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ORIGINAL PAPER

QUANTITATIVE CHANGES IN VARIOUS NUTRIENT RATIOS IN CULTIVATED PLANTS IN RELATION TO FERTILISATION*

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Abstract

The quality of plants can be assessed according to the basic quantitative nutrient ratios. Moreover, proportions and relationships between macronutrients in plant biomass can be an indicator of the composition of plants and nutrient limitation. The research deals with the influence of soil amendment with mineral, organic and mineral-organic fertilisation on quantitative changes in nutrient ratios determined for camelina, white mustard and spring barley cultivated on light soils in subsequent years. A 3-year pot experiment was conducted with two doses equivalent to 70 kg N ha^{-1} (I) and to 170 kg N ha^{-1} (II). Plant material was subjected to chemical analyses in order to assess the macronutrient content, after which mutual proportions of individual nutrients were calculated based on the acquired data. Changes in ratio values were visualised using statistical tools, i.e. multivariate analysis of variance MANOVA and standard analysis of variance ANOVA. Regardless of the applied fertilisation, white mustard showed the highest values of most calculated nutrient ratios, although the values of nutrient ratios were consistent with those given in the literature as adequate only for spring barley. Generally, the fertilisers applied in a dose equivalent to 170 kg N ha^{-1} resulted in higher values of nutrient ratios, which was observed especially in the case of NPK. The study clearly demonstrated that the plant species diversity as well as fertilisation play a crucial role in quantitative changes of nutrient ratios. It is worth stressing that both organic fertiliser and mineral-organic fertiliser similarly affected nutrient ratio values.

Keywords: fertilisation, plants, macroelements, quantitative ratios of nutrients, ANOVA.

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INTRODUCTION

The world population is increasing steadily and at present it is projected to reach over 9 billion by the year 2030 (Perea-Moreno et al. 2019). This is closely connected with the increase in available food supplies. However, food production needs to ensure high quality of products and must not be achieved at the expense of environmental degradation. Increasing crop yields in the 21st century has become an essential requirement for modern science and should meet the principles of sustainable agriculture. According to Velten et al. (2015), sustainable agriculture must produce adequate amounts of high-quality food, protect resources and be both environmentally safe and profitable. To fulfill these challenges, various agroecological practices have been developed, including the rational use of fertilisers, especially mineral ones. Karmakar et al. (2016), in a review article on the subject, pointed out that synthetic nitrogen fertilisers are a major cause of the decline of soil carbon. It is an unfavourable situation because reduction of soil carbon plays a considerable function in land degradation and unfavourable climate change. In this aspect, special attention is focused on organic amendments. Organic fertilisation is a method for substituting inorganic fertilisers and improving general soil fertility because organic fertilisers are acknowledged not only as a source of nutrients, but also as an essential pool of organic matter. The importance of nutrients and organic matter introduced with fertilisers should also be considered in the context of the impact on biochemical cycles of C as well as N and P, which are necessary nutrients for plants. Properly progressing cycles of elements in the soil affect their mutual quantitative balance, which in turn can change the quantitative nutrient ratios in plants. Alvares et al. (2018) stated that human activity has altered the global N and P cycles, which is particularly noticeable in the case of land used for agricultural purposes. Therefore, it is reasonable to analyse the impact of agricultural human activity, manifested by the application of various fertilisers on quantitative changes in nutrient ratios in plants, which has an indirect effect of biochemical cycles of both the elements introduced together with fertilisers and those naturally found in soil.

The main macronutrients such as nitrogen, phosphorous, potassium, magnesium and calcium are vital elements for metabolism and various physiological functions, that is processes determining plant development. However, plants have diverse nutrient requirements and for normal growth they need both specific amounts of nutrients and their proper balance. Crops are cultivated for many purposes and uses, such as industry, energy generation, food production, animal fodder or manure. In each case, the quality of yield is the most significant. The evaluation criteria as well as specific threshold nutrient concentrations in biomass are dependent on the intended final use of crops. Regardless of the above, both the nutrient content and quantitative ratios should be taken into consideration because they are of great

importance. In agricultural practice, nutrient concentrations are obligatorily determined in plant tissues and the quality of yield is evaluated accordingly (Dada et al. 2014, Bindraban et al. 2015, Agegnehu et al. 2016). Occasionally, quantitative ratios are assessed. Current studies on broadly understood plant nutrition and soil fertilisation in terms of nutrient ratios in plant tissues mainly focus on ecological stoichiometry. Many scientific papers concern the C:N:P ratio (Chen et al. 2016, Wu et al. 2017, Zeng et al. 2017), with less emphasis placed on the ratios of N:P, K:Mg, K:Na, Ca:P, Ca:Mg, K:(Ca+Mg) or (K+Na):(Ca+Mg). According to Chen et al. (2016), ecological stoichiometry is an important tool in studying nutrient cycling and a useful method to indicate limitation to plant growth, thus it is necessary to extend the evaluated range of nutrient ratios. It needs to be underlined that various quantitative ratios in plant biomass may be better indices of nutrient deficiency or their effective utilisation from applied fertlisers *per se*. However, balanced proportions between nutrients are important to ensure proper plant development and yield quality. The latter aspect is extremely significant in animal and human consumption. In general, it can be assumed that the plant biomass nutrient ratios may provide a better index of macronutrient deficiency than their concentrations.

As mentioned above, the effect of organic or mineral fertilisation on quantity and quality of crop yield expressed as nutrient concentrations in plant biomass is well documented. It is far more difficult to confirm the direct influence of various fertilisation variants on plant chemical composition expressed in quantitative nutrient ratios. In view of the above, the aim of this study was to evaluate the influence of different fertilisers applied in two doses on changes in values of quantitative nutrient ratios calculated for 3 subsequently cultivated plants. In addition, the multiplicity of ratios used has provided material for an in-depth analysis, including the interrelationships between individual nutrient ratios.

MATERIAL AND METHODS

Experimental design

A 3-year (2015-2017) pot experiment was conducted at the experimental station of the Poznan University of Life Sciences. The experimental facility is roofed over with wire mesh and thus ensures outdoor, natural conditions. In the experiment, light soil (loamy sand texture) classified as a *Haplic Luvisol* according to IUSS Working Group WRB (2014) was used. Samples were collected from a depth of 0 - 30 cm from an agricultural field. The soils and fertilisers were air-dried. Three different types of fertilization were used:

1. Organic fertilisers consisted of commercially available compost (OF), prepared by the aerated-pile method from a mixture of cow manure, green plant residues, household waste and wheat straw.

