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Low nitrogen and phosphorus effects on wheat Fe, Zn, phytic acid and phenotypic traits

In sub-Saharan Africa, crops are often grown under low nitrogen (N) and low phosphorus (P) conditions, which may impact on the nutritional components of the grains. The aim of this study was to investigate the effect of low N and low P and a combination of the two on iron (Fe), zinc (Zn) and phytic acid content in two commercial South African spring wheat cultivars (PAN3497 and SST806). Phenotypic traits were also investigated. Although cultivar effects were not significant, treatment effects were highly significant for the phenotypic and nutritional traits. Low P stress increased Fe and Zn levels, whereas low N stress had the opposite effect. In addition, low P stress inhibited phytic acid accumulation the most, suggesting that under this treatment, Fe and Zn were more available because of less interaction with phytic acid. Compared to the low N treatment, the low P treatment led to lower reductions in the number of tillers, plant height, stem thickness, number of seeds, weight of seeds and dry weight for both cultivars. While low P had positive effects on the nutritional value of wheat, the combination of low N and P treatment had a negative impact on most of the measured characteristics. Low N conditions had more negative effects on all measured characteristics than low P conditions and was very detrimental to wheat nutritional value and yield.

Significance:

- Results from this study emphasise the impact of fertilisation and the impact of insufficient nitrogen and phosphorus fertiliser on wheat productivity.
- Low nitrogen and phosphorus fertilisation impact grain microelement content and bioavailability which impact nutritional value.

Introduction

Nitrogen deficiency is one of the major crop production constraints in the world.¹ Plants require nitrogen (N) in large quantities to attain normal growth and development because N concentration is strongly related to photosynthetic rate and other photosynthetic parameters such as the electron transport rate and carboxylation capacity.² The estimated world supply of nitrogen as ammonia will be 170 761 thousand tons by 2020, of which only 5.5% will be used in Africa.³ Statistics indicate that the sub-Saharan region utilises very low levels of N for grain crop production, at an average of 11 kg/ha/year despite the 90 to 120 kg/ha/year recommended rates.⁴

Phosphorus (P) is the most widely used fertiliser after N.⁵ Its deficiency affects about 40% of the cultivated land of the world and causes loss of productivity and quality.⁶ As most of the P is stored in the grain, harvesting grain crops leads to continuous removal of the P from the soil. Consequently, P fertiliser application is required to address soil P deficiencies. Both N and P are essential macronutrients required for vegetative and reproductive plant growth.⁷⁻⁹ Small-scale farmers in sub-Saharan Africa often do not have access to fertiliser, due mainly to the cost, leading to poor N and P status of soils.¹⁰ Artificial fertilisers mainly contain N, P and potassium (K) while microelements are present in natural organic fertilisers such as compost.¹¹ Some industrial by-products and waste materials can be used as micro-fertilisers in case of iron (Fe) deficiency.¹²

Sub-optimal concentrations of Fe and zinc (Zn) in crops as well as in wheat grain cause micronutrient deficiencies in humans. Deficiency of Fe is a problem in most developing countries as a result of, amongst other things, inadequate intake, reduced absorption, and deficiency in the soil.¹³ Fe is a key component for infection resistance in humans.¹³ The World Health Organization (WHO) reported that 30% of the world population, specifically women and children, suffers from anaemia as a result of Fe deficiency.¹⁴ Furthermore, the WHO issued a statement that Zn deficiency ranks 11th among the 20 most important risk factors contributing to the burden of disease in the world.¹⁵ Zn deficiency is 5th among the 10 most important factors in developing countries, while Fe deficiency ranks 6th.¹⁶ Approximately 70–80% of the total P contained in cereal grains is in the form of phytate.¹⁷ The bioavailability of micronutrients for human uptake is limited by phytic acid concentration because it can make complexes with cations such as Zn²⁺, forming insoluble phytates (such as zinc phytate), which influence the bioavailability of Zn in grains.¹⁸ It has been reported that phytate content affects Fe bioavailability more than the total Fe content¹⁹, although this finding was contradicted by another study²⁰.

