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RESEARCH ARTICLE

Interactions of nitrogen and phosphorus in plant nutrition - Analysis of a 60-years old field experiment

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Abstract
Background and aims The aims are to determine to which extent different plant species increase nitrogen (N) acquisition in response to phosphorus (P) addition and P acquisition in response to N addition as well as to analyze resulting nutrient limitations.
Methods The field experiment is replicated at ten sites in Sweden and comprises three fertilization regimes (low, medium, and high) each consisting of a control and a N, P, and NP treatment. Yields as well as N and P contents of the yields of four species (winter wheat, spring barley, oat, and sugar beet) were analyzed over 60 years.
Results The relative increase in yield P in response to N fertilization was larger than the relative increase in yield N in response to P fertilization for three species. Synergistic NP co-limitation was observed for all species at some sites. The synergistic growth effect in response to NP addition occurred at more sites at high than at low fertilization. At one nutrient-poor site, all species in all fertilization regimes were (synergistically) NP co-limited. Sugar beet was (synergistically) NP co-limited at all sites in all fertilization regimes.
Conclusion Species differed in nutrient acquisition, but most species used added N more effectively to acquire P than they used added P to acquire N. Synergistic NP co-limitation was observed for all plant species at some sites, and it increased with fertilization rate, which is likely due to the limits to which plants can use N to acquire P and P to acquire N.

Keywords Nitrogen · Phosphorus · Nutrient interactions · Plant nutrition · Synergistic growth effect · Nutrient co-limitation

Introduction

Liebig's law of the minimum is still often used in ecology and plant sciences to explain dynamics of plant nutrient uptake, and it is also still implemented in many recent vegetation models (Goll et al. 2012; Mueller et al. 2012; Folberth et al. 2019) despite the fact that it ignores interactions among nutrients in plant nutrition (Davidson and Howarth 2007; Elser et al. 2007). Liebig's concept of plant nutrient limitation assumes that a plant is limited only by one nutrient at a time, namely by the specific nutrient that is least available relative to the plant's demand. The concept does not include interactions of nutrients or

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nutrient cycles. For instance, it does not capture the possibility that a plant allocates one nutrient in a way that allows it to acquire another nutrient, which might lead to multiple nutrient limitation (Bloom et al. 1985; Davidson and Howarth 2007; Ringeval et al. 2021). Furthermore, Liebig's concept cannot explain synergistic growth effects that are often observed in response to combined addition of two or more nutrients (Elser et al. 2007; Allgeier et al. 2011; Harpole et al. 2011; Fay et al. 2015). The main reason for the popularity of Liebig's law of the minimum despite these shortcomings seems to be that interactions among nutrients in plant nutrition are still not well understood (Davidson and Howarth 2007; Rietra et al. 2017; Duncan et al. 2018; Clayton et al. 2024), which calls for more detailed analyses.

Many plant species can allocate one nutrient in a manner that allows them to acquire another one (Bloom et al. 1985; Sumner and Farina 1986; Saito et al. 2008; Rietra et al. 2017; Clayton et al. 2024). For instance, plants can use nitrogen (N) to acquire phosphorus (P) through the production of phosphatase and P-transporters. Phosphatases catalyze the mineralization of organic P, thus rendering soil organic P plant-available, while P-transporter allow plants to take P up into their cells (Spohn et al. 2015, 2018). Phosphatases and P-transporters are proteins, and thus have a high N content. Plants can use N to produce these proteins, and thus invest N into the acquisition of P (Bloom et al. 1985; Smith and Jackson 1987; Zeng et al. 2012; Schleuss et al. 2020). In addition, plants can use added N or P to increase their root length or change their root morphology, which might increase nutrient uptake (Duncan et al. 2018; Weih et al. 2021). Several studies showed that plants increase phosphatase activity in soil when provided with N (Marklein and Houlton 2012; Schleuss, et al. 2020) which went along with increased plant P contents (Schleuss et al. 2020). In contrast, there is less evidence that plants also acquire more N in response to P addition (Schleuss et al. 2020; Vázquez et al. 2022, 2023).

Interactions between N and P in plant nutrition are likely also the reason for synergistic growth in response to combined N and P fertilization (Davidson and Howarth 2007; Elser et al. 2007; Harpole et al. 2011; Ågren et al. 2012). Synergistic plant growth responses to combined N and P addition are common in terrestrial and aquatic ecosystems (Elser et al. 2007; Allgeier et al. 2011; Harploe et al., 2022; Fay et al. 2015). The term synergistic (growth) response or synergistic (growth) effect refers to an increase in plant biomass or yield in response to combined N and P addition that is larger than the sum of the growth responses observed due to single N and single P addition (Davidson and Howarth 2007; Elser et al. 2007; Harpole et al. 2011; Ågren et al. 2012). The most likely explanation for the synergistic effect is that plants fertilized only with N use the added N partly to acquire P. Vice versa, plants fertilized only with P use the added P partly to acquire N (Bloom et al. 1985). In contrast, plants that are provided with both N and P do not use one nutrient to acquire the other, but allocate both nutrients directly to growth, which can lead to high growth causing the synergistic growth effect. Plants responding to NP addition with synergistic growth are classified as being synergistically NP-co-limited (Harpole et al. 2011). In contrast, if they grow significantly more in response to both N and P addition, without the effect of combined NP addition being larger than the sum of the effects of single N and single P addition, they are classified as NP co-limited (Harpole et al. 2011).

Many studies explored interactions of N and P and nutrient co-limitation in plant communities, for instance in natural grasslands (Harpole et al. 2011; Fay et al. 2015; Schleuss et al. 2020; Vázquez et al. 2023) and aquatic ecosystems (Elser et al. 2007; Allgeier et al. 2011). Most of these studies investigated growth responses to N and P addition of communities whose species composition varies among different sites (Elser et al. 2007; Allgeier et al. 2011; Harploe et al., 2011; Fay et al. 2015; Zeng et al. 2016; Schleuss et al. 2020; Vázquez et al. 2023). Consequently, it is not well understood whether nutrient co-limitation is a site-property or rather a species property. It might be that all species are NP co-limited when grown at a specific site because of the site's low nutrient availability. However, it might also be that some species are more prone to develop a nutrient co-limitation than others because of differences in their capacities to acquire nutrients. Field trials in which the same experiment with single species is replicated at several sites allow to elucidate whether NP co-limitation depends more strongly on site properties or on species properties.

Furthermore, the synergistic effect might depend on the fertilization rate, and it might only occur beyond a specific threshold of N and P fertilization rates. For instance, Zeng et al. (2016) reported that there is a critical nutrient threshold for the synergistic effect in phytoplankton in lake ecosystems, and synergistic growth can only be observed beyond this threshold. Accordingly, it can be hypothesized that synergistic NP co-limitation in plants becomes more dominant with increasing N and P fertilization rate or that it only occurs beyond a certain threshold.

The objective of this study is to answer four core questions about interactions among N and P in plant nutrition and nutrient co-limitation and to test the following four hypotheses.

1) Do plants use added N more effectively to acquire P than they use added P to acquire N?