2. Mineral - organic fertiliser (M-OF) was produced as a mixture of chicken manure, lignite, single superphosphate and potassium chloride.

3. Mineral fertilisers (NPK) were composed of ammonium nitrate (34% N), potassium chloride (60% K_2O) and single superphosphates (18% P_2O_5).

The basic chemical composition of the soils and fertilisers is presented in Table 1.

Table 1

Basic chemical composition of fertilisers and soil used in the experiment (g kg^{-1})

Property	Soil	Organic fertiliser	Mineral-organic fertiliser
pH	5.5	7.2	6.5
N_{tot}	1.3	10.4	22.0
C_{tot}	12.6	400.0	526.0
P*	0.28	2.63	30.2
K*	0.16	2.68	24.1
Mg*	0.022	4.59	8.90

* The values of nutrients presented for organic fertliser and mineral-organic fertiliser are total content and the ones for soils are available nutrients.

The experiment was conducted in PVC pots (volume 5 kg) and was set up in a randomised, factorial design with soil and the above amendments. The fertilisers were applied in two doses equivalent to 70 kg N ha^{-1} (I) and to 170 kg N ha^{-1} (II). The doses were calculated according to the N content in organic and mineral-organic fertilisers (Table 1) as well as the applied ammonium nitrate. The amounts of phosphorus and potassium were supplemented to the same level at each treatment in accordance with the nutritional requirements of cultivated plants. The experiment included the following treatments: T0 – soil control without fertilisation, T1 – soil with NPK I, T2 – with soil with NPK II, T3 – soil with OF I, T4 – soil with OF II, T5 – soil with M-OF I, T6 – soil with M-OF II.

Samples of 5 kg of dried soil were weighed in triplicate and mixed thoroughly with the doses of the fertilisers two weeks before plant cultivation. All treatments were made in three replicates. Each mixture was watered to 60% field capacity. The fertilisers were incorporated before planting each crop in the crop rotation cycle. Plants were watered daily with tap water, or as needed to maintain moisture level. Three crops, camelina (*Camelina sativa* L.), white mustard (*Sinapis alba* L.) and spring barley (*Hordeum vulgare* L.), were cultivated in the subsequent years of the experiment and were used as the test plants. The experiment was conducted at density of 10 plants. The plant growing period of the crops was typical for Polish conditions. The aerial plant matter was harvested at the onset of the flowering stage. After harvest, plant roots were thoroughly removed from the soil so as to cleanse the soil before the next fertilising treatment and sowing the seeds of another plant.

Analysis of plant materials

Plant material was dried at 60°C, ground and ashed in a furnace at 450°C for 6 h. The ash was dissolved in 5 mL of 6 mol dm^{-3} HCl (Ostrowska et al. 1991) and diluted to a constants volume with distilled water. The extracts were submitted to the determination of the K, Ca, Mg, Na content using atomic absorption spectrophotometry (AAS) in a Varian Spectra AA 220 FS apparatus. In the case of total nitrogen and phosphorus, plant samples were analysed separately, and different methods were applied. Total phosphorus (Ptot) content was measured colorimetrically by the vanadate--molybdate method, while total nitrogen (Ntot) was analysed by the Kjeldahl method. All the determinations of amounts of nutrients in the tested samples were performed in three replications. Based on the results, the following weight nutrient ratios were calculated: N:P, K:Mg, K:Na, Ca:P, Ca:Mg, K:(Ca+Mg) and (K+Na):(Ca+Mg). The mean content of nutrients in cultivated plants is presented in Table 2. The above nutrient ratios were selected

Table 2

Mean contents of nutrients in cultivated plants (g kg^{-1})

Treatment	Nutrients					
	N	P	K	Mg	Ca	Na
	Camelina					
T0	14.05	3.32	9.46	2.10	10.01	2.79
T1	14.86	3.27	11.28	2.11	16.11	2.75
T2	15.28	3.22	14.01	2.21	16.92	3.15
T3	27.02	3.11	7.83	1.93	13.96	2.82
T4	29.82	3.48	9.01	1.93	12.67	2.76
T5	26.87	3.07	8.12	1.71	7.30	3.14
T6	28.28	3.40	15.08	1.96	9.69	3.89
	White mustard					
T0	12.00	3.75	12.59	1.63	6.17	1.97
T1	14.39	3.72	16.27	1.61	9.39	1.83
T2	17.01	3.78	20.80	1.78	10.94	1.97
T3	16.73	3.97	14.29	1.65	5.91	2.38
T4	17.44	5.11	16.03	1.83	12.02	2.24
T5	15.73	3.49	13.51	1.71	7.59	1.83
T6	18.29	5.14	18.82	1.80	12.18	2.22
	Spring barley					
T0	18.76	3.91	9.29	1.65	3.94	1.57
T1	16.11	4.82	10.66	2.17	4.62	1.75
T2	17.80	5.30	15.31	2.31	4.94	2.35
T3	18.19	3.31	11.61	1.90	4.14	1.38
T4	15.32	4.86	14.92	2.68	5.05	1.77
T5	17.00	4.10	10.78	2.21	4.09	1.40
T6	15.00	5.29	14.41	2.68	4.71	1.93

for the research because of the literature reports where these ratios are considered to be useful parameters in the assessment of crop quality, and some are mandatory in routine chemical tests for agricultural purposes.

Statistical analysis

A multivariate analysis of variance MANOVA was carried out in order to determine whether the ratios of the nutrients taken together varied between the crop species (factor I) and whether they were influenced by different fertilisation variants (factor II). In addition, a two-way analysis of variance ANOVA with interactions was performed independently for each ratio (plant – factor I, fertilisation – factor II). In order to receive homogeneous groups for both factors: plants and fertilisation, a *post hoc* analysis, i.e. the Tukey's HSD test, was carried out. Pairwise comparisons of plants (and fertilisation) are illustrated in graphs with 95% confidence intervals for differences of averages in the values of elemental ratios. Let μ_i and μ_j denote expected values (means) for the *i*-th and *j*-th objects (e.g. the compared *i*-th and *j*-th plants or compared *i*-th and *j*-th fertilisation variants). Then, with 95% confidence it may be stated that the established confidence interval covers an unknown difference in mean values μ_i-μ_j of the two investigated objects. If the difference of μ_i-μ_j is a zero, then the investigated objects do not differ. Thus, when the confidence interval established for the difference of μ_i-μ_j includes a zero, we assume that the investigated objects do not differ significantly. Lengths of the confidence intervals presented in the graphs show the volume of common variance, i.e. variation within the investigated sample. The Principal Component Analysis (PCA) was applied to determine the interrelationships between the values of proportions of nutrients, and to characterise the effect (or its lack) exerted by some of the ratios on the others. Detailed statistical analysis was conducted with the support of RStudio software (R version 3.4.0) R Core Team (2017). The PCA package used is based on a correlation matrix.

