0.0121**	**6.2**	**0.0151**	**4.5**	**0.0367**	1.03	0.3142	0.06	0.8122	2.42	0.1241	**24.1**	**<0.0001**
K	**159**	**<0.0001**	**26.6**	**<0.0001**	**12.1**	**0.0008**	**7.2**	**0.0092**	1.63	0.2059	2.97	0.0889	**28.2**	**<0.0001**	**146**	**<0.0001**
Sp × K	1.70	0.1958	**11.1**	**0.0013**	0.25	0.6206	**9.3**	**0.0031**	0.53	0.4708	2.58	0.1125	2.84	0.0962	**8.7**	**0.0042**
N × K	**56.4**	**<0.0001**	**16.4**	**0.0001**	3.32	0.0723	0.10	0.7530	0.55	0.4612	**7.3**	**0.0087**	0.27	0.6057	**50**	**<0.0001**
Sp × N × K	**14.1**	**0.0003**	**26.3**	**<0.0001**	0.25	0.6206	**15.8**	**0.0002**	0.71	0.4030	**7.1**	**0.0095**	2.42	0.1241	**23**	**<0.0001**
P × K	**24.4**	**<0.0001**	2.72	0.1031	1.35	0.2497	0.61	0.4383	0.40	0.5274	**7.0**	**0.0097**	0.02	0.8972	**22**	**<0.0001**
Sp × P × K	**13.1**	**0.0005**	**19.4**	**<0.0001**	1.35	0.2497	**11.8**	**0.0010**	0.01	0.9034	**11.6**	**0.0011**	2.84	0.0962	**18.0**	**<0.0001**
N × P × K	**190**	**<0.0001**	0.56	0.4579	**12.1**	**0.0008**	**67**	**<0.0001**	0.11	0.7437	**32.1**	**<0.0001**	**15.1**	**0.0002**	**158**	**<0.0001**
Sp × N × P × K	**6.7**	**0.0118**	**5.9**	**0.0179**	0.69	0.4100	0.28	0.6001	0.36	0.5514	0.75	0.3905	**5.4**	**0.0224**	0.15	0.7030

Table 2. Means ± standard error (n = 6) of the yield, yield components, plant height, and oil concentration in the seed.

			Grain Yield	Yield per Pod	Number of Branches	Number of Pods	Pods per Branch	Plant Height	Oil Concentration in the Seed	Total Oil Yield
			[kg seed ha^{-1}]	[mg seed pod^{-1}]	[n brances plant^{-1}]	[n pods m^{-2}]	[n pods n branc^{-1}]	[cm]	[kg oil (100 kg seed^{-1})]	[kg oil ha^{-1}]
Evening Primrose	No N	No P	No K	538 ± 8	59.95 ± 1.96	2.50 ± 0.22	901.3 ± 25.3	23.3 ± 2.3	64.9 ± 1.3	24.13 ± 0.02	130 ± 2
			With K	760 ± 7	60.78 ± 1.34	3.17 ± 0.17	1252.1 ± 24.3	24.8 ± 1.4	68.8 ± 0.6	24.32 ± 0.06	185 ± 2
		With P	No K	845 ± 5	65.53 ± 0.72	3.83 ± 0.17	1289.8 ± 13.0	21.0 ± 1.1	71.8 ± 0.5	24.53 ± 0.03	207 ± 1
			With K	827 ± 18	65.77 ± 1.98	3.83 ± 0.31	1260.2 ± 26.6	21.0 ± 1.9	71.4 ± 0.7	24.73 ± 0.08	205 ± 5
	With N	No P	No K	923 ± 8	65.44 ± 0.68	4.67 ± 0.21	1411.3 ± 20.7	18.9 ± 1.0	76.5 ± 0.7	24.70 ± 0.04	228 ± 2
			With K	872 ± 8	63.28 ± 0.56	5.00 ± 0.45	1378.9 ± 16.9	17.7 ± 1.5	75.3 ± 1.4	24.85 ± 0.03	217 ± 2
		With P	No K	981 ± 9	65.49 ± 0.86	5.50 ± 0.22	1500.3 ± 30.0	17.0 ± 0.9	81.3 ± 1.4	25.05 ± 0.13	246 ± 2
			With K	1134 ± 13	71.85 ± 0.35	6.50 ± 0.22	1578.6 ± 16.6	15.1 ± 0.6	86.0 ± 1.3	25.42 ± 0.10	288 ± 2
Rapeseed	No N	No P	No K	1502 ± 7	88.09 ± 1.73	3.33 ± 0.21	1708.1 ± 32.0	32.3 ± 2.3	83.8 ± 1.5	33.95 ± 0.07	510 ± 2
			With K	1806 ± 27	103.95 ± 1.98	3.67 ± 0.33	1740.5 ± 42.2	30.5 ± 2.7	87.8 ± 0.7	34.38 ± 0.04	621 ± 9
		With P	No K	1872 ± 12	93.44 ± 2.98	4.33 ± 0.21	2015.7 ± 75.7	29.0 ± 1.4	98.8 ± 1.0	34.57 ± 0.04	647 ± 4
			With K	1909 ± 23	102.99 ± 0.72	4.17 ± 0.17	1853.8 ± 25.7	27.7 ± 1.2	89.3 ± 1.4	34.50 ± 0.06	659 ± 9
	With N	No P	No K	1952 ± 10	84.91 ± 1.34	5.33 ± 0.21	2301.7 ± 37.5	26.9 ± 1.2	102.1 ± 0.9	34.67 ± 0.07	677 ± 3
			With K	1952 ± 10	89.97 ± 1.31	5.17 ± 0.31	2172.2 ± 37.5	26.5 ± 2.0	104.8 ± 1.5	34.57 ± 0.09	675 ± 5
		With P	No K	2033 ± 15	84.29 ± 0.72	6.67 ± 0.33	2412.4 ± 20.0	22.6 ± 1.1	112.5 ± 1.6	34.73 ± 0.08	706 ± 6
			With K	2069 ± 20	78.23 ± 1.51	8.17 ± 0.31	2647.1 ± 34.4	20.2 ± 0.8	115.7 ± 1.0	34.93 ± 0.06	723 ± 8