H1: The relative increase in yield N (in percentage) in response to P fertilization is larger than the relative increase in yield P in response to N fertilization (because plants can use N very effectively for mobilizing P from soil organic phosphorus through the release of phosphatases).

2) Does the extent of P acquisition in response to N addition and N acquisition in response to P addition differ among plant species?

H2: The capacity of plants to use N to acquire P, and vice versa, to use P to acquire N differs strongly among plant species (due to differences in their capacities to mobilize and take up nutrients).

3) Is the occurrence of synergistic NP-co-limitation site-specific or plant species-specific?

H3: NP co-limitation is plant species-specific rather than site-specific (due to differences among plant species in their capacity to take up nutrients).

4) Does occurrence and size of the synergistic growth effect in response to NP addition depend on the fertilization rate?

H4: The occurrence and size of the synergistic growth effect increases with increasing fertilization rate.

To test these four hypothesis, I analyzed a long-term field experiment that has been replicated at ten contrasting sites in southern and central Sweden for more than 60 years. The experiment comprises three fertilization regimes (low, medium, and high), each consisting of a control and a N, P, and NP treatment.

Material and methods

Field sites and experiment

The field experiment is replicated at ten sites in south and central Sweden with contrasting soil properties (Tables 1 and 2). More information about the ten sites can be found in Kirchmann et al. (1996), Kirchmann and Eriksson (1993), Carlgren and Mattsson (2001), Kirchmann et al. (2005), and Kirchmann et al. (2013). The experiments in southern Sweden started in 1957 and the experiments in central Sweden started in 1963 or 1966. The experimental design includes two crop rotations, one without manure application and

Table 1 Location and geographical properties of the ten sites (Carlgren and Mattsson 2001; Kirchmann et al. 2005)

Site name	Number	Latitude	Longitude	Altitude a.s.l. (m)	MAT (°C)	MAP (mm)	Soil type
Fjärdingslöv	M-1–1957	54°24´ N	13°14´ E	30	7.7	550	Haplic Phaeozem
Orop	M-2–1957	55°49´ N	13°30´ E	75	8.3	769	Haplic Phaeozem
Örja	M-4–1957	55°53’ N	12°52’ E	10	8.3	593	Eutric Cambisol
Ugglarp	M-5–1957	55°38´ N	13°25´ E	65	7.7	686	Haplic Phaeozem
Ekebo	M-6–1957	55°59’ N	12°52’E	59	8.2	622	Eutric Cambisol
Kungsängen	C-7–1963	59°50´ N	17°40´ E	4	6.0	543	Gleyic Cambisol
Fors	C-8–1963	60°20´ N	17°29´ E	25	5.5	613	Calcaric Phaeozem
Vretakloster	E-9–1966	58°29´ N	13°08´ E	47	6.4	527	Haplic Phaeozem
Högåsa	E-10–1966	58°30´ N	15°27´ E	80	6.4	527	Arenic Umbrisol
Bjertorp	R-94–1966	58°14´ N	13°08´ E	90	6.5	593	Not determined

Table 2 Soil chemical properties in the uppermost 20 cm of the soils at the ten sites, including total organic carbon (TOC), total nitrogen (TN), and total organic P (TOP). The chemical soil properties are means calculated across all ten treatments and across all years of the experiments. The TOC:TN ratio is calculated as a mass:mass ratio. Data on soil texture and bulk density is taken from Poeplau et al. (2016)

Site name	Texture			Bulk density ($g\ cm^{-3}$)	pH	TOC ($g\ kg^{-1}$)	TN ($g\ kg^{-1}$)	TOC:TN	P-AL ($mg\ kg^{-1}$)	P-HCl ($mg\ kg^{-1}$)	TOP ($g\ kg\ -1$)	K-AL ($mg\ kg^{-1}$)
	Clay (%)	Silt (%)	Sand (%)									
Fjärdingslöv	14	24	62	1.66	7.3	12.6	1.3	9.7	65.7	256.8	0.18	76.8
Orop	12	29	59	1.51	6.1	36.0	3.2	11.3	44.5	415.8	0.34	87.1
Örja	23	25	52	1.72	7.1	18.1	2.6	7.0	68.6	307.4	-	123.9
Ugglarp	12	25	63	1.5	6.0	13.3	1.4	9.5	51.7	359.6	0.15	85.2
Ekebo	18	35	47	1.44	6.4	47.1	3.3	14.3	79.6	380.5	0.37	135.9
Kungsängen	56	40	4	1.31	6.7	21.8	2.3	9.5	45.5	576.0	0.29	152.8
Fors	18	58	24	1.49	7.7	20.6	1.6	12.9	129.4	765.5	0.30	99.1
Vretakloster	48	44	8	1.43	8.6	19.2	1.9	10.1	73.8	402.6	0.29	262.1
Högåsa	7	15	78	1.38	7.2	19.3	1.4	13.8	54.9	334.1	0.25	128.6
Bjertorp	30	54	16	1.37	6.4	18.5	1.7	10.9	44.8	373.7	0.28	134.2

the other with manure application and two years of pasture. The experiments were laid out with a total of 16 different combinations of fertilization in each of the two crop rotations in a replicated (n = 2) randomized splitsplit-block design for a total of 64 plots per site (Carlgren and Mattsson 2001). Each plot has a size of 125 m^2. The experiment is maintained by the Swedish University of Agricultural Sciences (SLU) and has the number R3-9001.

For the present study, only the rotation without manure application and without ley was analyzed. This crop rotation consists of the following crops grown in the following order; spring barley (*Hordeum vulgare* L.), oil seed (*Brassica napus* L.), winter wheat (*Triticum aestivum* L.), and sugar beet (*Beta vulgaris* L.) in southern Sweden. In central Sweden the crop rotation consists of the following crops grown in the following order; spring barley, oat (*Avena sativa* L.), oil seed, winter wheat, oat, and winter wheat (winter wheat and oat are grown twice in this rotation). In each year, the same crop is grown in all nutrient addition treatments at one site. In the present study, all plant species for which there were at least eight observations on N and P in yields per site were analyzed (i.e., eight different years in which the plant species was cultivated and analyzed at each site). These species were winter wheat, spring barley, oat, and sugar beet.

For the present study, ten fertilization treatments were grouped into three fertilization regimes (low, medium, and high). Specifically, the low fertilization regime contained the control which did not receive any fertilizer, a N treatment with low N addition, a P treatment with replacement P addition, and a NP treatment with low N addition and replacement P addition. Replacement P addition refers to a P addition that equals the amount of P that is removed during harvest. The medium fertilization regime contained the control which did not receive any fertilizer, a N treatment with medium N addition, a P treatment with replacement + low P addition, and a NP treatment with medium N addition and replacement + low P addition. The high fertilization regime contained the control which did not receive any fertilizer, a N treatment with high N addition, a P treatment with replacement + high P addition, and a NP treatment with high N addition and replacement + high P addition. The exact fertilizer application rates are adapted to the location (south and central Sweden) and for N also to the nutrient demand of each crop (Table S1). The ten experimental treatments have the numbers 211, 212, 213, 214, 221, 222, 231, 233, 241, and 244.