RESULTS

Results of the multivariate analysis of variance MANOVA indicate that both the plant factor and the fertilisation factor had a highly significant effect on values of all observed nutrients jointly (Table 3). The interaction between the experimental factors also had a significant effect on changes in the investigated nutrient values. Results of independent analyses indicate that values of individual ratios were significantly dependent on the plant species, being different for each of the crops (Table 4). Also, the effect of every fertilisation variant statistically significantly modified values of the discussed nutrient ratios. The interaction between the factors was not significant only for the K:Mg ratio (Table 4). Quantitative changes in the

Table 3

Results of multivariate analysis of variance MANOVA for the ratios

Sources	Df	Wilks statistic	Approx *F* value	*p*-value
Plant	2	0.013	87.009	< 2.2e-16
Fertilisation	6	0.038	8.861	< 2.2e-16
Plant × fertilisation	12	0.010	6.528	< 2.2e-16
Residuals	84			

Df – degrees of freedom

Table 4

Values of *F* statistics from two-factorial analyses of variance with interaction for ratio (results of 7 separate analyses of variance)

Sources	Df	N:P	K:Mg	K:Na	Ca:P	Ca:Mg	K:(Ca+Mg)	(K+Na):(Ca+Mg)
Plant	2	265.74***	49.63***	89.65***	254.35***	97.04***	142.26***	107.39***
Fertilisation	6	28.88***	5.71***	6.28***	16.26***	6.12***	7.36***	6.67***
Interaction	12	24.96***	1.50	6.08***	9.55***	3.44***	6.57***	7.12***

Significance: *** 0.001, ** 0.01, * 0.05, Df – degrees of freedom

means of nutrient ratios resulting from the interaction of these factors are presented in a graph (Figure 1). The lines representing the type of applied fertilisation (T0-T6) run parallel, with no intersection, thus definitely showing a lack of interaction of the factors and no effect on values of a given nutrient ratio in relation to individual plant species for the K:Mg ratio. An opposite situation is observed in the case of the other nutrient ratios. For each of the other six ratios the lines on the graphs are not parallel, that is they intersect. This shows the significance of interactions between the factors (plant×fertilisation) in relation to the modification of quantitative changes in values of nutrient ratios. In this context, we need to stress the significant role of mineral fertilisation with a high dose of nitrogen (T2) in obtaining higher average values of the K:Na ratios (10.89) in mustard, Ca:Mg (7.42) and Ca:P (5.04) in camelina as well as K:(Ca+Mg) (2.12) and (K+Na):(Ca+Mg) (2.45) in barley. At the same time, organic fertilisation with the highest dose of nitrogen (T6) applied to barley resulted in the lowest average values of N:P (2.93), Ca:P (0.91) and Ca:Mg (1.78). Camelina was also characterised by the lowest mean values of the ratio K:Na (2.62) at organic fertilisation with a low N dose (T5), while the ratios K:(Ca+Mg) (0.79) and (K+Na):(Ca+Mg) (1.02) were the lowest in the treatment with no fertilisation (T0) – Figure 1.

Data in Table 5 (with results of comparisons applying the Tukey's test) give averaged values of ratios for individual crop species, irrespective of the applied fertilisation variant. Cultivated plants differed significantly, forming separate groups "*a*", "*b*" and "*c*", in terms of the K:Mg, Ca:P, K:(Ca+Mg) and (K+Na):(Ca+Mg) ratios. Generally, the highest mean values of K:Mg (9.68)