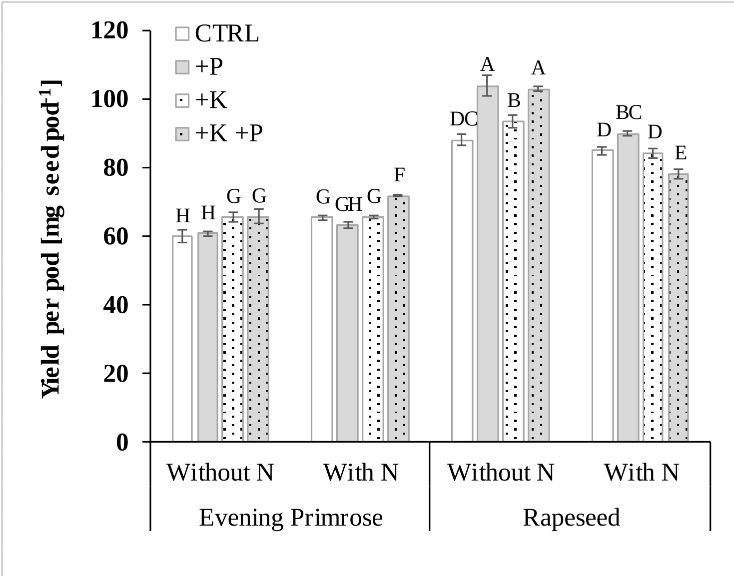

Figure 2. Least squares means (LSmeans) and standard error of the LSmeans of the grain yield per pod of Oenothera biennis L. local genotype (Evening Primrose) and Brassica napus L cv. serw 1 (Rapeseed) grown in 2 growing seasons in a P and K deficient soil and fertilized with 142.9 kg N ha^{-1}, 171.4 kg K_2O ha^{-1}, or 36.9 kg P_2O_5 ha^{-1}. Means with a letter in common cannot be considered different according to a conservative Tukey-grouping applied to the p differences of the LSmeans. N = 6. Please note that error bars are standard error estimates of the LSmeans and not the computed standard error, which can be found in Supplementary Materials Table S1. CTRL indicates a lack of P and K applications.