N was applied in the form of calcium ammonium nitrate (nitrochalk). P was applied as superphosphate until 1991, and thereafter as triple superphosphate. P was always added together with K (Table S1) which was applied as potassium chloride (Carlgren and Mattsson 2001). Since N was added in the form of calcium ammonium nitrate, all N treatments include calcium addition. Further, the P treatments do not only include K addition, but also calcium and chloride addition since P was added in the form of superphosphate and K in the form of potassium chloride.

For the present study, I analyzed data on yields collected from 1957 to 2021, data on soil chemical properties collected from 1957 to 2019, and data on N and P in yields collected from 1973 to 2021.

Measurements

Crop yields have been measured every year since the beginning of the experiment. For this purpose, grains and beets were oven-dried at 65 °C, and subsequently the mass was determined. The P content of the yield was assessed by boiling the milled grains or beets in 65% HNO_3, and the filtered digest was analyzed for total P using inductively coupled plasma-optical emissions spectroscopy (ICP-OES; Avio 200, Perkin Elmer). The N content of the yield was determined using an elemental analyzer (TruMac CN, LECO). Before the 1980s, total N was analyzed using a Kjeldahl procedure after a wet oxidation in H_2SO_4 and P was analyzed photometrically using a molybdenum blue-based method (John 1970). The analyses started in 1973, and all analyses were conducted in the year of sampling.

Soil samples were collected from all treatments at a soil depth of 0–20 cm every six year (right before application of fertilizers). Soil samples were air dried, and sieved (<2 mm) and roots were removed. The soil organic C and total N concentrations were analyzed using an elemental analyzer (TruMac CN, LECO). The pH was determined in water (at a soil:water ratio of 1:3) using a Pt electrode (Aquatrode Plus Pt1000, Metrohm). Plant available P (P-AL) and K (K-AL) were extracted from the soils by acid ammonium lactate according to the method of Egnér et al. (1960). Briefly, 5 g soil were shaken at room temperature for 1.5 h in a solution containing 0.1 M ammonium lactate and 0.4 M acetic acid, with a pH adjusted to 3.75. The suspension was filtered (0.2 µm) before analysis using ICP-OES (Avio 200, Perkin Elmer). For P-HCl, a 100-ml glass flask containing 2 g soil and 50 ml 2 M HCl was immersed in a boiling water bath (100 °C) and swirled after 30, 60 and 90 min. After 2 h, the extraction was interrupted by immersion in a cool water bath (approximately 10 °C). The suspension was filtered (0.2 µm) before analysis using ICP-OES (Avio 200, Perkin Elmer).

Soil total organic P was determined according to Saunders and Williams (1955) and Williams and Saunders (1956) as specified in Pansu and Gautheyrou (2007). Briefly, each sample was separated in two aliquots, each of 1 g. The first aliquot was directly extracted in 0.5 M H_2SO_4 on a horizontal shaker for 16 h. The other aliquot was ignited at 550 °C for 2 h and subsequently extracted in H_2SO_4 in the same way as the non-ignited sample. Inorganic P was measured in the extracts by the molybdenum blue method according to Murphy and Riley (1962) using a continuous flow system (AA500, Seal). Soil total organic P was calculated as the difference between inorganic P in the ignited and non-ignited sample. P in the non-ignited sample is considered as total P (TP).

Calculations and definitions of nutrient limitations

For many years, only means across the two replicates of each treatment at each site were saved for further analysis. Therefore, I calculated the means across the two replicates of all other years for each site and treatment, and only analyzed these means in the following (i.e., one observation per site and treatment per year). This was done in order to give the same weight to the data from all years.

I tested if there was a significant synergistic effect in each of the three fertilization regimes (low, medium, and high), separately for the four plant species. For this purpose, the yield in the control was subtracted from the yield in the N, P, and NP treatments. This was done separately for each site, crop, and year. The difference between the yield in the N treatment and the control ($\Delta \mathrm{Yield}_N$) was calculated as well as the difference between the yield in the P treatment and the control ($\Delta \mathrm{Yield}_P$). Next, the sum of these two differences in each of the three fertilization regimes (low, medium and high) was calculated

($\Delta\text{Yield}_N + \Delta\text{Yield}_P$). Subsequently, this sum of the two differences was compared to the difference between the NP treatment and the control (ΔYield_{NP}) in each of the three fertilization regimes. This was done by t-test, whereby $P < 0.05$ was considered to indicate a statistically significant difference. If the effect of combined NP addition (ΔYield_{NP}) was significantly ($P < 0.05$) larger than the sum of the effects of single N and P addition ($\Delta\text{Yield}_N + \Delta\text{Yield}_P$), a crop at this specific site was classified as being ***synergistically NP co-limited*** in this fertilization regime. If there was a significant synergistic effect, the percentage of the synergistic difference in yields as percentage of the yield in the NP treatment (Yield_{NP}) was calculated in order to quantify the relative size of the synergistic effect, as follows.

In order to analyze the treatment effects at different sites (among which yields differ) together, response ratios were calculated. For this purpose, the yield in the N, P, and NP treatments was divided by the yield in the control. This was done separately for each site, crop, and year. Subsequently, I compared the response ratios of the three nutrient addition treatments (N, P, and NP), separately for different species and the three fertilization regimes. In addition, I compared the response ratios of the three fertilization regimes (low, medium, and high), separately for different species and the three fertilization treatments. These comparisons were done using Kruskal–Wallis test followed by Dunn post hoc test (using the R package FSA, version 0.9.4), whereby $P < 0.05$ was considered to indicate statistically significant differences.

$$\text{Relative size of the synergistic effect (\%)} = \left(\Delta\text{Yield}_{NP} - \left(\Delta\text{Yield}_N + \Delta\text{Yield}_P\right)\right)/\text{Yield}_{NP} * 100$$

The nutrient limitation was further classified. For this purpose, I tested if there were significant differences among the yields in the four treatments (control, N, P, and NP) in each of the three fertilization regimes (low, medium, and high), and this was calculated separately for each crop at each site across all years. The comparison was done using Kruskal–Wallis test followed by Dunn post hoc test (using the R package FSA, version 0.9.4), whereby $P < 0.05$ was considered to indicate statistically significant differences. A crop at a specific site that was not synergistically co-limited was classified as ***NP co-limited*** when the yield was significantly larger in the N, P, and NP treatment than in the control or when the yield was only significantly larger in the NP treatment than in the control but not in the N and P treatment. Further, a crop at a specific site that was not synergistically NP co-limited or NP co-limited was classified as ***N limited*** when the yield was significantly larger in the N treatment than in the control. Likewise, a crop at a specific site that was not synergistically NP co-limited or NP co-limited was classified as ***P limited*** when the yield was significantly larger in the P treatment than in the control. Furthermore, a crop at a specific site was classified as not being nutrient limited when the yield in the N, P and NP treatment was not significantly different from the control (no significant response).

