

## Soil mineral nitrogen and the rating of $\text{CaCl}_2$ extractable nutrients

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### ABSTRACT

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It was assumed that the determination of the mineral nitrogen ( $N_{\min}$ ) content in the 0.01 mol/L  $\text{CaCl}_2$  could rely on measurements of single form  $\text{NO}_3^-$ -N,  $\text{NH}_4^+$ -N or both, and even including other extractable nutrients. This hypothesis was verified based on some primary data from 17 fields: ten with oilseed rape and seven with maize as indicator crops during three consecutive seasons in a production farm in Górzno, Poland. The contents of  $\text{NO}_3^-$ -N,  $\text{NH}_4^+$ -N, P, K, Mg and pH were measured in soil prior to the spring vegetation start and after a crop harvest (autumn). Phosphorus in spring and  $\text{NH}_4^+$ -N in autumn, were variables discriminating against the number of clusters. It was higher in cropping sequences (CSs) with maize than with oilseed rape. The reliability of  $N_{\min}$  determination and distribution between clusters in spring based only on  $\text{NO}_3^-$ -N was fully corroborated for maize CSs. In autumn, irrespective of the CS, the decisive factor in  $N_{\min}$  prediction and distribution over clusters was the  $\text{NH}_4^+$ -N pool. This study resulted in the rating of  $\text{CaCl}_2$  extractable nutrients, indicating their availability status, shortage or excess, on the background of the  $N_{\min}$  temporary rating.

**Keywords:** ammonium; cluster analysis; *Zea mays*; nitrate; *Brassica napus*; subsoil

The measurement of soil mineral nitrogen ( $N_{\min}$ ) at the beginning of winter crop regrowth or just before spring crops sowing is the standard in N recommendation for the last 40 years (Wehrmann and Scharpf 1986). The biggest advantage of this method is data on the content of inorganic N forms ( $\text{NO}_3^-$ -N;  $\text{NH}_4^+$ -N) and their distribution with soil depth. It is, however, time- and labour-consuming procedure. Two approaches may be considered leading to overcome this limitation. The first assumes a reduction of  $N_{\min}$  determination only to  $\text{NO}_3^-$ -N (Luce et al. 2011). The second one relies on the fact that the extraction power of 0.01 mol/L  $\text{CaCl}_2$  solution is closely linked to the soil solution strength. It can be used for determination of both  $N_{\min}$  and other nutrients (Van Erp et al. 1998, Balík et al. 2003). Its importance was recognized about 100 years ago. In spite of that, there is a

deep gap in knowledge about resources of easily available nutrients in the subsoil. The importance of the subsoil N for crops, for example for wheat, is well recognized (Haberle et al. 2006). Kuhlmann (1990) based on data from 34 field experiments in Germany showed that the subsoil was responsible for 34% K supply to spring wheat. The other pools of nutrients, as recently reported by Łukowiak et al. (2016) for P and Mg, are efficiently exploited by plants. All these studies are based on the response of a particular crop to a single nutrient, whereas the soil solution is composed of different nutrient pools, interacting each other (Bouldin 1989). The cropping sequence is one of the most important factors affecting N management during the main vegetative season and between seasons. The  $N_{\min}$  remaining after crop harvest undergoes numerous processes, decisive for the environment

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safety (Heumann et al. 2013). The quick and reliable procedures of the post-harvest  $N_{\min}$  pools determination are important to develop an efficient strategy of the environment protection during winter period.

The minor objective of the conducted study was to evaluate associative relationships between nutrient contents extracted in the  $CaCl_2$  solution. The key objective was to assess the impact of  $N_{\min}$  contents on the rating of  $CaCl_2$  extractable nutrients, based on data from 17 cropping sequences, dominated by winter oilseed rape or silage maize.

## MATERIAL AND METHODS

This study was carried out at the Górzno farm, located in the central-western Poland (51°74'N, 17°83'E). The farm has 400 ha of agricultural land, dominated by arable soils classified as typical Luvisols. The basic soil properties are included in Table 1. The cropping system is composed of 2–4 crops in a sequence. For purposes of this work, all the fields were divided into two cropping sequences

(CSs), with oilseed rape (OSR) and maize (SM) as main crops. All procedures, including farmyard manure and NPK application, were performed by farmers according to good agricultural practices. During the study, total annual precipitation ranged from 507 mm to 655 mm and average yearly temperature from 8.9°C to 11.1°C. In general, the risk of intensive nitrate leaching was low.