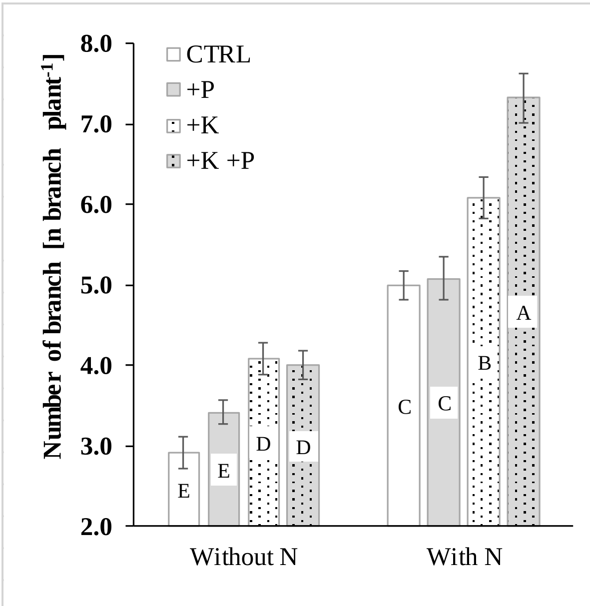

Figure 3. Least squares means (LSmeans) and standard error of the LSmeans of the number of branches per plant in Oenothera biennis L. local genotype (Evening Primrose) and Brassica napus L cv. serw 1 (Rapeseed) grown in 2 growing seasons in a P and K deficient soil and fertilized with 142.9 kg N ha^{-1}, 171.4 kg K_2O ha^{-1}, or 36.9 kg P_2O_5 ha^{-1}. Data are means of the species. Means with a letter in common cannot be considered different according to a conservative Tukey-grouping applied to the p differences of the LSmeans. N = 12. Please note that error bars are standard error estimates of the LSmeans and not the computed standard error, which can be found in Supplementary Materials Table S1. CTRL indicates a lack of P and K applications.

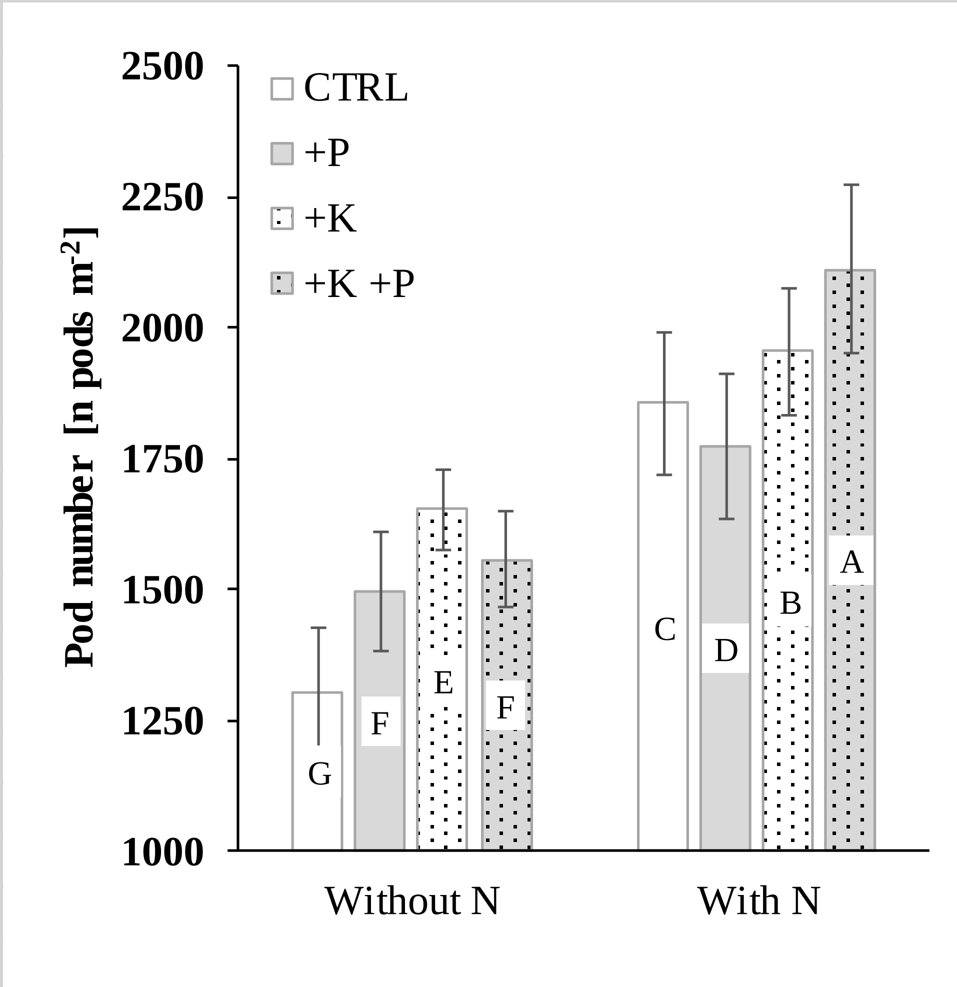

Figure 4. Least squares means (LSmeans) and standard error of the LSmeans of the number of pods per unit area in Oenothera biennis L. local genotype (Evening Primrose) and Brassica napus L cv. serw 1 (Rapeseed) grown in 2 growing seasons in a P and K deficient soil and fertilized with 142.9 kg N ha^{-1}, 171.4 kg K_2O ha^{-1}, or 36.9 kg P_2O_5 ha^{-1}. Data are means of the species. Means with a letter in common cannot be considered different according to a conservative Tukey-grouping applied to the p differences of the LSmeans. N = 12. Please note that error bars are standard error estimates of the LSmeans and not the computed standard error, which can be found in Supplementary Materials Table S1. CTRL indicates a lack of P and K applications.