Although studies on the effect of low N and P content in the soil have been conducted extensively, the main focus of these studies was on crop yields. Information on how these macronutrients affect micronutrients in wheat is not evident. Therefore, the aim of this study was to investigate the effect of low N and low P as well as a combination of the two on Fe, Zn and phytic acid content in two commercial South African spring wheat cultivars with excellent baking quality. The results of this study will shed light on the quality of these cultivars under low N, P and a combination of the two. The effects of these treatments on the phenotypic characteristics of the two wheat cultivars further elucidated how the cultivars responded under these conditions.

Materials and methods

Greenhouse trials

Two commercial, South African spring wheat cultivars with excellent baking quality, PAN3497 and SST806 (the commercial standard cultivar for spring wheat baking quality in South Africa), were sown in 2-L pots filled with 2 kg soil in a greenhouse. A randomised complete block design was used with two factors: treatment and cultivar. Soil from a depth of 1.5 m was obtained from Bainsvlei, Bloemfontein, South Africa (29.05° S, 26.11667° E). The soil had very low nutrient content as indicated in Table 1.

Four treatments were applied to the two cultivars, with three replications: 15 pots per replication in 2016 and 20 pots per replication in 2017. Each pot contained three plants. The trials were carried out from June to the end of October 2016 (winter time) and during the same time in 2017. Greenhouse temperatures were set to 18 °C at night (21:00–06:00) and 22 °C during the day. Low N and low P stress and a combination of the two were induced according to the protocol given in Table 2. These treatments were tested against an optimal control. The treatments were initiated at three-leaf stage. Before this, plants were irrigated with deionised water. Once a week, pots were flushed with deionised water to prevent salt build-up. Treatments were applied twice a week (250 mL nutrient solution per pot). The electrical conductivity was maintained at 1.5 mS/cm² to tillering stage and at 1.80 mS/cm² after tillering.

All treatments received the same micronutrient fertilisation as follows: 3.45 mg/L C₁₀H₁₃FeN₂O₈, 0.30 mg/L MnSO₄, 0.13 mg/L ZnSO₄, 0.62 mg/L H₃BO₃, 0.05 mg/L CuSO₄, 0.02 mg/L Na₂MoO₄. After ripening, the seeds were harvested and milled into whole flour using a laboratory mill (IKA A10 Yellowline analysis grinder, Merck Chemicals Pty Ltd. Mountainview, CA, USA). These whole flour wheat samples were used for the determination of Fe, Zn and phytic acid.

Total iron and zinc analysis

Total Fe and Zn were extracted according to the dry-ashing method.²¹ Wheat flour (1 g) was placed in glazed, high-form porcelain crucibles and ashed in a furnace at 550 °C for 3 h. A few drops of HNO₃ (55 %, v/v) were added to the samples for digestion. The samples were then placed

on a hot sand-bath to completely dry, after which they were returned to the oven for 1 h at 550 °C for further ashing. After cooling, 10 mL of 1:2 HNO₃ was added to the samples for further digestion. The samples were returned to the hot sand-bath until they became warm (100 °C). The samples were then transferred to 100-mL volumetric flasks and filled to the mark with distilled water. Mineral concentrations were measured in triplicate using an atomic absorption spectrophotometer (Agilent Technologies 300 Series AA).