Furthermore, it was assessed to which extent the four plant species change N acquisition in response to P addition, and vice versa, change P acquisition in response to N addition. For this purpose, I first calculated the N and P stocks of the yield (called yield N and yield P, in g m^{-2}) by multiplying the yield with its N and P contents (i.e., the N and P contents of the grains or beets, respectively). Subsequently, I calculated the change in yield P due to N addition (relative to yield P in the control treatment) in percentage. For example, an increase in yield P by 30% means that the yield P in the N treatment was 30% larger than in the control treatment. Likewise the change in yield N due to P addition was calculated. These percentages were compared among the low, medium, and high fertilization regimes using Kruskal–Wallis test followed by Dunn post hoc test (using the R package FSA, version 0.9.4), whereby $P < 0.05$ was considered to indicate statistically significant differences. In addition, it was tested if yield N and yield P in the different treatments were significantly ($P < 0.05$) different from the control treatment. This was tested by t-test separately for each crop and each treatment. All data analyses were conducted using R (version 4.2.1; R Core Team 2021).

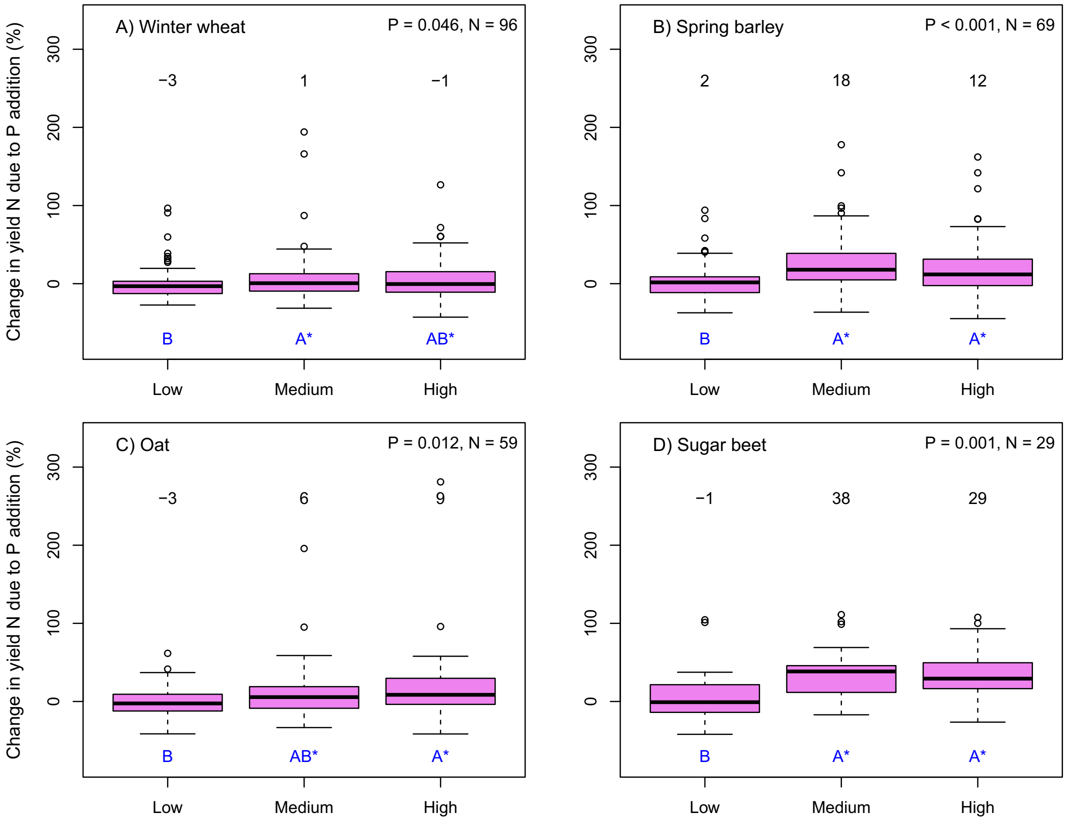


Fig. 1 Change in yield nitrogen (yield N) of the four plant species winter wheat (**A**), spring barley (**B**), oat (**C**), and sugar beet (**D**) due to phosphorus (P) addition in the three fertilization regimes (low, medium, and high). The change is given in percentage in relation to the yield N in the control treatment. Yield N (in g m^{-2}) was calculated by multiplying the yield with its N content. The number of observations (N) and the P value of the Kruskal–Wallis test is given in the right corner of each panel. Black numbers depict the median. Different blue capital letters indicate significant ($P < 0.05$) differences according to the Dunn post hoc test, tested separately for each crop. Blue asterisks indicate significant ($P < 0.05$) differences compared to the control treatment

Results

In response to P addition, sugar beet increase yield N by 38% and 29%, in the medium and high fertilization regime, respectively, compared to the control (Fig. 1D). Similarly, in response to P addition spring barley increased yield N by 18% and 12% in the medium and high fertilization regime, respectively, compared to the control (Fig. 1B). Further, oat only increased yield N by less than 10%, and winter wheat did not significantly increase yield N in response to P addition (Fig. 1C and A). In the high P addition treatment, the increase in yield N due to P addition was not significantly higher than in the medium P treatment in any plant species (Fig. 1). Furthermore, in the low P treatment, yield N was not significantly different form the control (see blue asterisks in Fig. 1). In contrast, in the NP treatment, yield N increased significantly from the low, to the medium, to the high fertilization regime (see blue capital letters in Fig. S1), and yield N in all three fertilization regimes (low, medium, high) was significantly different from the control (see asterisks in Fig. S1).

Winter wheat, spring barley, and oat increased substantially yield P when provided with N (Fig. 2). Specifically, in the low N addition treatment, yield P was 31% to 45% higher than in the control across all three plant species (winter wheat, spring barley, and oat; Fig. 2A-C). In the medium N addition