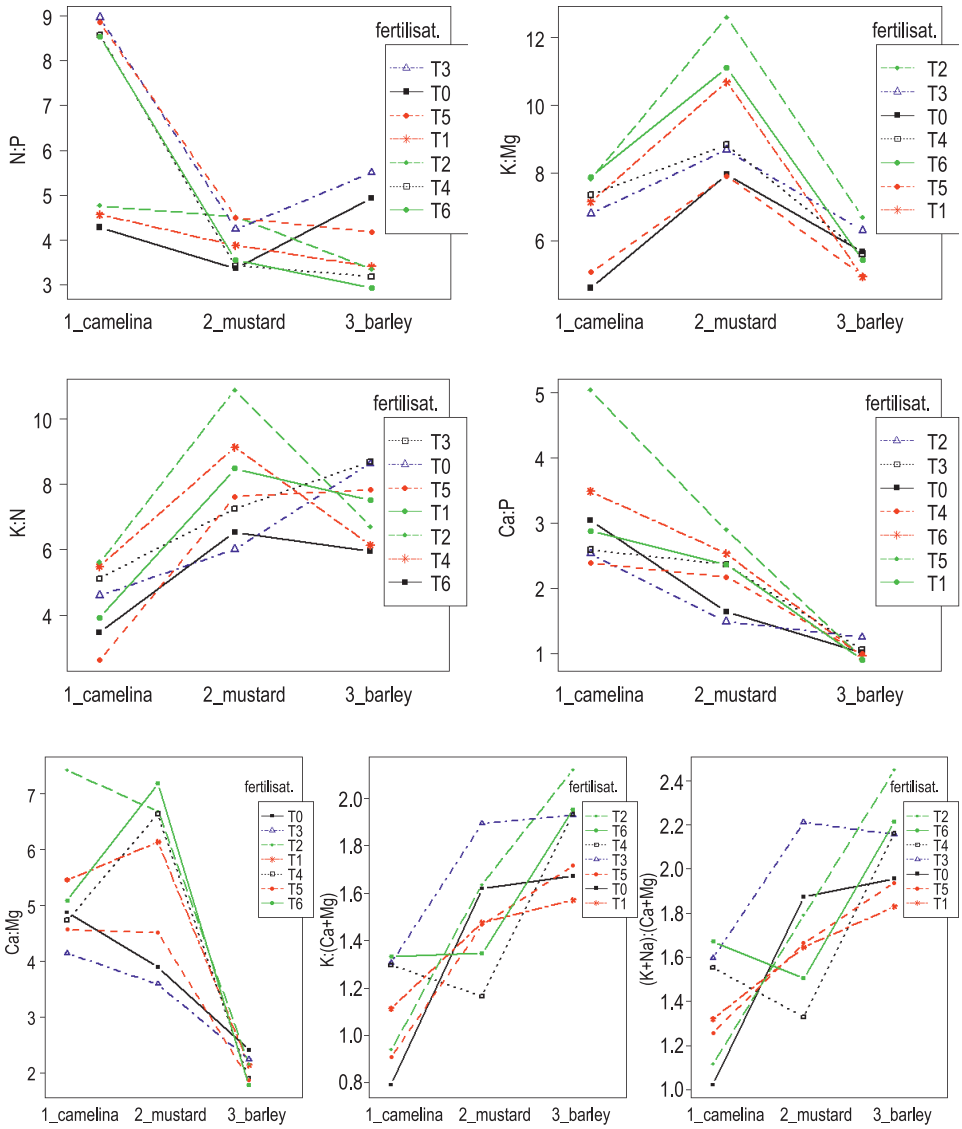


Fig. 1. The average values of 7 ratios, for 2 experimental factors: factor 1 – plants (camelina, mustard, barley) and factor 2 – fertilisation types (T0 – T6). The lines represent changes in average values depending on factors

and K:Na (7.99) were recorded in mustard. In turn, the highest mean values of ratios in camelina and barley were found for N:P (6.94) and Ca:P (3.14) as well as K:(Ca+Mg) (1.84) and (K+Na):(Ca+Mg) (2.10). Moreover, barley was another crop, next to camelina, in which low average values of nutrient ratios were recorded most frequently.

Table 5

Average values of the ratios in the plants, irrespective of fertilisation

N:P			K:Mg			K:Na			Ca:P		
Plant	mean	group	plant	mean	group	plant	mean	group	plant	mean	group
Camelina	6.94	*a*	mustard	9.68	*a*	mustard	7.99	*a*	camelina	3.14	*a*
Mustard	3.93	*b*	camelina	6.67	*b*	barley	7.35	*a*	mustard	2.21	*b*
Barley	3.93	*b*	barley	5.66	*c*	camelina	4.40	*b*	barley	1.02	*c*

Ca:Mg			K:(Ca+Mg)			(K+Na):(Ca+Mg)		
Plant	mean	group	plant	mean	group	plant	mean	group
Mustard	5.52	*a*	barley	1.84	*a*	barley	2.10	*a*
Camelina	5.18	*a*	mustard	1.52	*b*	mustard	1.72	*b*
Barley	2.07	*b*	camelina	1.10	*c*	camelina	1.36	*c*

Homogeneous groups for plants – results of HSD Tukey's test

In the next step of the statistical analysis, differences between plants were tested with respect to average (mean) values of the ratio tested. The study was conducted in pairs, testing the significance of mean differences (mustard-camelina, mustard-barley, camelina-barley). A confidence interval was determined for each pair of plants. The results show that the difference between average values of N:P calculated for mustard and barley is not significant (the determined intervals include the zero value). An opposite situation may be observed when assessing the significance of differences between mean N:P values recorded for pairs of crop species: camelina and barley, and mustard and camelina. When interpreting the other results, we need to stress a lack of significant differences between K:Na values calculated for mustard and barley and between Ca:Mg values for mustard and camelina. Analogous analyses were conducted in order to identify homogeneous groups for the applied fertilisation variants irrespective of the plant species (Table 6). As can be seen, mineral fertilisation with a high nitrogen dose (T2) most frequently resulted in the highest average values of such ratios as K:Mg (9.04), K:Na (7.74), Ca:P (2.96) and Ca:Mg (5.42). In turn, organic fertilisation with a low nitrogen dose (T3) resulted in the highest average values of N:P (6.24), K:(Ca+Mg) (1.71) or (K+Na):(Ca+Mg) (1.99). We need to stress here that values of all the analysed nutrient ratios were comparable and did not differ statistically in the case of organic and organic--mineral fertilisers with high nitrogen doses (T4, T6) (Table 6). Also, organic and organic-mineral fertilisers with low nitrogen doses (T3 and T5) similarly affected modifications in average values of the following nutrient ratios: N:P, K:Mg, K:Na, Ca:P or Ca:Mg, as confirmed statistically. In many cases, a lack of fertilisation (T0), or organic (T3) or mineral fertilisation with a low N dose (T1) resulted in low mean values of all investigated nutrient ratios (except for the ratio K: Mg) – Table 6.