Phytic acid determination

Phytic acid concentration was determined using a rapid colourimetric procedure based on the reaction between ferric acid and sulfosalicylic acid according to the method described by Dragičević et al.²² with modifications. Ground flour samples (0.25 g) were placed in glass tubes containing 10 mL of trichloroacetic acid (TCA) (5 %, v/v) and placed on a mechanical shaker for 1 h, vortexed at 10-min intervals. The extract (5 mL) was transferred into 15-mL tubes and centrifuged at 12 000 g for 20 min. The supernatant (0.5 mL) was transferred into a clean glass tube and 1.5 mL WADE reagent (0.3%, w/v, FeCl₃ + 6H₂O; 3%, v/v, 5"-sulfosalicylic acid) was added. The samples were then centrifuged at 12 000 g for 10 min. Absorbance was read at 500 nm with a Helios gamma spectrophotometer (Erlangen, Germany). When phytate is present, the Fe ion present in the WADE reagent binds to the phosphate ester instead of reacting with sulfosalicylic acid, resulting in a decrease in pink colour intensity. The phytic acid concentration was calculated from the phytic acid standard.²³

The phytic acid standard solution was made from phytic acid sodium salt hydrate from rice (Sigma-Aldrich, P-8810, molecular weight: 660.04 g/mol). A series of standard phytic acid solutions was made from the stock standard solution by appropriate dilutions, with the addition of extraction solutions to simulate conditions similar to the ones in the samples. The concentrations of phytic acid in this series were as follows: 10, 50, 100, 150, 200, 250, 300, 350 and 400 μmol/100 mL.

Phytic acid:iron and phytic acid:zinc molar ratios

The contents of phytic acid, Fe and Zn were converted into moles by division through their molar mass or atomic weight (phytic acid: 660.04

Table 1: Characteristics of the applied soil and the measured nutrient content

pH	N (%)	P (mg/kg)	K (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	Na (mg/kg)	S (mg/kg)	Zn (mg/kg)	C (%)	Sand (%)	Clay (%)	Silt (%)
4.1	0.004	26.5	103.4	136	38.9	1.8	1.62	0.71	0.04	96	4	0

Table 2: Fertiliser applied over two years to two wheat cultivars in four treatments in a greenhouse experiment

Chemical (mg/L)	Optimal		Low N		Low P		Low N and P	
	BT	AT	BT	AT	BT	AT	BT	AT
KNO ₃	261	313	0	0	228	273	0	0
K ₂ SO ₄	210	252	210	252	196	235	196	235
KCl	0	0	193	231	56	67	223	268
NH ₄ H ₂ PO ₄	87	104	87	104	0	0	0	0
Ca(NO ₃) ₂	758	909	0	0	797	956	0	0
CaCl ₂	0	0	353	424	0	0	446	446
MgSO ₄	348	418	348	418	369	443	443	443

BT, before tillering; AT, after tillering



g/mol, Fe: 55.85 g/mol, Zn: 65.4 g/mol). The molar ratios of phytic acid:Fe and phytic acid:Zn were calculated.

Phenotypic traits

The measured traits were recorded for each plant in a pot at maturity, and values were averaged. These traits included number of tillers per plant, plant height, length of the main ear, stem thickness (main tiller, measured by a ruler), seeds per plant, seeds per main ear, seed weight of main ear, aboveground dry weight and dry leaf area per plant (main stem).

Statistical analysis

Analyses of variance (ANOVA) were performed on the data for both genotypes, four treatments and two years as a three-factor analysis.²⁴ ANOVA was also done for the two cultivars separately as well as for the two years combined, in order to determine the effects of treatments on the measured parameters within each cultivar. Differences were tested at a $p < 0.05$ level of significance. The Tukey test and least significant difference test were used for means separation.

Results

The ANOVA showed that cultivar effect was not significant for nutritional traits (Fe, Zn and phytic acid) (Table 3). The effect of treatment and year was highly significant for Fe, Zn and phytic acid contents. There was a highly significant interaction between cultivar and treatment, and cultivar and year for Zn, and between treatment and year for phytic acid.

Although SST806 recorded higher values for all nutritional traits than did PAN3497, the two cultivars were not significantly different (Table 4).