The seed oil concentration varied similarly to the seed yield, but differences among treatments were less pronounced (Figure 5).

In particular, in the non-N fertilized Evening Primrose, application of K increased seed oil concentration more than P, and the application of both P and K increased the seed oil concentration compared to the application of only P or only K. In the Rapeseed, the role of P and K on the seed oil concentration was similar to that exerted on the grain yield. In the N fertilized Evening Primrose, K but not P alone increased the seed oil concentration, whereas in the N fertilized Rapeseed, the role of K and P were scarce and evident only when both fertilizers were applied. Oil yield in Rapeseed was 206% more than Evening Primrose and the role of the fertilizers was similar in both species (Figure 6).

In particular, under non-N fertilized conditions, the application of P increased oil yield by 25.9% compared to no fertilizer application and such an increase was more evident when K was applied (+33.6% to 34.9%) irrespective of the P application. Under N fertilization, application of only K, but not only P, slightly increased oil yield (+5.2% compared to the non-N fertilized, non-P fertilized treatment) and such an increase was more evident when both P and K were applied (+11.7% compared to the N fertilized, non-P, non-K fertilized treatment).

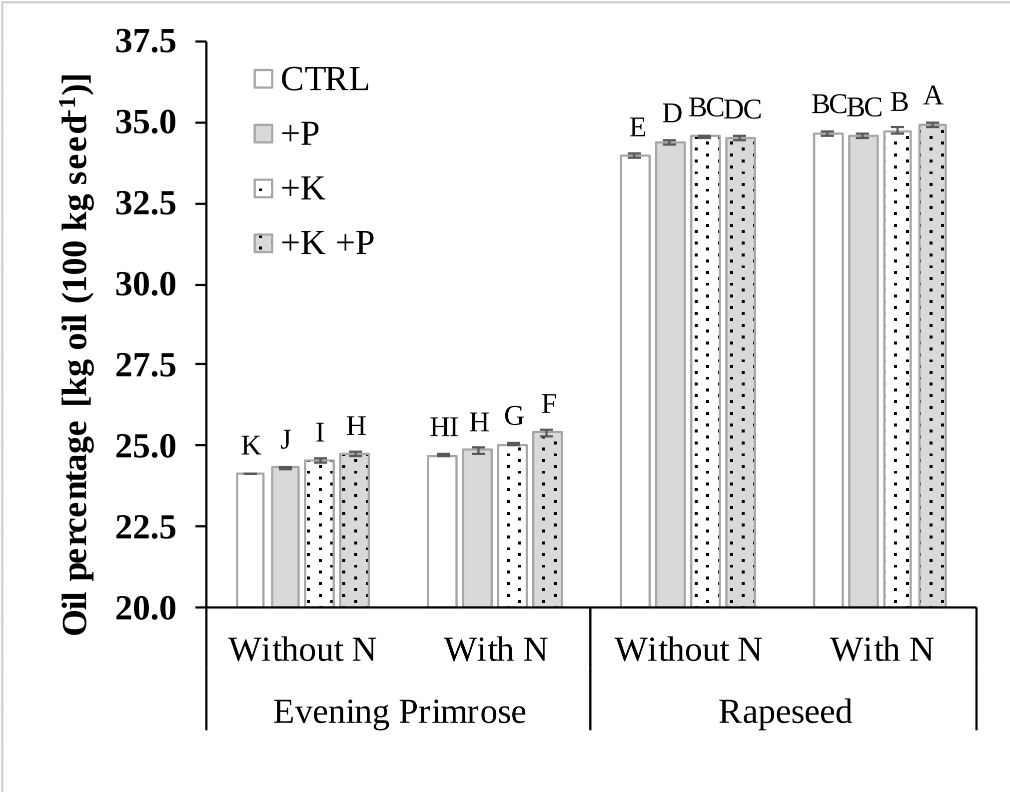

Figure 5. Least squares means (LSmeans) and standard error of the LSmeans of the oil concentration in the seed of Oenothera biennis L. local genotype (Evening Primrose) and Brassica napus L cv. serw 1 (Rapeseed) grown in 2 growing seasons in a P and K deficient soil and fertilized with 142.9 kg N ha^{-1}, 171.4 kg K_2O ha^{-1}, or 36.9 kg P_2O_5 ha^{-1}. Means with a letter in common cannot be considered different according to a conservative Tukey-grouping applied to the p differences of the LSmeans. N = 6. Please note that error bars are standard error estimates of the LSmeans and not the computed standard error, which can be found in Supplementary Materials Table S1. CTRL indicates a lack of P and K applications.