The composite soil samples were collected from each field twice a year, at the beginning of each spring season, before fertilizers application (acronym: Spring) and after crop harvest, in summer or autumn depending on the crop (Autumn). One sample represents an area of 4.0 ha, and the total number of samples was adjusted to field size (Table 1). They were taken at three depths: 0.0–0.3; 0.3–0.6, and 0.6–0.9 m. The total number of soil samples for OSR and SM fields were 918 and 720, respectively. The mineral forms of nitrogen ( $NH_4^+$ -N and  $NO_3^-$ -N), P, K, Mg and pH were determined in 0.01 mol/L  $CaCl_2$  solution (soil/solution ratio 5:1; m/v). Concentrations of  $NH_4^+$ -N,  $NO_3^-$ -N and P were determined by colorimetric

Table 1. Field characteristics and cropping sequence

Field	Size (ha)	Soil texture <sup>1</sup>	Soil ( $C_{org}$ g/kg)	Cropping sequence (2004–2008)	Acronym
1	15	LS	0.79	W <sup>2</sup> - <b>R-R-OSR-W</b>	OSR-CS
2	7	SL	1.03	<b>OSR-W-R-OSR-W</b>	
3	17	S	0.89	<b>OSR-W-OSR-W-OSR</b>	
4	15	S	0.66	<b>OSR-W-OSR-W-OSR</b>	
5	14	S/LS	0.57	<b>OSR-W-OSR-W-OSR</b>	
6	10	S	0.68	<b>OSR-W-OSR-W-OSR</b>	
7	10	S	0.61	<b>OSR-W-OSR-W-OSR</b>	
8	41	LS	0.82	<b>OSR-W-OSR-W-OSR</b>	
9	47	SL	0.97	<b>OSR-OSR-W-OSR-W</b>	
10	23	S/LS	0.74	<b>OSR-OSR<sup>f</sup>-W<sup>f</sup>-OSR<sup>f</sup>-W</b>	
11	55	LS	0.86	<b>ON-R<sup>f</sup>-SB-SM<sup>f</sup>-W</b>	SM-CS
12	13	LS	0.77	<b>SM-SM-W-OSR-W</b>	
13	15	LS	0.86	<b>SM-SM-W-OSR-W</b>	
14	15	LS	0.77	<b>SM-SM-SM-SB-SM</b>	
15	32	LS	0.85	<b>SM-SM-SM<sup>f</sup>-SM<sup>f</sup>-SM</b>	
16	26	S/LS	0.56	<b>SM-SM-SM-SM<sup>f</sup>-SM</b>	
17	32	LS	0.65	<b>ON-ON-ON-ON-ON</b>	

<sup>1</sup>soil texture: S – sand; LS – loamy sand; SL – sandy loam; <sup>2</sup>OSR – winter oilseed rape; SM – silage/grain maize; WW – winter wheat; WR – winter rye; SB – spring barley; ON – onion; f – farmyard manure applied. The years of full study are indicated in bold

method using flow injection analyses (FIAstar5000, FOSS Tecator AB, Höganäs, Sweden). The P concentration in the extract was measured by using the molybdenum blue method. Concentrations of cations in collected extracts were analysed by AAS (SpectrAA 250 Plus, Varian, Mulgrave, Australia). Soil nutrient content of  $N_{\min}$  (the sum of  $NH_4^+$ -N and  $NO_3^-$ -N) were expressed in mg/kg. The pH was measured by using the pH-meter CX-742 (Elmetron, Zabrze, Poland).

The relationships between variables representing soil properties were analysed by principal factor analysis (PFA), irrespective of the soil depth. Factors (PFs) whose eigenvalues were  $\geq 1$  were considered in further analysis. To find the clear structure of PF loadings, a rotation of a factor's axis was undertaken by the 'normalized Varimax' method. The soil characteristics whose PF loadings explained 50% of factor variability (loadings  $> 0.7$ ) were used for interpreting the PFs. The new set of variables (PFs) and standardized  $N_{\min}$  values ( $N_{\min St}$ ) were used in the cluster analysis (CA). The division of data into clusters was conducted by the non-hierarchical method of  $k$ -means. The Euclidean distance between centroids was applied. The significance of the difference between isolated clusters was assessed by variance analysis using the Fisher's test at the  $P = 0.05$  level. To classify the original variables (real contents of a particular nutrient and pH) and evaluate their suitability for  $N_{\min}$  prediction, the studied observations were assigned to a particular group of data, according to the results of PF analysis. In the next step, the means ( $\bar{x}$ ) and standard deviations (SD) for each group

(cluster) were computed. Thus, the nutrient ranges ( $x_1 - x_2$ ) were computed as follows:  $x_1 = \bar{x} - SD$  and  $x_2 = \bar{x} + SD$ . Statistica 12 software was used for all statistical analyses (StatSoft, Inc. 2013).