Under low N conditions, Fe concentration was significantly reduced, but under low P, as well as low N and P treatments combined, Fe content was not significantly higher than under control conditions (Table 5). Zn content increased significantly with low P inducing the highest (33.79 mg/kg) and low N inducing the lowest (20.54 mg/kg) increase. Phytic acid concentrations under both treatments were not significantly different from that of the control. The low P and combination of low N and P treatments produced significantly lower phytic acid content compared to the control, and low N with low P produced the highest reduction (5.03 and 5.31 mg/kg). The molar ratio of phytic acid:Fe was increased under low N conditions, but was significantly decreased under low P (7.13) and a combination of low N and P (4.43). The phytic acid:Zn molar ratio was decreased under low N, low P and a combination of the two but the effect was by far the highest under low P conditions with a 23.15 reduction, followed by that under a combination of low N and P (15.06).

For the two cultivars separately (Figures 1 and 2), PAN3497 Fe content was reduced in all treatments compared to the control. Low N treatment caused the most substantial reduction (21.13 mg/kg) compared to the low P and low N with P combination treatments. Similarly, for SST806, Fe content was reduced (18.08 mg/kg) under low N conditions. However, under low P and a combination of low N and low P stress, there were slight increases in Fe content compared to the control. The Zn content for PAN3497 increased in all treatments compared to the control, with the low P treatment inducing the most significant increase (35.37 mg kg).

Table 3: Analysis of variance for Fe, Zn and phytic acid concentrations in a trial of two wheat cultivars with four treatments over two years

	Cultivar (C)	Treatment (T)	Year (Y)	CxT	CxY	TxY	CxTxY
Fe	11.15	197.44**	382.45**	27.39	55.19	6.38	14.86
Zn	0.26	597.08**	0.01	45.27**	173.09**	96.61	0.05
Phytic acid	0.21	10.26**	5.35**	0.24	0.24	2.92**	0.06

** $p \leq 0.01$

Table 4: Average values of four treatments for the measured characteristics over two years separated by two cultivars

	PAN3497	SST806	Least significant difference (0.05)
Fe	24.86	25.82	1.85
Zn	23.58	23.70	1.09
Phytic acid	5.89	6.02	0.18
Phytic acid:Fe molar ratio	20.26	21.12	
Phytic acid:Zn molar ratio	23.36	26.06	

Table 5: Average values of two cultivars for the measured nutritional traits over two years separated by treatments

	Control	Low N	Low P	Low N and P	Least significant difference (0.05)
Fe (mg/kg)	26.25	19.60	28.61	26.90	2.61
Zn (mg/kg)	17.68	20.54	33.79	22.56	1.54
Phytic acid (mg/kg)	6.92	6.56	5.03	5.31	0.25
Phytic acid:Fe molar ratio	22.28	27.77	15.15	17.84	
Phytic acid:Zn molar ratio	38.08	26.62	14.93	23.02	

Although a similar pattern was observed for SST806, the low N treatment induced slightly higher Zn than that of PAN3497. Contrary to Fe and Zn, phytic acid content was similar for the two cultivars under the different treatments. In both cultivars, low P and a combination of low N and P caused a significant decrease in phytic acid.

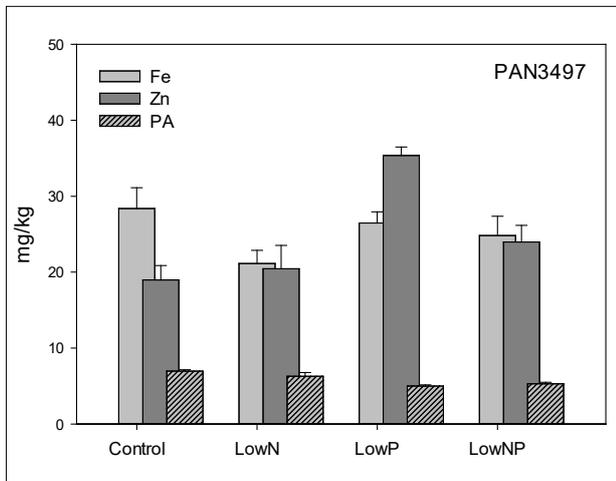


Figure 1: The Fe, Zn and phytic acid contents of PAN3497 under four treatments over two years. Values are means \pm s.d. ($n=6$).