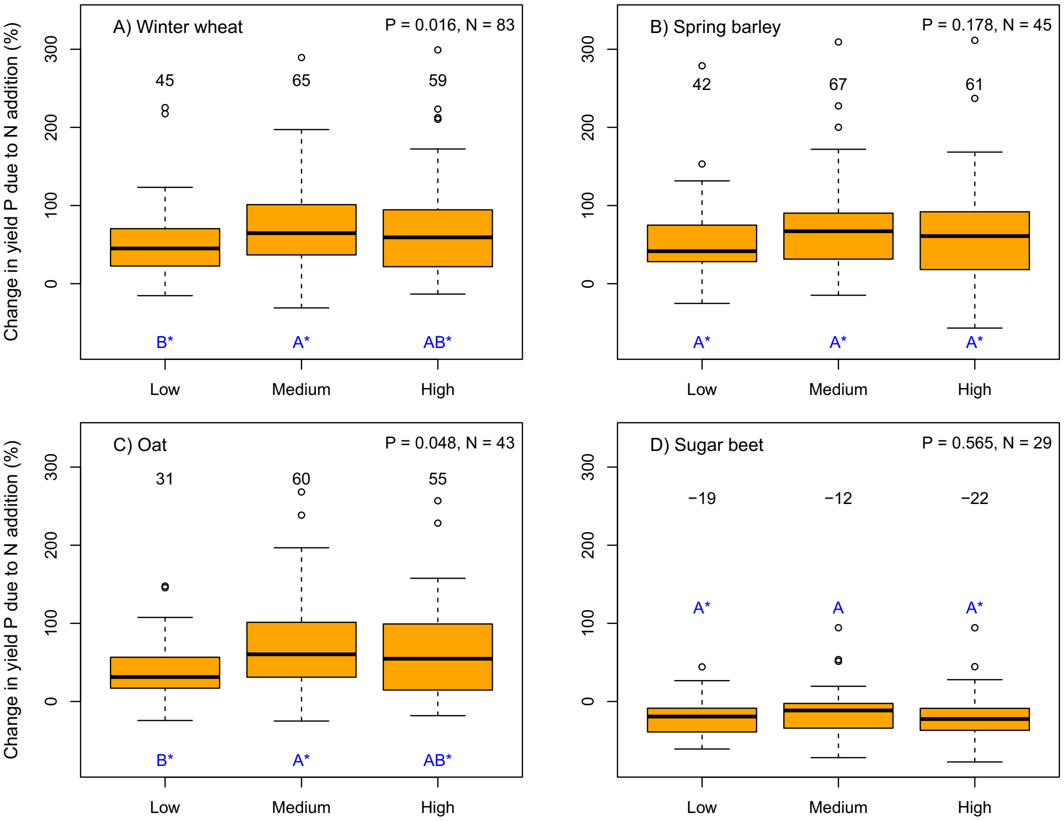
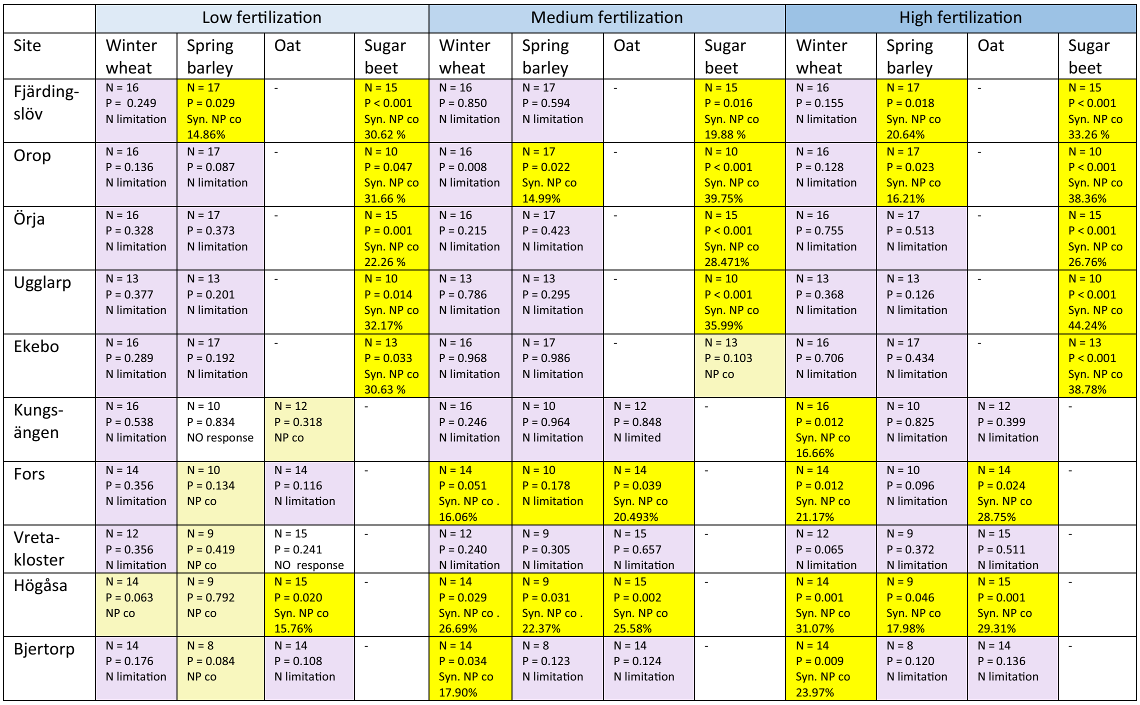


Fig. 2 Change in yield phosphorus (yield P) of the four plant species winter wheat (**A**), spring barley (**B**), oat (**C**), and sugar beet (**D**) due to nitrogen (N) addition in the three fertilization regimes (low, medium, and high). The change is given in percentage in relation to the yield P in the control treatment. Yield P (in g m^{-2}) was calculated by multiplying the yield with its P content. The number of observations (N) and the P value of the Kruskal–Wallis test is given in the right corner of each panel. Black numbers depict the median. Different blue capital letters indicate significant ($P < 0.05$) differences according to the Dunn post hoc test, tested separately for each crop. Blue asterisks indicate significant ($P < 0.05$) differences compared to the control treatment

treatment, yield P was 60% to 67% higher than in the control across the three plant species (winter wheat, spring barley, and oat; Fig. 2A-C). In contrast, sugar beet decreased yield P by up to 22% in the three N treatments compared to the control (Fig. 2D). Yet, in the NP treatments, yield P of sugar beet was 89, 211, and 301% higher in the low, medium and high fertilization regime, respectively, compared to the control (Fig. S2). Winter wheat, spring barley, and oat had very similar yield N:P ratios, while the yield N:P ratio of sugar beet was less than half than that of winter wheat, spring barley, and oat (Table S2).

Sugar beet was synergistically NP co-limited at all sites and in all three fertilization regimes (except for one site in the medium fertilization regime where sugar beet was only NP co-limited; Fig. 3). Winter wheat was N limited at all sites in the low fertilization regime (except for one site), and the number of sites at which winter wheat was (synergistically) NP co-limited was higher in the medium and high fertilization regime (Fig. 3). Spring barley and oat were N limited at some sites and (synergistically) NP co-limited at other sites (Fig. 3). The maximum size of the synergistic effect in response to NP addition (across all species) equaled 32.2% of the yield in the NP treatment in the low fertilization regime, and 39.7% and 44.2% in the medium and high fertilization regime, respectively. The largest synergistic effects in all three fertilization regimes were