3.2. Fatty Acid Composition of the Seed Oil

In contrast to the yield traits, oil composition showed almost no effects by the fertilization factors and mostly varied by the plant species (Table 3 and Figure 7, raw data in Supplementary Materials Table S2). No Palmitoleic (C16:1) and Lignoceric (C24:0) acids were found in the Evening Primrose seed oil. Seed oils of both species were mostly characterized by C18 fatty acids, with Rapeseed enriched in oleic acid (C18:1) and Evening Primrose in Linoleic acid (C18:2) and similar amounts of C18:0 and C18:3.

The C18:1 showed differences by the fertilization, with Sp × N × P × K showing and $F = 4.8$ and a $p = 0.031$. Differences in the C18:1 percentage occurred only in Evening Primrose, where the addition of N + K slightly increased the C18:1 ratio by 0.28% compared to the plots fertilized with only N or only K (Supplementary Materials Figure S1). The addition of N + P + K also increased the C18:1 ratio by 0.14%, but such a difference was not evident according to the statistical analysis.

In general, the FAME ratios highly correlated with each other, with $|r| > 0.7$ (Supplementary Materials Table S3), thus no multivariate analysis was performed.

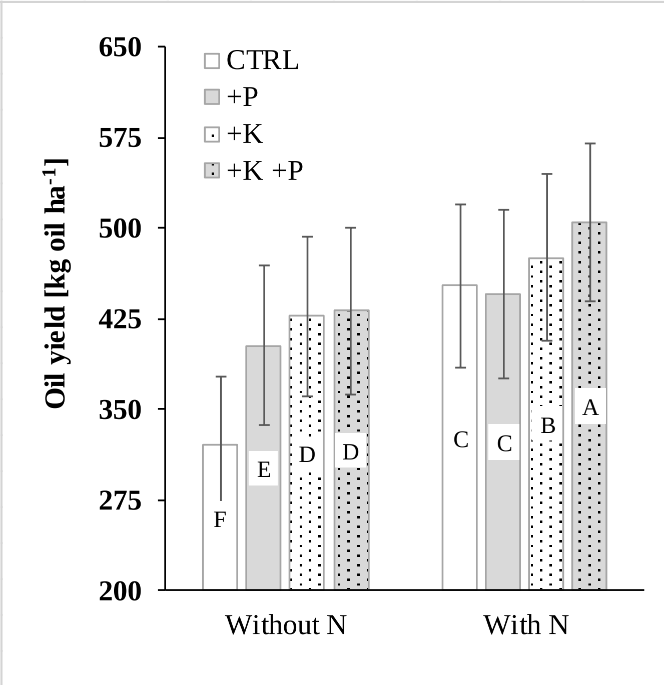

Figure 6. Least squares means (LSmeans) and standard error estimate of the LSmeans of the oil yield number of pods per unit area in Oenothera biennis L. local genotype (Evening Primrose) and Brassica napus L cv. serw 1 (Rapeseed) grown in 2 growing seasons in a P and K deficient soil and fertilized with 142.9 kg N ha^{-1}, 171.4 kg K_2O ha^{-1}, or 36.9 kg P_2O_5 ha^{-1}. Data are means of the species. Means with a letter in common cannot be considered different according to a conservative Tukey-grouping applied to the p differences of the LSmeans. N = 12. Please note that error bars are standard error estimates of the LSmeans and not the computed standard error, which can be found in Supplementary Materials Table S1. CTRL indicates a lack of P and K applications.