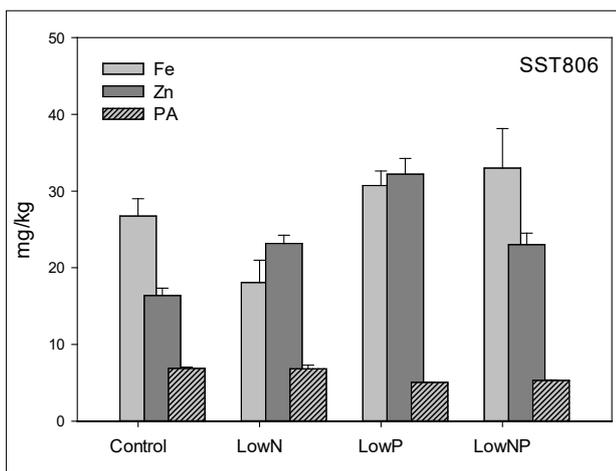


Figure 2: The Fe, Zn and phytic acid contents of SST806 under four treatments over two years. Values are means \pm s.d. ($n=6$).

The results of the ANOVA showed that cultivar effect (as seen from significance of F ratios of mean squares) was significant ($p \leq 0.05$) for main ear, seeds number per main ear, seeds per plant, above ground dry weight and dry leaf area (Table 6). Furthermore, there was a large treatment contribution to variation in the case of plant height, main ear length, main stem thickness, seeds per main ear, seeds per plants and dry leaf area. Year contributed significantly to variation in seeds per main ear and number of tillers. Dry leaf area showed large cultivar \times treatment and cultivar \times treatment \times year interactions.

To better understand the effect of treatment on morphological parameters without genotype playing a role, values were averaged for two cultivars (Tables 7 and 8). Cultivars did not differ significantly for measured phenotypic characteristics, but differences between treatments were significant. For both cultivars, the controls produced the highest values for all phenotypic characteristics and characteristics varied substantially between different treatments. Plants under the low P treatment had significantly higher numbers of tillers than those under low N and low N and P combined treatments for both cultivars. For both cultivars, plant height was reduced by all the treatments, where low N treatment led to the highest reduction in length. The main ear length, stem thickness and seeds per main ear were reduced the most under low N treatment, with low P having the least effect. Total seeds per plant were the least affected by the low P treatment for both cultivars although the weight was not significantly different from that under the low N and P combination for SST806. For the aboveground dry weight, both cultivars showed the largest reduction under the low N treatment. The low N treatment led to the lowest reduction in dry leaf area, but a combination of low N and P stress caused the highest reduction in this parameter for both cultivars, which was unexpected.

Discussion

Wide variation in Fe and Zn concentrations has been recorded in wheat grain.²⁵⁻²⁷ The average Fe concentration was reported to be between 30 mg/kg and 73 mg/kg²⁸, in contrast to this study where it was low – ranging between 19.6 mg/kg and 28.61 mg/kg. The range of Zn concentration was reported to be between 20.4 mg/kg and 30.5 mg/kg in wheat grains.²⁹ In this study, Zn concentration varied between 17.68 mg/kg and 33.79 mg/kg across the different treatments. This variation in the Fe and Zn contents could be affected by the treatments and the different cultivars used because their concentrations are determined by genetic and environmental factors.³⁰

In this study, the effect of cultivar on the measured characteristics was negligible, although SST806 had slightly higher values for all the traits. The effects of the treatments were highly significant for all measured nutritional characteristics. Zn content showed a significant cultivar with treatment interaction, indicating that the two cultivars did not react the same to treatments in terms of Zn content.