	Low fertilization				Medium fertilization				High fertilization			
Site	Winter wheat	Spring barley	Oat	Sugar beet	Winter wheat	Spring barley	Oat	Sugar beet	Winter wheat	Spring barley	Oat	Sugar beet
Fjärding-slöv	N = 16 P = 0.249 N limitation	N = 17 P = 0.029 Syn. NP co 14.86%	-	N = 15 P < 0.001 Syn. NP co 30.62 %	N = 16 P = 0.850 N limitation	N = 17 P = 0.594 N limitation	-	N = 15 P = 0.016 Syn. NP co 19.88 %	N = 16 P = 0.155 N limitation	N = 17 P = 0.018 Syn. NP co 20.64%	-	N = 15 P < 0.001 Syn. NP co 33.26 %
Orop	N = 16 P = 0.136 N limitation	N = 17 P = 0.087 N limitation	-	N = 10 P = 0.047 Syn. NP co 31.66 %	N = 16 P = 0.008 N limitation	N = 17 P = 0.022 Syn. NP co 14.99%	-	N = 10 P < 0.001 Syn. NP co 39.75%	N = 16 P = 0.128 N limitation	N = 17 P = 0.023 Syn. NP co 16.21%	-	N = 10 P < 0.001 Syn. NP co 38.36%
Örja	N = 16 P = 0.328 N limitation	N = 17 P = 0.373 N limitation	-	N = 15 P = 0.001 Syn. NP co 22.26 %	N = 16 P = 0.215 N limitation	N = 17 P = 0.423 N limitation	-	N = 15 P < 0.001 Syn. NP co 28.471%	N = 16 P = 0.755 N limitation	N = 17 P = 0.513 N limitation	-	N = 15 P < 0.001 Syn. NP co 26.76%
Ugglarp	N = 13 P = 0.377 N limitation	N = 13 P = 0.201 N limitation	-	N = 10 P = 0.014 Syn. NP co 32.17%	N = 13 P = 0.786 N limitation	N = 13 P = 0.295 N limitation	-	N = 10 P < 0.001 Syn. NP co 35.99%	N = 13 P = 0.368 N limitation	N = 13 P = 0.126 N limitation	-	N = 10 P < 0.001 Syn. NP co 44.24%
Ekebo	N = 16 P = 0.289 N limitation	N = 17 P = 0.192 N limitation	-	N = 13 P = 0.033 Syn. NP co 30.63 %	N = 16 P = 0.968 N limitation	N = 17 P = 0.986 N limitation	-	N = 13 P = 0.103 NP co	N = 16 P = 0.706 N limitation	N = 17 P = 0.434 N limitation	-	N = 13 P < 0.001 Syn. NP co 38.78%
Kungs-ängen	N = 16 P = 0.538 N limitation	N = 10 P = 0.834 NO response	N = 12 P = 0.318 NP co	-	N = 16 P = 0.246 N limitation	N = 10 P = 0.964 N limitation	N = 12 P = 0.848 N limited	-	N = 16 P = 0.012 Syn. NP co 16.66%	N = 10 P = 0.825 N limitation	N = 12 P = 0.399 N limitation	-
Fors	N = 14 P = 0.356 N limitation	N = 10 P = 0.134 NP co	N = 14 P = 0.116 N limitation	-	N = 14 P = 0.051 Syn. NP co . 16.06%	N = 10 P = 0.178 N limitation	N = 14 P = 0.039 Syn. NP co 20.493%	-	N = 14 P = 0.012 Syn. NP co 21.17%	N = 10 P = 0.096 N limitation	N = 14 P = 0.024 Syn. NP co 28.75%	-
Vreta-kloster	N = 12 P = 0.356 N limitation	N = 9 P = 0.419 NP co	N = 15 P = 0.241 NO response	-	N = 12 P = 0.240 N limitation	N = 9 P = 0.305 N limitation	N = 15 P = 0.657 N limitation	-	N = 12 P = 0.065 N limitation	N = 9 P = 0.372 N limitation	N = 15 P = 0.511 N limitation	-
Högåsa	N = 14 P = 0.063 NP co	N = 9 P = 0.792 NP co	N = 15 P = 0.020 Syn. NP co 15.76%	-	N = 14 P = 0.029 Syn. NP co . 26.69%	N = 9 P = 0.031 Syn. NP co . 22.37%	N = 15 P = 0.002 Syn. NP co 25.58%	-	N = 14 P = 0.001 Syn. NP co 31.07%	N = 9 P = 0.046 Syn. NP co 17.98%	N = 15 P = 0.001 Syn. NP co 29.31%	-
Bjertorp	N = 14 P = 0.176 N limitation	N = 8 P = 0.084 NP co	N = 14 P = 0.108 N limitation	-	N = 14 P = 0.034 Syn. NP co 17.90%	N = 8 P = 0.123 N limitation	N = 14 P = 0.124 N limitation	-	N = 14 P = 0.009 Syn. NP co 23.97%	N = 8 P = 0.120 N limitation	N = 14 P = 0.136 N limitation	-

Fig. 3 Nutrient limitations of the four plant species at the ten sites in the three different fertilization regimes (low, medium, and high) calculated across all years. The colors indicate the different nutrient limitations. Violet: N limitation. Light yellow: NP co-limitation (NP co), bright yellow: Synergistic NP-co-limitation (Syn. NP co). In addition, the figure shows the number of observations (N), the P value of the synergistic effect (synergistic NP co-limitation if $P < 0.05$). If there is a synergistic NP-co-limitation, the size of the synergistic effect is given in percentage (%) relative to the yield in the NP treatment

observed for sugar beet. Furthermore, the site Högåsa was the only site at which all species in all fertilization regimes were either NP co-limited or synergistically NP co-limited. At all other sites, some species were N limited while others were (synergistically) NP co-limited.

The effects of N, P, and NP addition on yield were higher in the medium than in the high fertilization regime in all four plant species, but they did not differ significantly between the medium and the high fertilization regime (except for oat in the NP treatment; see blue lowercase letters in Fig. 4). The effect of N on yield was smaller in sugar beet than in the other species, and in sugar beet the effect of N was not significantly different from the effect of P on yield (Fig. 4 D, H, L), in contrast to the other three species for which the effect of P on yield was smaller than the effect of N (Fig. 4). The yield of all species was significantly higher in the NP treatment than in the N treatment, except for winter wheat and oat in the low fertilization regime.

Discussion

Stronger increase in P acquisition due to N addition than in N acquisition due to P addition

In accordance with the first hypothesis, the relative increase in yield P in response to N addition was larger than the relative increase in yield N in response to P addition in winter wheat, spring barley, and oat (Figs. 1 and 2). The reason for the strong effect of N addition on yield P is likely that plants use N to produce phosphatase (Marklein and Houlton 2012; Schleuss, et al. 2020) and P-transporters (Smith and Jackson 1987; Zeng et al. 2012). Phosphatases catalyze the mineralization of organic