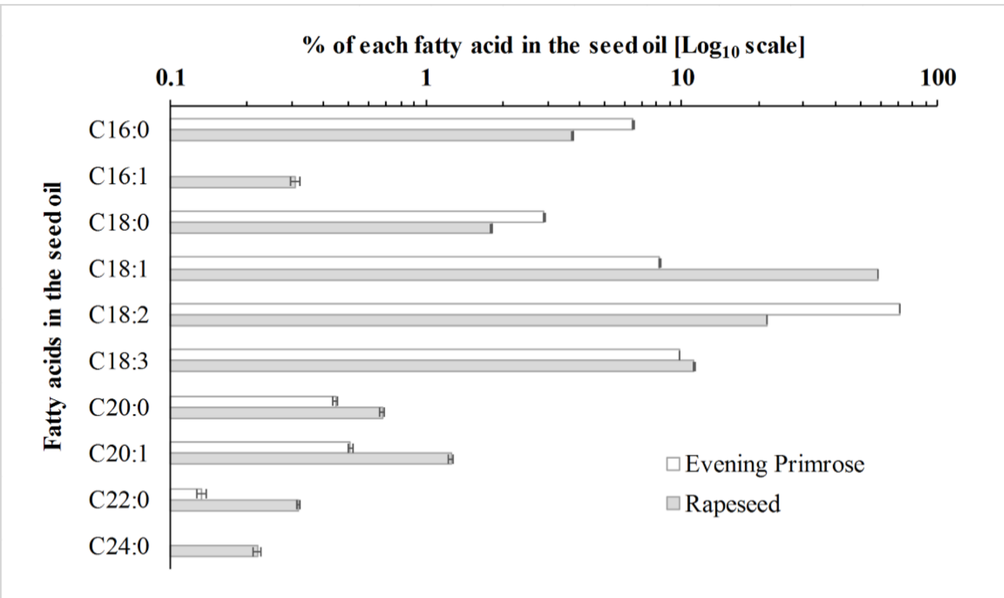

Figure 7. Concentration of each fatty acid in the seed oil of Oenothera biennis L. (Evening Primrose) and Brassica napus L cv. serw 1 L. local genotype (Rapeseed) grown in 2 growing seasons in a P and K deficient soil and fertilized with 142.9 kg N ha^{-1}, 171.4 kg K_2O ha^{-1}, or 36.9 kg P_2O_5 ha^{-1}. Data are means ± S.E. of the N, P, and K fertilization treatments. Species differed by each fatty acid (see Supplementary Materials Table S3). N = 48. Please note that error bars are standard error estimates of the LSmeans and not the computed standard error, which can be found in Supplementary Materials Table S2.

Table 3. F statistics and p-values of the general linear mixed model applied to seed oil composition. Factors were the plant species (Sp) and each of the three main nutrients added as a fertilizer (N, P, K). Plant species were Evening Primrose or Rapeseed and each fertilizer factor had two treatments: either added or not. Fertilizers were nested into Sp. When the *p*-values were lower than 0.05, *F* and *p* are shown in bold. * n.a. is for not applicable.

Effect	C16:0		C16:1		C18:0		C18:1		C18:2		C18:3		C20:0		C20:1		C22:0		C24:0	
	F	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***
Sp	**4589**	**<0.0001**	n.a.*	n.a.	**2073**	**<0.0001**	9×10^5	**<0.0001**	**1×10^6**	**<0.0001**	**972**	**<0.0001**	**156**	**<0.0001**	**397**	**0.0003**	**571**	**<0.0001**	n.a.	n.a.
N	**4.0**	**0.05**	2.36	0.13	**4.5**	**0.04**	0.90	0.35	0.53	0.47	1.06	0.31	0.08	0.78	0.16	0.69	0.09	0.77	0.00	0.96
Sp × N	3.09	0.08	n.a.	n.a.	0.08	0.78	0.88	0.35	0.44	0.51	0.89	0.35	0.00	0.96	1.26	0.26	0.12	0.73	n.a.	n.a.
P	0.73	0.40	0.02	0.90	0.60	0.44	1.24	0.27	2.48	0.12	0.64	0.43	0.03	0.87	0.11	0.74	0.04	0.85	0.05	0.83
Sp × P	0.45	0.50	n.a.	n.a.	0.87	0.36	3.00	0.09	3.10	0.08	0.84	0.36	0.25	0.62	0.02	0.90	1.46	0.23	n.a.	n.a.
N × P	0.95	0.33	0.02	0.90	2.82	0.10	0.39	0.54	1.62	0.21	0.17	0.68	1.13	0.29	0.32	0.57	0.78	0.38	0.60	0.44
Sp × N × P	0.00	0.99	n.a.	n.a.	2.11	0.15	1.85	0.18	0.62	0.43	0.57	0.45	2.08	0.15	0.30	0.59	1.87	0.18	n.a.	n.a.
K	0.09	0.77	0.72	0.40	0.06	0.80	0.04	0.84	0.00	0.97	0.01	0.94	0.15	0.70	0.70	0.41	0.88	0.35	0.44	0.51
Sp × K	0.32	0.57	n.a.	n.a.	0.30	0.59	0.25	0.62	0.22	0.64	1.48	0.23	0.14	0.71	0.26	0.61	0.21	0.65	n.a.	n.a.
N × K	1.26	0.26	1.47	0.23	0.17	0.68	0.24	0.63	0.91	0.34	1.58	0.21	1.33	0.25	1.10	0.30	0.02	0.89	0.44	0.51
Sp × N × K	0.37	0.54	n.a.	n.a.	0.07	0.79	0.45	0.50	0.51	0.48	1.18	0.28	0.41	0.53	2.00	0.16	0.00	0.98	n.a.	n.a.
P × K	1.95	0.17	0.01	0.93	0.08	0.78	0.02	0.88	0.61	0.44	0.21	0.65	0.56	0.46	0.00	0.99	0.04	0.85	0.15	0.70
Sp × P × K	2.98	0.09	n.a.	n.a.	0.02	0.90	0.03	0.87	1.15	0.29	0.43	0.51	0.09	0.76	0.91	0.34	0.06	0.81	n.a.	n.a.
N × P × K	0.98	0.33	0.50	0.49	0.20	0.66	0.01	0.91	0.08	0.78	1.69	0.20	2.15	0.15	0.04	0.84	0.00	0.98	0.69	0.41
Sp × N × P × K	0.17	0.68	n.a.	n.a.	1.55	0.22	**4.8**	**0.031**	0.57	0.45	2.43	0.12	2.63	0.11	0.52	0.47	0.06	0.81	n.a.	n.a.