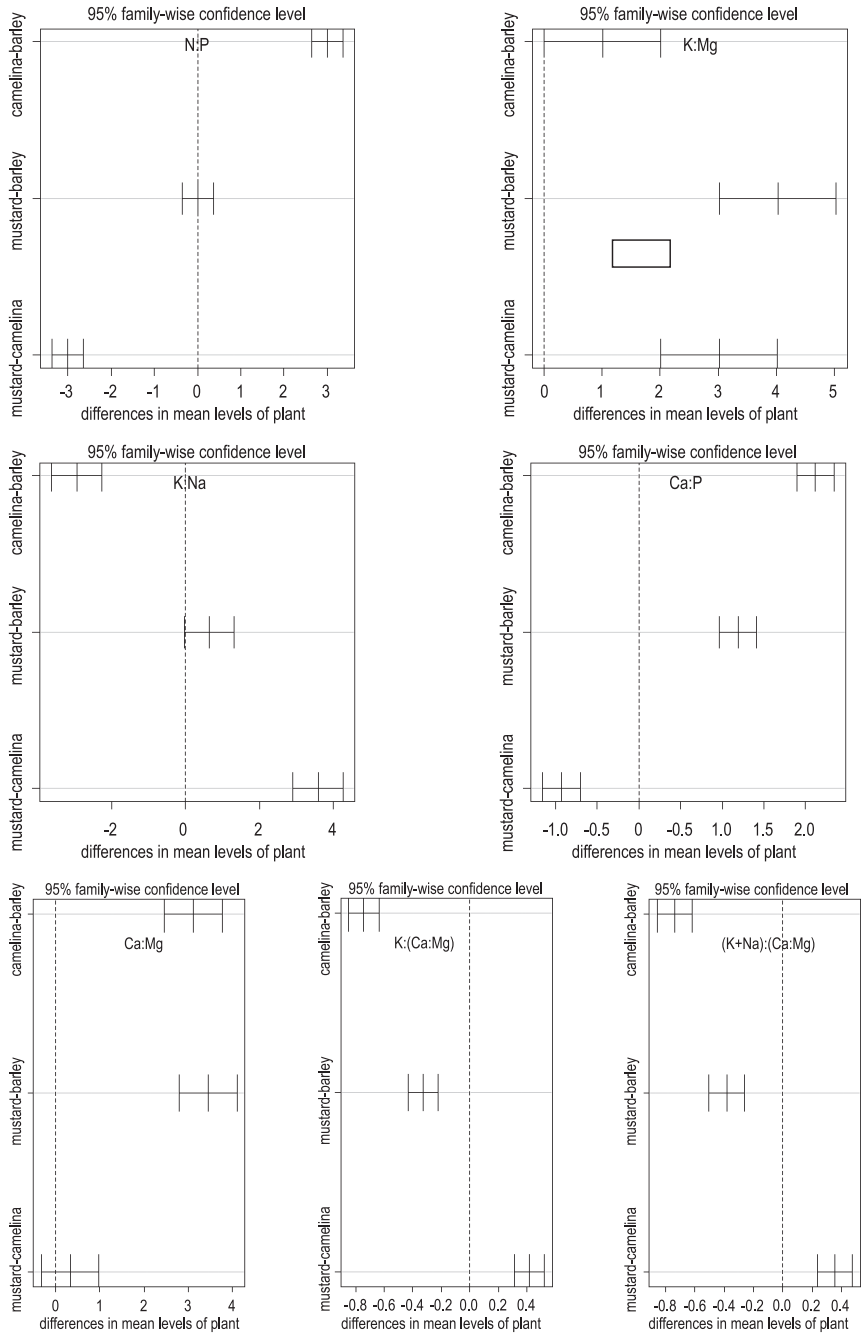


Fig. 2. Confidence intervals for differences in mean levels of values of the ratios for plants. Confidence intervals do not contain zero – means for plants are significantly different, confidence intervals contain zero – the plants do not differ in terms of the mean values of the ratio tested. The zero value is marked on the graph with dashed lines

Table 6

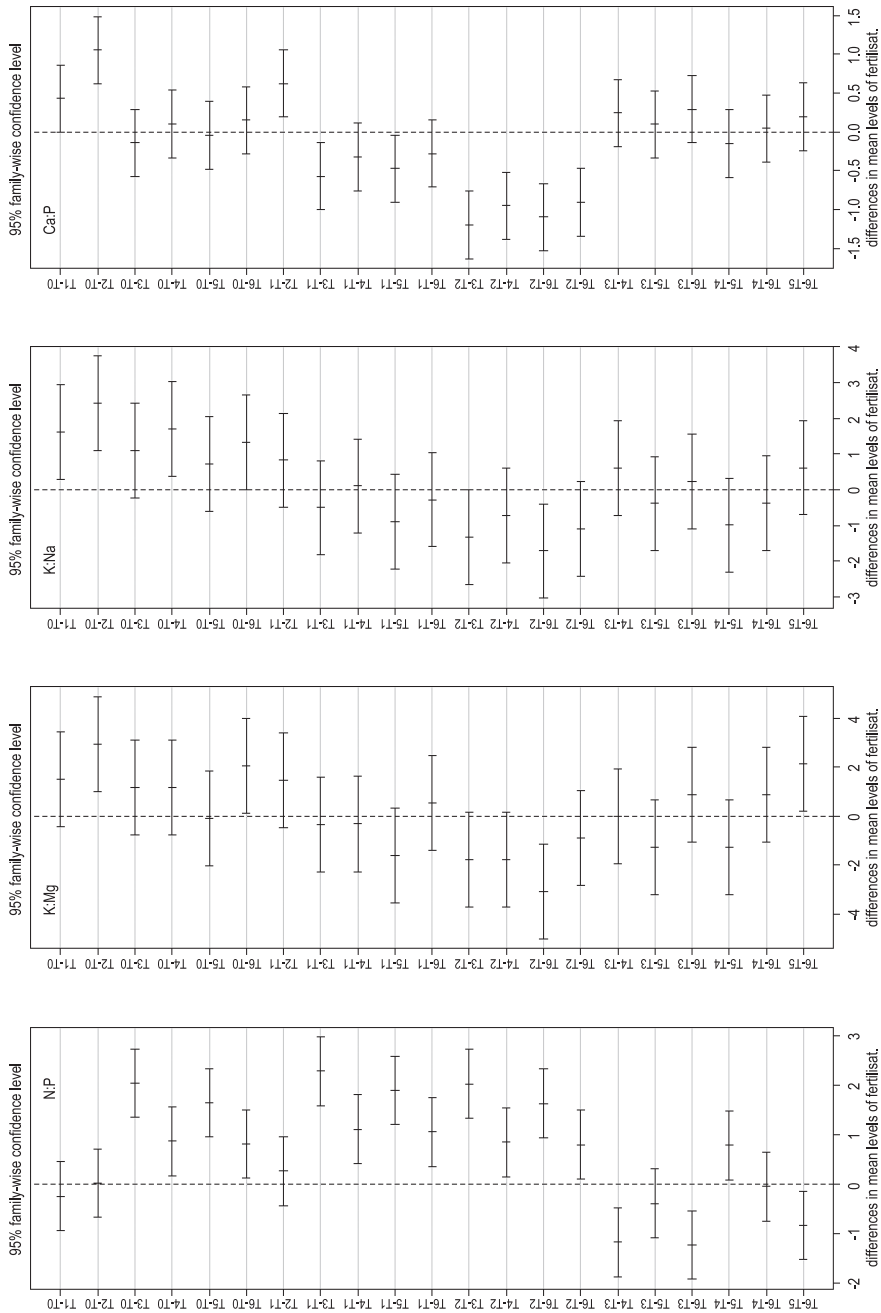
Average values of the ratios in the plants and homogeneous groups for particular fertilisation treatment (averaged for types of fertilisation applied)

N:P			K:Mg			K:Na			Ca:P		
Fertilisation	mean	group	fertilisation	mean	group	fertilisation	mean	group	fertilisation	mean	group
T3	6.24	*a*	T2	9.04	*a*	T2	7.74	*a*	T2	2.96	*a*
T5	5.85	*a*	T6	8.14	*a*	T4	7.02	*ab*	T1	2.33	*b*
T4	5.06	*b*	T1	7.58	*ab*	T1	6.92	*ab*	T6	2.05	*bc*
T6	5.01	*b*	T4	7.26	*ab*	T6	6.64	*ab*	T4	2.01	*bc*
T2	4.21	*c*	T3	7.26	*ab*	T3	6.41	*bc*	T0	1.90	*bc*
T0	4.20	*c*	T0	6.08	*b*	T5	6.03	*bc*	T5	1.86	*c*
T1	3.95	*c*	T5	5.98	*b*	T0	5.32	*c*	T3	1.76	*c*