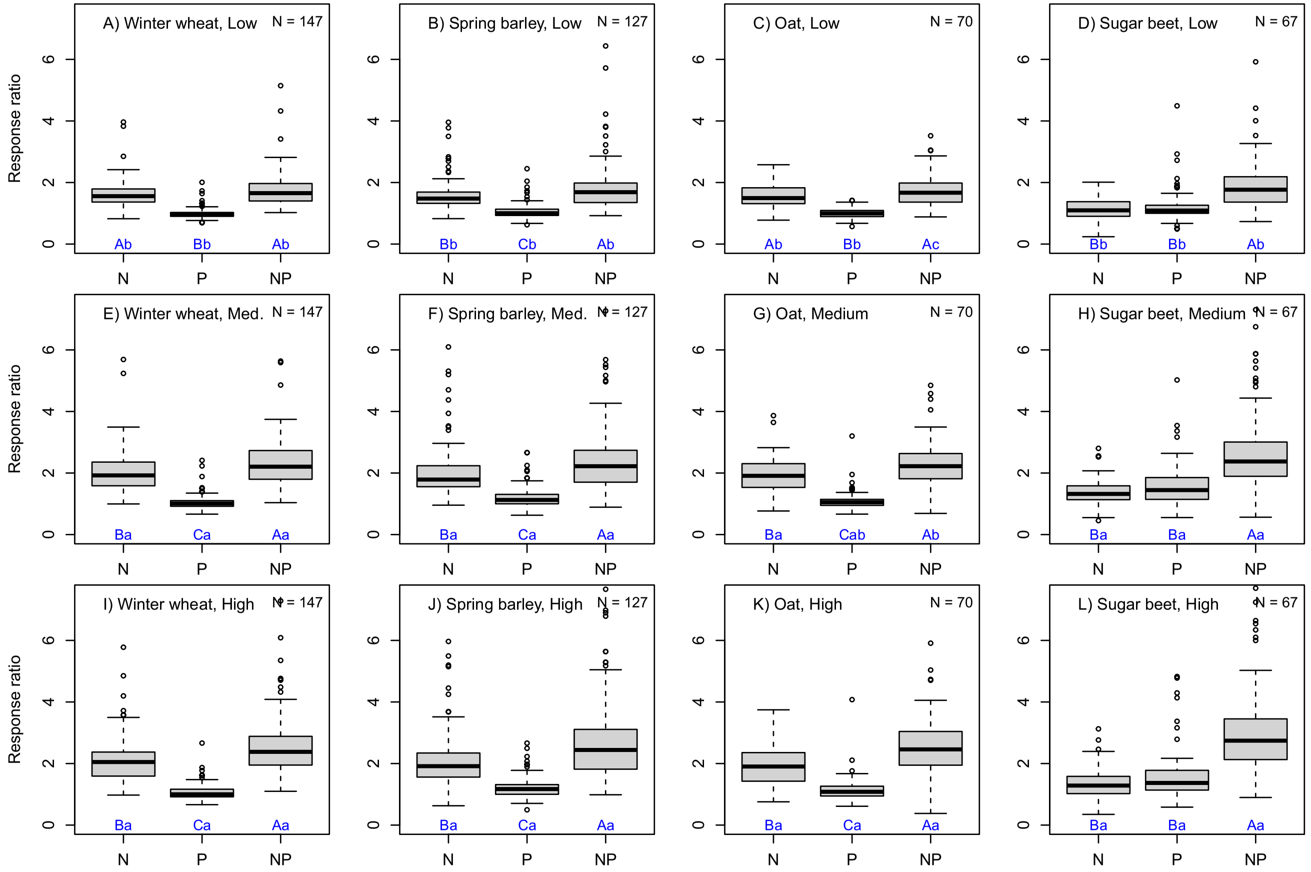


Fig. 4 Response ratios of the yields of the four plant species (winter wheat, spring barley, oat, and sugar beet) in the nitrogen (N), phosphorus (P), and nitrogen-phosphorus (NP) treatments in the three fertilization regimes (low, medium, and high) calculated across all years and sites. The number of observations (N) is given in the right corner of each panel. Different blue capital letters indicate significant ($P < 0.05$) differences among the three fertilization treatments (N, P, and NP) according to the Dunn post hoc test, tested separately for each plant species in each fertilization regime. Different blue lowercase letters indicate significant ($P < 0.05$) differences among the three fertilization regimes (low, medium, and high) according to the Dunn post hoc test, tested separately for each plant species in each fertilization regime

P, which renders organic P plant-available, while P-transporters facilitate plant P uptake. Plants can use N to produce these proteins (phosphatases and P-transporters), and thus invest N into the acquisition of P (Bloom et al. 1985). This is supported by studies showing that plants increase soil phosphatase activity when provided with N, leading to increased plant P uptake compared to the control (Schleuss et al. 2020). In addition, plants might have used added N to increase root length and change the root morphology, leading to increased nutrient uptake (Duncan et al. 2018). The strong effect of N addition on plant P acquisition in winter wheat, spring barley, and oat (Fig. 2) is in accordance with previous studies (for reviews see Aulakh and Malhi (2005) and Rietra et al. (2017)).

The increase in yield N due to P addition was lower than the increase in yield P due to N addition in all species except for sugar beet. Further, winter wheat did not increase yield N in response to P addition (Figs. 1 and 2). P is required for many metabolic processes as it forms part of ATP, NADH, DNA, and RNA. Thus, in conditions of low P availability, P fertilization might facilitate faster metabolic processes, and in particular faster and larger production of compounds that are involved in N acquisition, such as proteases that hydrolyze organic N in soil or N transporters that allow plants to take up N (Aulakh and Malhi 2005; Rietra et al. 2017). Yet, the results show that not all plant species used additional P to acquire N, and if they did, the increase in yield N was smaller than the effect of additional N on yield P, with the exception of sugar beet (Figs. 1 and 2).

One reason why the increase in yield P due to N addition was larger than the increase in yield N due to P addition in most species is that yield was more strongly limited by N than by P in most species (Fig. 4). The exception from this general trend was sugar beet, which increased yield N in response to P addition much more strongly than it increased yield P in response to N addition. This is in accordance with the fact that sugar beet was the only species that was not N limited at any site but (synergistically) NP co-limited at all sites and in all fertilization regimes (Fig. 3).

Increases in yield N and yield P due to the addition of P and N did not differ significantly between the low and the medium fertilization regime (Figs. 1 and 2). One reason for this is likely that the yield did not differ significantly between the low and the medium fertilization regime, when comparing specific treatments (see lowercase letters in Fig. 4). The latter is in agreement with previous studies showing that yields do no increase further with raising nutrient addition beyond the maximum yield response of a given species (Hoogmoed et al. 2018; Lollato et al. 2019). Another reason is that there is likely a limit to the amount of N and P that plants can mobilize from soil during one vegetation period (Spohn et al. 2018, 2020). Specifically, it might be that higher nutrient mobilization in the high fertilization regime (compared to the medium fertilization regime) was too N- or P-costly for the plants. In contrast, all plant species increased yield N and yield P strongly with increasing fertilization rate in the NP treatment (Figs S1 and S2). Specifically, all plant species produced significantly higher yield N in the high than in the medium NP treatment (Fig. S1), and one plant species (winter wheat) also produced significantly higher yield P in the high than in the medium NP treatment (Fig. S2). This suggests that the reason why N and P uptake was not higher in the high P and N treatments than in the medium P and N treatments, respectively, is less related to plant growth and yield and more to nutrient availability.