Table 6: Analysis of variance for morphological traits in a trial of two wheat cultivars with four treatments over two years

	Cultivar (C)	Treatment (T)	Year (Y)	CxT	CxY	TxY	CxTxY
Tillers	88.74	250.61	66.51*	30.52	89.90*	45.60	52.26
Plant height (cm)	13.89	235.11*	45.67	33.52	45.69	22.11	19.82
Main ear length (cm)	68.20*	513.68*	28.65	35.11	61.12*	48.36	16.52
Main stem thickness (mm)	165.44	495.133*	15.48	29.35	87.11	66.24	24.51
Seeds/main ear	85.35*	134.34*	58.04*	22.11	29.12	37.58	31.89
Seeds/plant	58.22*	363.05*	34.58	17.46	53.64*	87.11*	55.19
Main ear seed weight (g)	35.11	225.18	12.15	65.15	72.32	46.52	28.16
Aboveground dry weight (g)	156.73*	201.07	47.18	26.345	113.58*	61.98	34.58
Dry leaf area/main plant	125.11*	112.835*	288.92	87.11*	126.12	25.69	64.11*

* $p < 0.01$

Table 7: Average values for measured characteristics in PAN3479 with four treatments over two years

	Control	Low N	Low P	Low NP
Tillers	2.67±0.80c	1.03±0.16a	1.37±0.52b	1.06±0.24a
Plant height (cm)	63.89±5.06c	57.31±6.75a	59.74±6.67b	58.18±8.15b
Main ear length (cm)	8.60±0.87c	5.72±1.29a	6.01±1.11b	5.91±1.52ab
Main stem thickness (mm)	5.78±0.62c	3.81±0.84a	4.26±0.64b	3.81±0.93a
Seeds/main ear	49.55±9.96c	22.12±9.18a	29.05±8.83b	26.43±11.80b
Seeds/plant	93.80±39.49c	22.45±9.75a	35.76±19.91b	32.11±12.09b
Main ear seed weight (g)	2.02±0.43c	0.84±0.40a	0.98±0.29b	0.98±0.43b
Aboveground dry weight (g)	3.94±0.74c	2.09±1.00a	2.26±0.68b	2.20±1.01ab
Dry leaf area/main plant	56.47±24.02c	13.62±6.18a	17.06±8.39b	12.51±6.21a

Values in columns are means ± standard deviation (n=225; except for dry leaf area n=30)

Letters indicate a significant difference among the treatments; p≤0.05

Table 8: Average values for measured characteristics in SST806 with four treatments over two years

	Control	Low N	Low P	Low NP
Tillers	2.57±0.87c	1.01±0.11a	1.32±0.54b	1.05±0.22a
Plant height (cm)	60.09±6.61c	57.55±7.07a	57.77±6.75a	58.68±9.58b
Main ear length (cm)	8.81±0.97d	5.52±1.41a	6.32±1.26c	5.96±1.56b
Main stem thickness/plant (mm)	5.47±0.68d	3.58±0.79a	4.21±0.65c	3.70±0.96b
Seeds/main ear	50.85±13.37c	25.05±12.05a	33.13±10.86b	27.78±13.44a
Seeds/plant	86.91±39.56c	25.21±12.31a	37.93±18.01b	28.38±15.19a
Main ear seed weight (g)	2.02±0.50c	0.90±0.61a	1.03±0.35b	0.91±0.47a
Aboveground dry weight (g)	3.98±0.83d	2.02±0.97a	2.38±0.77c	2.21±1.10b
Dry leaf area/main plant	62.08±27.96d	15.38±6.73b	17.91±7.95c	12.65±6.14a

Values in columns are means ± standard deviation (n=225; except for dry leaf area n=30)

Letters indicate a significant difference among the treatments; p≤0.05

There was also a cultivar by year interaction, showing that the trend for Zn content for the two years differed for the two cultivars. There was a treatment by year interaction for phytic acid, showing that the ranking for phytic acid for the different treatments differed for the two years. Only the low N treatment reduced Fe content significantly, and low P stress increased the concentrations of Fe and Zn. With P deficiency or limitation, roots secrete high amounts of organic acids, resulting in the mobilisation of and more intensive uptake of microelements by plants. The hyphae of arbuscular mycorrhizal fungi help to absorb more nutrients from the soil under P-deficient soil conditions.³¹ Fe and Zn were found to be negatively correlated in some studies.³²