Ca:Mg			K:(Ca+Mg)			(K+Na):(Ca+Mg)		
Fertilisation	mean	group	fertilisation	mean	group	fertilisation	mean	group
T2	5.42	*a*	T3	1.71	*a*	T3	1.99	*a*
T6	4.69	*ab*	T2	1.57	*ab*	T6	1.80	*ab*
T1	4.57	*abc*	T6	1.54	*abc*	T2	1.79	*ab*
T4	4.42	*abc*	T4	1.47	*bc*	T4	1.68	*b*
T0	3.72	*bc*	T1	1.39	*bc*	T5	1.62	*b*
T5	3.65	*bc*	T5	1.364	*bc*	T0	1.62	*b*
T3	3.32	*c*	T0	1.361	*c*	T1	1.60	*b*

Results of analyses with Tukey's HSD test

Confidence intervals for the difference between mean values of quantitative ratios of nutrients (for the analysed fertilisation variants) are additionally presented in a graph (Figure 3). It may be concluded which fertilisation variants significantly differed in terms of nutrient values (the intervals do not contain a zero). Figure 4 presents the mutual effect of all investigated seven nutrient ratios in the system of the first two principal components: Dim1 and Dim2. Overall, the first two principal components explain almost 80% all variation. Values of all ratios show comparable variation (as indicated by comparable lengths of arrows), while the smallest variation in values was recorded for N:P. Our analysis of data presented in Figure 4 confirms the existence of relationships between individual ratios. An inversely proportional dependence was shown for N:P in relation to K:Na (the value of the correlation coefficient r=–0.52), which indicates an increase in the N:P value at a simultaneous decrease in K:Na or vice versa. At the same time, changes in values of the aforementioned ratios had no effect on variation in Ca:Mg values, as indicated by low values of correlation coefficients r=0.09 and r=0.06. In contrast, a strong correlation confirmed statistically (r=0.99)


95% family-wise confidence level
Ca:P
differences in mean levels of fertilisat.
-1.5 -1.0 -0.5 0.0 0.5 1.0 1.5
T1-T0 T2-T0 T3-T0 T4-T0 T5-T0 T6-T0 T2-T1 T3-T1 T4-T1 T5-T1 T6-T1 T3-T2 T4-T2 T6-T2 T6-T2 T4-T3 T5-T3 T6-T3 T5-T4 T6-T4 T6-T5
95% family-wise confidence level
K:Na
differences in mean levels of fertilisat.
-3 -2 -1 0 1 2 3 4
T1-T0 T2-T0 T3-T0 T4-T0 T5-T0 T6-T0 T2-T1 T3-T1 T4-T1 T5-T1 T6-T1 T3-T2 T4-T2 T6-T2 T6-T2 T4-T3 T5-T3 T6-T3 T5-T4 T6-T4 T6-T5
95% family-wise confidence level
K:Mg
differences in mean levels of fertilisat.
-4 -2 0 2 4
T1-T0 T2-T0 T3-T0 T4-T0 T5-T0 T6-T0 T2-T1 T3-T1 T4-T1 T5-T1 T6-T1 T3-T2 T4-T2 T6-T2 T6-T2 T4-T3 T5-T3 T6-T3 T5-T4 T6-T4 T6-T5
95% family-wise confidence level
N:P
differences in mean levels of fertilisat.
-2 -1 0 1 2 3
T1-T0 T2-T0 T3-T0 T4-T0 T5-T0 T6-T0 T2-T1 T3-T1 T4-T1 T5-T1 T6-T1 T3-T2 T4-T2 T6-T2 T6-T2 T4-T3 T5-T3 T6-T3 T5-T4 T6-T4 T6-T5

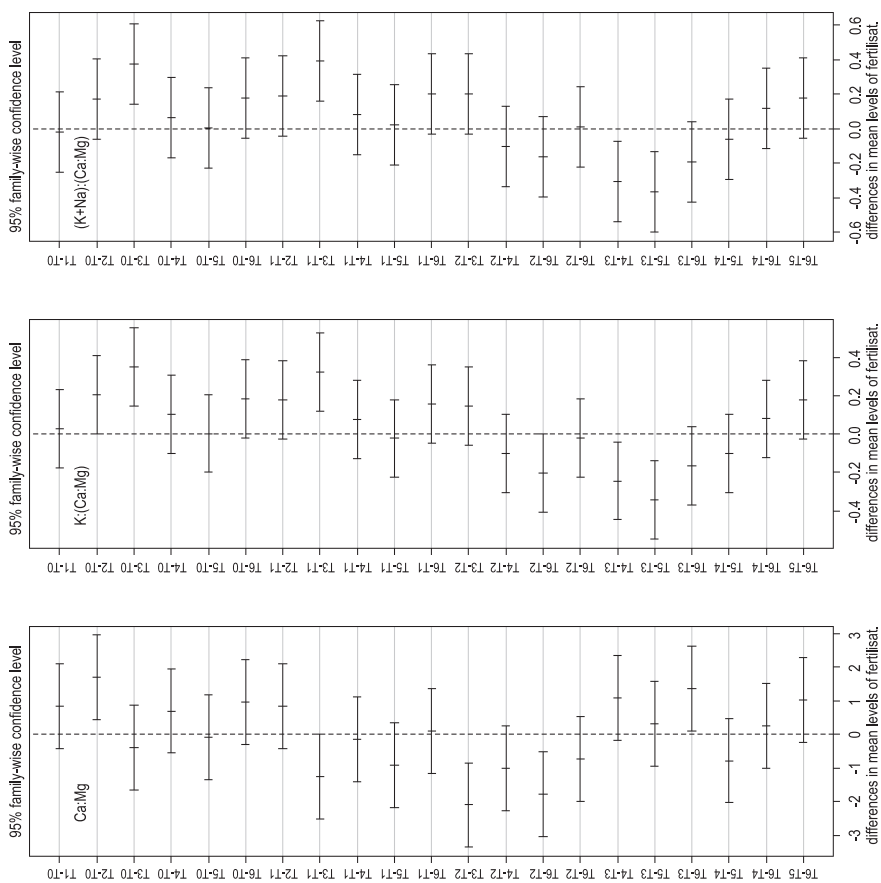


Fig. 3. Confidence levels for differences in mean levels of values of the ratios for fertilisation types. Confidence intervals do not contain zero – means for fertilisations are significantly different