Species differ in the extent to which they use N to acquire P and P to acquire N

The results support the second hypothesis that plant species differ strongly in the extent to which they increase P acquisition in response to N addition and, vice versa, increase N acquisition in response to P addition. The largest contrast among plant species was found with respect to the effect of N addition on yield P. Sugar beet decreased yield P in the three N treatments compared to the control (Fig. 2D), while all other plant species (winter wheat, spring barley, and oat) substantially increased yield P in response to N addition (Fig. 2A-C). It seems that sugar beet has no or only very limited capacities to use N for the acquisition of P, and it can be speculated that this is due to a low production of phosphatases, a low capacity to increase the expression of P-transporters or a low capacity to adjust its root length and morphology. Sugar beet had a lower yield N:P ratio than the other three plant species (Table S2). Thus, P uptake of sugar beet was generally high in relation to N uptake, and it could be that further plant P uptake was limited by the soil properties. The reason why yield P of sugar beet in the N treatments was decreased relative to the control is likely that sugar beet changed its N allocation, and used less N in the beet (which forms the yield), and more N in other parts of the plant. For instance, it might be that sugar beet allocated more P to its roots to enhance processes of P acquisition in response to N application. This is supported by a study showing that the root-to-shoot ratio of sugar beet changed in response to changes in N and P availability (Hadir et al. 2020). It is also in accordance with studies observing that sugar beet adjusts allocation of N according to the N availability (Draycott and Christenson 2003). The fact that sugar beet very efficiently took up P in the NP treatment (Fig. S1) demonstrates that N addition did not inhibit P uptake in sugar beet. The fact that sugar beet did not use added N to increase yield P is likely the main reason why the effect of N on yield was smaller for sugar beet than for the other plant species (Fig. 4). In contrast to sugar beet, the three other species, winter wheat, spring barley, and oat used the added N to strongly increase yield P (see previous section).

Barley and sugar beet substantially increased yield N in response to P addition, while oat only increased yield N by less than 10%, and winter wheat did not increase yield N in response to P addition (Fig. 1). This further supports the hypothesis that species strongly differ in the extent to which they use one nutrient to acquire another one. The underlying processes might be that barley and sugar beet invested additional P effectively into the transcription of genes coding for proteases and N-transporters or into the production of roots since these process require P, for instance for the synthesis of RNA and phospholipids (Aulakh and Malhi 2005; Rietra et al. 2017). In contrast, winter wheat did not use added P to enhance its N acquisition. This in accordance with a review about nutrient interactions in wheat reporting that the addition of P had no significant effect on plant N uptake, yield N or yield protein content in wheat (Duncan et al. 2018).

The findings indicate that crops which effectively use N to acquire P and P to acquire N can be beneficial to improve yields in agroecosystems in which nutrients are not supplied in the optimal ratio (for instance due to economic reasons). In more general terms, the results suggest that plant growth in ecosystems with non-balanced nutrient supply is higher if the ecosystems are dominated by plant species that effectively use N for P acquisition and P for N acquisition (Bloom et al. 1985).

Is nutrient limitation species- or site-specific?

Concerning the third hypothesis, the results suggest that the occurrence of synergistic co-limitation depends both on site properties and on plant species properties. One plant species (sugar beet) was consistently (synergistically) NP co-limited at all sites and in all three fertilization regimes, while at one site (Högåsa) all species in all fertilization regimes were consistently (synergistically) NP co-limited (Fig. 3). The soil at this site (Högåsa) has an outstandingly high sand content as well as relatively low N and P contents and a comparatively high C:N ratio (Table 2). The high sand content and the low nutrient contents of the soil have been described to limit crop production at the site Högåsa (Kirchmann et al. 2005). These soil properties likely also lead to the consistent synergistic NP co-limitation of all four plant species when grown at this site. In addition, sugar beet was likely NP co-limited at all sites due to its ability to use N to acquire P (see previous sections).

Synergistic growth effect more frequent at high than at low fertilization

The results support the fourth hypothesis that the synergistic growth effect in response to NP addition occurred at a larger number of sites in the high than in the low fertilization regime with respect to winter wheat (Fig. 3). The reason why winter wheat responded to NP addition with synergistic growth at a larger number of sites at high than at low fertilization is very likely that the species did not use additional P for N acquisition from soil (Fig. 1). Thus, winter wheat took up more N and P in the NP treatment (Fig. S1 and S2) than in the N and P treatments (Figs. 1 and 2), leading to larger yields in the NP treatments.

Furthermore, the size of the synergistic growth effect in sugar beet increased with increasing N and P fertilization (Fig. 3), in accordance with the fourth hypothesis. The reason for this increase in the size of the synergistic effect with increasing fertilization is likely also the increase in the difference in nutrient acquisition between the N and the NP treatments (as well as the P and the NP treatments) with increasing fertilization rate, resulting mostly from the fact that yield P in sugar beet was not elevated in response to N addition (Fig. 2). The findings are in accordance with a study showing that a synergistic growth response in barley only occurred beyond a critical level of P fertilization (Clayton et al. 2024) and a study about phytoplankton reporting that synergistic NP co-limitation occurs more frequently at high than at low N and P addition rates (Zeng et al. 2016).

The most likely explanation for the synergistic effect in response to NP addition is that plants which are provided with both N and P do not need to invest one nutrient to acquire the other but can allocate both nutrients directly to growth. The reason why the synergistic growth effect was stronger in the high than in the low fertilization regime is likely that high N and P availability in the soil allows the plant to more easily gain a biomass stoichiometry that is optimal for growth. This decreases the probability that catabolic or anabolic processes are N, P or NP limited, and thus allows for high growth. In particular, high N and P availability decreases the probability that nutrient uptake is N or P limited. If the nutrient concentrations are lower, (transient) nutrient limitations of individual processes might occur, resulting in overall lower plant growth.

Taken together, the synergistic growth effect in response to NP addition occurred more frequently at high than at low fertilization rates in winter wheat and it was larger at high than at low fertilization rates in sugar beet. The reason for this are likely the differences in nutrient availability between the NP treatment and the N and P treatments. Yet, in the two species that substantially increased both N and P uptake when provided with P and N, respectively, the synergistic effect was not stronger at high than at low fertilization rate.

In addition to interactions of N and P, also interactions with calcium and potassium might occur since calcium was added together with N and potassium was added together with P (see section `Field sites and experiment´). Interactions of N and P with several other elements in plant nutrition are known to occur (Sumner and Farina 1986; Saito et al. 2008; Rietra et al. 2017). However, these interactions cannot be analytically isolated based on the experiment analyzed here.

Conclusions

Synergistic NP co-limitation was observed for all four plant species at some of the ten sites, which is likely due to the limited extent to which plants can use N to acquire P and P to acquire N. Most plant species, except for sugar beet, used N more effectively to acquire P than they used P to acquire N. Further, most plant species were N limited at most sites, and only sugar beet was (synergistically) NP co-limited at all sites in all three fertilization regimes. The synergistic growth effect was stronger in the high than in the low fertilization regime in two plant species.

The study shows that plant N and P acquisition processes are strongly interrelated, which is important for understanding interactions of the element cycles but also for improving nutrient use efficiency in agroecosystems. Synergistic growth effects due to combined nutrient additions should, for example, be considered for fertilizer recommendations. Furthermore, the interactions of N and P should be included in conceptual and numeric models of plant nutrition. The findings imply that crops which effectively use N to acquire P and P to acquire N can be beneficial to improve yields when nutrients are not supplied in the optimal ratio. In more general terms, the results suggest that plant growth in ecosystems with non-balanced nutrient supply is higher if the ecosystems are dominated by plants species that effectively use N for P acquisition and P for N acquisition.

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Author contributions MS conceptualized the study, analyzed data and wrote the manuscript.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests The author has no relevant financial or non-financial interests to disclose.

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