Phytic acid, on the other hand, was less reduced under low N stress than under low P stress (Table 5), indicating that availability should increase under low P stress conditions. The highly reduced phytic acid concentration under low P stress was probably due to the fact that most phosphorus in the plant is in the form of phytate^{19,33}, meaning that reduced availability of P would lead to reduced phytic acid. It has been reported that high N treatment reduced phytic acid and increased Fe content in rice.³⁴ Similarly in pearl millet, cultivars with low phytic acid had high Fe and Zn bioaccess.³² This finding is in contrast with that of

our study, however, where the low P treatment reduced phytic acid and enhanced Fe content, although it was cultivar dependent. Significant correlations between N fertilisation and Fe and Zn concentration in wheat grains has been previously reported.³⁵ Phytic acid was reduced by increasing N concentration, with the effect of varieties being significant in barley. In contrast to the current study, under low P conditions, one cultivar of barley had the highest phytic acid content.³⁶ Although phytic acid is the main reservoir of P in plants, it possesses anti-nutritional characteristics¹⁷ which are associated with decreased bioavailability of Fe and Zn^{18,19}. Therefore, the current results indicate that in areas experiencing low P stress, Fe and Zn will be more available, which can combat malnutrition and improve human health. This statement is further supported by the lowest phytic acid:Fe and phytic acid:Zn molar ratios under low P stress (Table 5), emphasising higher bioaccessibility of Fe and Zn under low P stress. In agreement, more Zn was available under such low P supply.³² It has been reported that 55% of Zn was absorbed when the phytic acid:Zn ratio was less than 5, while 35% of Zn was absorbed when the ratio was between 5 and 15, and only 15% was absorbed when the ratio was higher than 15.¹⁶ Low N stress benefitted cultivars selectively because it led to an increase in Zn content for SST806 only (Figure 2), showing that cultivars responded differently



under this treatment compared with under low P stress, which increased Zn content for both cultivars. For all nutritional traits, under combined low N and P stress, it appears that low N effect dominated effects of low P.

PAN3497 and SST806 were not significantly different for the measured phenotypic traits, irrespective of the treatment, which is in agreement with the nutritional quality data. Reduced tillering caused by these treatments, especially under low N stress, shows that the earlier a tiller is formed, the more kernels it will produce.³⁷ Reduction of other parameters, such as plant height and dry mass, under N and P deficiencies substantiates the fact that they correlate with grain yield positively.³⁸ As expected, low N, P and a combination of low N with P reduced the phenotypic performance of both cultivars substantially because both macronutrients are essential for vegetative and reproductive growth.⁷ Similar to nutritional quality results, the effect of low N masked that of low P and low N and low P combined stress, even though low P alone caused the lowest reductions in phenotypic parameters, pointing out the importance of N in these cultivars.

To conclude, our study has revealed that the cultivars were not significantly different for most nutritional and phenotypic traits under various treatments. What is interesting is that, under low P stress, micronutrients (Fe, Zn) increased to levels higher than those of the control while phytic acid was substantially reduced, leading to higher bioavailability of Fe and Zn in these cultivars. Although growing wheat under low P will reduce phenotypic traits and yield, there may be an unexpected benefit for combatting Zn and Fe deficiencies in the areas where the soil is deficient in these macronutrients.

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Competing interests

We declare that there are no competing interests.

Authors' contributions

B.T. and M.J.M. performed the experiments and collected data. M.L. supervised the experiment. M.L. and L.S. completed the data statistical analysis. B.T., M.J.M. and M.L. wrote the manuscript. All authors read and approved the final manuscript.

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