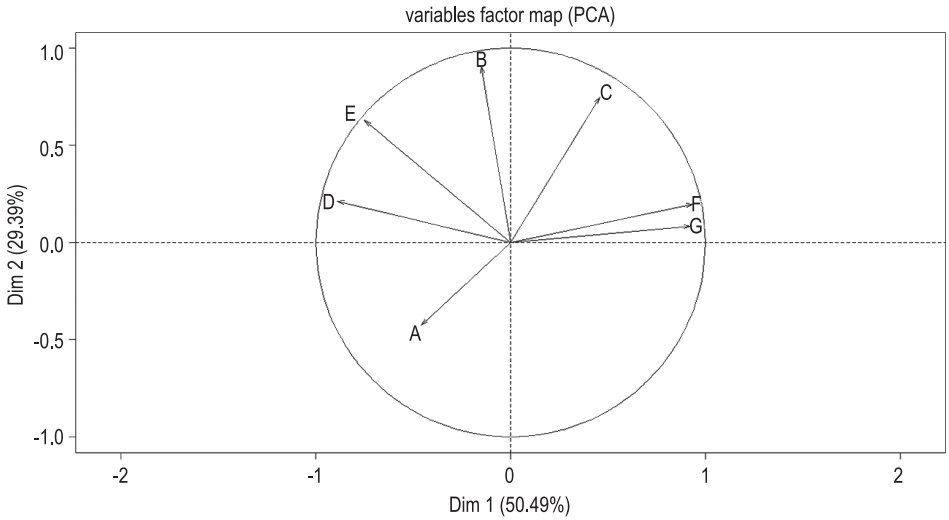


Fig. 4. Correlation plot of the ratios in system of first two principal components: first component – Dim1, second component – Dim2: A – N:P, B – K:Mg, C – K:Na, D – Ca:P, E – Ca:Mg, F – K:(Ca+Mg), G – (K+Na):(Ca+Mg).

The first principal component explains about 50.5% of the total variation, and the second principal component an additional 29.4% (the first two principal components explain nearly 80% of the total variance)

is observed in the case of K:(Ca+Mg) and (K+Na):(Ca+Mg). Also, Ca:Mg values were correlated with Ca:P (r=0.77) and K:Mg (r=0.73).

DISCUSSION

Plant stress can be caused not only by the deficiency of a single nutrient, but also by inadequate relationship between nutrients. In field conditions, for large populations of crops, a diagnosis of plant nutritional status could be based on the methods such as DRIS – Diagnosis and Recommendation Integrated System (Mercik et al. 1993). However, in small-scale pot experiments, the nutritional status of plants is described by the macronutrient content in plant biomass and is expressed by mutual quantitative ratios of nutrients. The literature data dedicated to the issue of nutrient ratios (expressed as ionic, molar or weight ratios) mainly deal with grassland (Filipek-Mazur, Tabak 2016, Zeng et al. 2017, Feng et al. 2019), trees (Wu et al. 2017, Li et al. 2019*a*), leguminous and herbaceous plants (Rady et al. 2016, Grzegorczyk et al. 2017) and occasionally crops (Barczak, Nowak 2013, Ostrowska, Kozera et al. 2017, Porębska 2017). The numerous plant species and varied results obtained by the above authors hinder comparisons and interpretation of data. Nevertheless, some authors (Filipek-Mazur, Tabak

2016, Grzegorczyk et al. 2017, Yan et al. 2017, Zeng et al. 2017) referred to optimal proportions of nutrients in plants biomass, whose values should be as follows: N:P = 14-16:1; K:Mg = 2-6:1; K:Na = 5:1; Ca:P = 2:1; Ca:Mg = 2-3:1; K:(Ca+Mg) = 1.62-2.2:1; (K+Na): (Ca+Mg) = 1.9-2.1:1. It is worth emphasising that the so-called optimal ratio values are treated as helpful indicators in evaluation of nutrient limitation, or the nutritional value of plants for animals and human. Generally, these values are given for biomass of tested plants.

Regarding N:P values, various ranges of this ratio are cited in the literature, e.g. Zeng et al. (2017) gave the range between 12.63 and 15.67 for grasslands, while Wu et al. (2017) determined it at 27.3 to 61.7 for trees. Slightly different thresholds of N:P values were presented by Yan et al. (2017). According to those authors, who used metadata of 3441 species, the N:P values should range between 14 to 16 or between 10 to 20. The N:P values obtained in this study and ranging from 3.9 to 6.9 were considerably lower than the above data. However, independently of different ranges of N:P thresholds given in the literature, low values of N:P might indicate either nitrogen deficiency in plant biomass or ineffective utilisation of this nutrient by cultivated plants. However, no nitrogen deficiency was observed during the growing period of plants. Additionally, the concentrations of N and P in plant tissue were adequate, which was shown in the earlier work (Jakubus, Bakinowska 2019). Thus, it is difficult to confirm this assumption with absolute certainty. It cannot be excluded that the given N: P ranges should be verified and adjusted more specifically to arable crops, taking into account their development phases. The findings obtained in this experiment correspond to young plants in the flowering stage, whereas the literature data refer to plants mainly in the maturation stage or very close to it, thus leading to some problems in their interpretation.

The present research results clearly demonstrate that the plant species diversity should be taken into consideration when interpreting changes in values of quantitative nutrient ratios. While data presented in Table 3 proved a significant effect of the experimental factors on quantitative nutrient ratios, the individual crop species played a crucial role. It is closely connected with genetic variation of plants, which results in their different nutrient requirements, nutrient uptake and finally their effective utilisation. Based on data presented in Table 5, we can conclude that the cultivated plants varied significantly, showing different values for the same nutrient ratios. An exception was found only for the N:P ratio, Ca:Mg ratio and K:Na ratio. Assuming that the cited thresholds of nutrient ratios are optimal and reflect an adequate nutritional status of plants, proper values of K:Mg, Ca:Mg, Ca:P, K:(Ca+Mg) and (K+Na):(Ca+Mg) were observed only for spring barley. Numerous authors have proved that various plant species markedly differ in the terms of their nutrient ratios (Ostrowska, Porębska 2017, Wu et al. 2017, Zeng et al. 2017, Li et al. 2019). In the present study, the lowest values of the analysed nutrient ratios were obtained either for spring