4. Discussion

Production of biomass and seed and its constituents under arid and semiarid conditions may be limited by both the nutrient supply and drought stress [34–37]. In such environments, the ability of the species to be grown in the winter and their earliness are a prerequisite for achieving high yields and adequate quality [36–38], especially for grain species that show shattering habits at increasing temperatures. In addition, a tradeoff between yield and quality may occur at varying water [39] and nutrient availabilities [35].

In the present study, Evening Primrose yield was similar to the mean reported by Fieldsend [8], which implies that the area under study has potential for the growth of this species. Evening Primrose produced 54.4% less seed compared to Rapeseed, but differences in yield between the two species reduced when N fertilizer was applied, suggesting a lower ability of the Evening Primrose to grow under non-optimal N conditions when compared to Rapeseed. In experiments not directly comparing these species, a similar response to N was found in Evening Primrose [20,40], Canola [41,42], and Carinata [43]. Evening Primrose and Rapeseed usually show a similar total biomass production [44,45], thus the main reason for yield differences is likely due to a low harvest index of the Evening Primrose. This depends on the lack of breeding improvements [20,46], and especially seed shatter that can strongly compromise yield in arid and semiarid areas due to an early seed loss primed by the high temperatures. Indeed, we found that Rapeseed produced 59.4% more pods and 119% more grain than Evening Primrose, suggesting that grain per pod had similar importance of the pod per area in determining the yield differences between the species. In the present work, Evening Primrose responded to the application of multiple nutrients better than Rapeseed. Similar behaviour in Evening Primrose was found by other authors [47,48], however, the effect of the single nutrients was not checked. In particular, P mostly controlled yield at high N availability in the present experiment, and K at low N availability. Said-Al Ahl and Ramadan [40] showed that Evening Primrose yield under high N availability was limited by the Zn availability for the plants, whereas in Rapeseed, Said-Al Ahl et al. [49] showed that yield response to the P availability was limited by drought. This suggests, from one side, that Evening Primrose may have a higher earliness than Rapeseed. On the other side, it implies that the response to P and K applications in both species differ when varying the N availability. K stimulated branching of both species under both low and high N availability and yield per pod under low N only. This may have affected the harvest index by influencing the seed load per branch [50]. Other species in the family *Onagranacea* showed an increased abundance in high P mining areas, suggesting their scarce ability to take up P [51]. Mohammadi et al. [52] found that Evening Primrose did not show a similar response to the application of N + P compared to the effect of the soil beneficial microbes (either arbuscular mycorrhizal fungi or free living nitrogen fixing bacteria). This implies that N and P uptake in Evening Primrose may depend on other nutrients supplied by the microbes, especially Zn and Fe, which has an uptake frequently coupled with P [33,53,54]. Other authors found scarce effect of the N and K availabilities on camelina [35], whereas Ali et al. [55] found a similar effect on borage seed yield to that in the present study.

Seed oil concentration and oil yield were similar but more pronounced, the response to the treatments compared to the response for the seed yield. Rapeseed oil concentration was 9.5% to 10.1% more than Evening Primrose. Rapeseed produced 119.4% more seed and 206.0% more oil than Evening Primrose, which implies a better ability of this species to accumulate oils in the conditions under study. Such a difference was more pronounced under N limiting conditions than in the N fertilized treatments. In addition, K favored Evening Primrose oil yield when no N or P was added, whereas P favored the oil yield in Evening Primrose more than Rapeseed in almost all conditions. However, little variation was found in the seed oil concentration among treatments, thus oil yield mostly depended on seed yield. A similar result of the N and P interaction was found by Cheema et al. [41] in canola. Additionally, Said-Al Ahl et al. [49] found that P increased oil yield in Rapeseed when applied at a fourfold rate than the present study. Ghasemnezhad and Honermeier [20] found a slight reduction of the seed oil concentration of Evening Primrose when increasing N concentration from 167 ppm to 333 ppm, whereas yield increased mostly when increasing N concentration from 83 ppm to 167 ppm. The latter authors [21] found a scarce effect of the variation of the soil N concentration on the seed oil concentration of Evening Primrose below 100 ppm. This implies that the N supply stimulates Evening Primrose oil accumulation after the species had satisfied its N needs for the grain yield. Since soil N concentration was low in the present study, this could explain the lack of N effects on the seed oil accumulation. In such conditions, the application of K but not P allowed an increase in crop performances in terms of oil accumulation. These results agree with Ali et al. [55] in borage, who found that half of the dose of K applied in the present study was sufficient to increase seed yield but not seed oil concentration. Indeed we also found that application of K, which strongly contributes to a regulation of the transpiration, increased oil concentration and yield more in Evening Primrose than Rapeseed. In addition, we cannot exclude that the application of sulfur through the K fertilizer may also have inadvertently stimulated the oil yield, as seen by Jankowski et al. [56], or sustained the K uptake [24]. In contrast to K, the scarce effect of the application of P may have been due to the timing and method of application used in the present experiment, which may have reduced its benefit. In particular, the high soil pH very likely leads to a P sequestration, and surface applications may have strongly reduced the P availability [57,58]. A potential effect on the oil concentration and yield may have been seen with higher fertilizer application, however, further increasing nutrient concentration in the soil in the area under study may have lead to additional stresses, especially drought stresses, due to a stimulation of the transpiration, as also seen in other grain species in similar conditions [59]. Nonetheless, we found that the nutrient supply scarcely affected the lipid composition, which appeared strongly related to other stresses or genetic aspects in many species, including Evening Primrose [31,60–62].

5. Conclusions

Evening Primrose showed a lower yield potential than Rapeseed. Nonetheless, Evening Primrose better responded to the increase in nutrient availability compared to Rapeseed. According to our hypothesis, P modulated seed and oil yield at high N availability and, in contrast to our expectation, K modulated it at low N availability. These variations occurred irrespective of an effect on the seed oil accumulation. A role of the S within the K fertilizer in affecting the seed oil concentration and composition may have occurred, but notably, N + K marginally increased the oleic acid concentration compared to the sole N and sole K applications, whereas P increased it only when both N and K were also applied.

In conclusion, these results suggest that P can be more important to increase yield in Evening Primrose than Rapeseed in arid conditions. Additionally, these results indicate that Evening Primrose responded to the N application more sharply in terms of seed rather than oil yields.

Evening Primrose can be a valuable option to achieve a high income from industrial oils in arid and semiarid areas, especially when considering its high market price, which is usually 4 to 50 fold higher than many other species with similar traits. However, breeding is needed to reduce seed loss.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy11071271/s1. Table S1: Means (above) and standard deviations (below) of the raw data per year. N = 3. Table S2: Means ± standard error (n = 6) of seed oil composition. Columns indicate the fatty acid and its unsaturation, the values are the ratio of each fatty acid peak area on the total peak area (TPA), expressed as a percentage. Table S3: Correlation among traits using the raw data (n = 96). Figure S1: Least squares means (LSmeans) and standard error of C18:1 fatty acid in the seed oil of *Oenothera biennis* L. local genotype (Evening Primrose) and *Brassica napus* L. cv. serw 1 (Rapeseed) grown in 2 growing seasons in a P and K deficient soil and fertilized with 142.9 kg N ha^{-1}, 171.4 kg K_2O ha^{-1}, or 36.9 kg P_2O_5 ha^{-1}. Means with a letter in common can't be considered different according to a conservative Tukey-grouping applied to the p differences of the LSmeans. N = 6.

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