barley or camelina, while the highest values of K:Mg, K:Na, and Ca:Mg were calculated for white mustard (Table 5). These differences should be interpreted in terms of nutrient requirements of these plants and their root system structure, because white mustard develops a vigorous and extensive root system, which can explore large soil volumes, adsorb more nutrients, thus helping the plant to increase crop yield and nutrient use efficiency. In contrast, camelina and spring barley are characterised by a small and less developed root system, as a result of which the intensity of nutrient uptake by these plants is smaller. This is consistent with the results presented by Fageria et al. (2008), who stated that the shape and size of a root system influence the rate and pattern of nutrient uptake from soil. The highest values of K:(Ca+Mg) and (K+Na):(Ca+Mg) as well as K:Mg and K:Na were found for spring barley and white mustard respectively (Table 5), and this may be explained by the "luxury consumption" of potassium, where K is absorbed by plants in amounts greater than required for the optimum yield (Herencia et al. 2011). It may also be associated with large accumulation of potassium, especially in the reproductive phase (boot stage), as was proven by Rogers et al. (2019), who studied the distribution of nutrients in barley. Also, the antagonistic effect of K^+ to Ca^{2+}, Mg^{2+} and Na^+ ions should be considered in this interpretation. The above natural phenomena could cause meaningfully higher values of ratios with potassium for mustard and barley, particularly in plants cultivated on soil amended with mineral fertiliser (T2).

The effect of fertilisation on quantitative nutrient ratios has not been fully clarified because the literature data provide contradictory information. Generally, the level of nitrogen fertilisation significantly differentiates nutrient ratios and results in a reduction of the values (Ostrowska, Porębska 2017). On the other hand, Rady et al. (2016) and Kozera et al. (2017) demonstrated that higher values of K:Na were due to the mineral or mineral-organic fertilisation applied. Simultaneously, under the same experimental conditions, the values of Ca:Mg or Ca:P were too narrow (Kozera et al. 2017). Our findings indirectly confirmed the above reports. Generally, fertilisers applied according to the higher dose of N caused higher values of most analysed nutrient ratios, particularly K:Mg, K:Na, Ca:P and Ca:Mg. In this context, the crucial effect was ascribed to mineral fertiliser (T2) – Table 6. It is worth noticing that both organic fertiliser and mineral-organic fertiliser similarly affected all analysed nutrient ratio values, which was confirmed by statistical analysis. This is definitely connected with the role of the introduced organic matter in shaping the rate and direction of biogeochemical cycles of these elements, which are essential macronutrients for plants. Organic fertiliser (T3) played a significant role in the modification of quantitative nutrient ratios, as shown for N:P, K: (Ca+Mg) and (K+Na): (Ca+Mg). These findings are interesting because organic fertilisers are characterised by a slow release rate of nutrients while undergoing mineralisation process, manifested as a decrease in the organic matter content and an increase in available nutrients, previously immobilised in the organic form. For this purpose, some

authors (Mahdy 2011, Agegnehu et al. 2016, Rosenani et al. 2016, Anwar et al. 2017) indicate a positive aspect of organic fertilisers, while other researchers (Herencia et al. 2011, Dada et al. 2014, Papafilippaki et al. 2015, Rady et al. 2016) point to the negative influence of such amendments on yield responses. However, it needs to be remembered that the mineralisation process mainly depends on the soil texture, moisture regime, microbiological activity and the quantity of organic matter incorporated into soil (Järvan et al. 2017, Moretti et al. 2017, Zhang et al. 2017). Obviously, the decomposition process is more effective and quicker in light soil, where physical properties were more favourable and can accelerate mineralisation after the application of compost. Such soil was used in the present experiment, and it may be assumed that the efficiency of organic fertiliser might be comparable to that of mineral one. Nevertheless, complementary applications of organic amendments with fast nutrient release mineral fertilisers are recommended and, as confirmed by the results obtained in this experiment (Table 6), it is an interesting procedure, which in practice can give the same results in terms of the highest N:P values (5.85 and 6.24). The study conducted by Li et al. (2019b) showed that the combination of vermicompost with inorganic N and P could be used to minimise loss of N and P after inorganic fertiliser addition, thereby providing longer-lasting nutrient supply for plants. In this respect, it also needs to be stressed that the application of organic as well as mineral-organic fertiliser contributed to the values of K:Mg (5.98-8.14) and Ca:P (1.76-2.05). However, only the values of 5.98 for K:Mg as well as 2.01 and 2.05 for Ca:P were comparable with the literature data given as optimal for plant nutrition (Filipek-Mazur, Tabak 2016, Grzegorczyk et al. 2017, Feng et al. 2019,). In summary, it can be stated that the nutrient ratios might serve as one of the parameters in the evaluation of the nutritional state of plants, although some thresholds should be verified and adjusted to specific growth stages of plants.

Another interesting aspect of this experiment was the PCA performed (Figure 4). This method is very useful in the interpretation and visualisation of mutual relationships and interactions between values of quantitative nutrient ratios. The results indirectly indicate an antagonistic effect between such nutrients as N, P and K, Na. The values of K:Na decreased with an increase in N:P values. At the same time, a synergistic effect was shown for Ca and Mg. It was manifested in a simultaneous increase in values of individual nutrient ratios calculated for the above nutrients. This is partly connected with the synergistic interaction between Ca and Mg found by Matula (1992, cited after Bindraban et al. 2015) in the case of barley. The cited author stated that the observed and confirmed relationships between the nutrients reflect their biogeochemical cycles determining transformations in soil and potential uptake by plants. Moreover, Bindraban et al. (2015) and Feng et al. (2019) stated that antagonistic or synergistic interactions among nutrients may occur during uptake from the soil and can influence on values of quantitative nutrient ratios in plant tissues.

CONCLUSIONS

The results show that changes in values of quantitative nutrient ratios are influenced by both plants and fertilisation factors. It was confirmed that genetic variation of plants plays a crucial role in the interpretation of results relating to varied values of nutrient ratios. Also, the applied fertilisation had a significant influence on changes in values of nutrient ratios and, as proven in the present study, the type of fertilisation is of great importance. It was found that the higher dose of nitrogen applied with mineral fertiliser strongly determined higher values of K:Mg, K:Na, Ca:P and Ca:Mg. A similar influence of organic and mineral-organic fertilisation on values of all nutrient ratios was statistically confirmed. Independently of a fertilisation variant, adequate values of the analysed ratios (within the thresholds given in literature as proper for plant nutrition) were found only for spring barley.

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