## RESULTS AND DISCUSSION

**Nutrient content.** The  $N_{\min}$  content, as the key variable, was much higher at autumn compared to the beginning of the growing season (Table 2). The effect of cropping sequence on  $N_{\min}$  variability was stronger in fields with maize compared to those with oilseed rape. The response of  $NO_3^-$ -N to the key experimental factors, as evaluated by its average content and SD, was weakly impacted by CS. In contrast, a different response pattern was observed for  $NH_4^+$ -N. In spring, its content was much lower compared to  $NO_3^-$ -N, but at the same time, the variability was much greater. In autumn, the content of  $NH_4^+$ -N almost tripled, irrespectively of the CS, compared to spring.

The content of  $CaCl_2$ -extractable phosphorus, despite constant averages, was highly variable. In the OSR-CS, the average P content did not change much during the season, whereas in soils cropped with maize, it decreased by 1/3 during the season. This study clearly indicates that SM-CSs were P exhaustive, leading to the depletion of its easily available soil resources (Łukowiak et al. 2016). The averages of K content were much higher in autumn compared to spring. At the same time, the average content of Mg was higher compared to K. The increase of its content in autumn compared to

Table 2. Statistical overview of nutrient content (mg/kg) and soil reaction (pH)

Properties	Spring				Autumn			
	OSR-CS		SM-CS		OSR-CS		SM-CS	
	mean	SD	mean	SD	mean	SD	mean	SD
$N_{\min}$	8.1	1.11	7.4	5.24	13.5	7.57	13.4	6.30
$NO_3^-$ -N	4.8	3.52	4.8	4.39	4.6	3.15	5.5	4.31
$NH_4^+$ -N	3.3	2.79	2.6	2.05	8.9	6.91	7.9	5.23
P	7.2	7.77	10.9	11.57	7.0	6.77	6.9	7.47
K	9.4	3.56	8.0	4.39	10.4	5.97	9.1	5.39
Mg	11.2	4.09	11.4	4.05	11.1	4.25	12.7	5.12
pH	7.0	0.42	6.8	0.48	6.7	0.58	6.6	0.76

OSR – oilseed rape; CS – cropping sequence; SM – silage maize; SD – standard deviations

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Table 3. Loadings of principal factors (PFs)

Soil property	OSR-CS			SM-CS		
	PF <sub>1</sub>	PF <sub>2</sub>	PF <sub>3</sub>	PF <sub>1</sub>	PF <sub>2</sub>	PF <sub>3</sub>
<b>Spring</b>						
NO <sub>3</sub> <sup>-</sup> -N	0.69	0.25	-0.08	<b>0.71</b>	-0.05	–
NH <sub>4</sub> <sup>+</sup> -N	-0.05	<b>0.78</b>	0.17	0.47	0.20	–
P	<b>0.83</b>	0.03	-0.05	0.60	-0.41	–
K	0.56	-0.29	0.50	0.70	0.24	–
Mg	-0.11	0.11	<b>0.89</b>	0.18	<b>0.70</b>	–
pH <sub>CaCl2</sub>	-0.22	-0.69	0.12	-0.01	<b>0.78</b>	–
<b>Autumn</b>						
NO <sub>3</sub> <sup>-</sup> -N	<b>0.79</b>	0.26	-0.08	<b>0.84</b>	0.10	-0.14
NH <sub>4</sub> <sup>+</sup> -N	0.06	-0.11	<b>0.89</b>	-0.07	-0.02	<b>0.97</b>
P	<b>0.80</b>	-0.05	-0.02	<b>0.85</b>	-0.13	0.06
K	0.12	<b>0.86</b>	-0.09	0.41	0.65	0.10
Mg	-0.17	0.60	0.60	0.00	<b>0.81</b>	-0.19
pH <sub>CaCl2</sub>	0.47	-0.31	0.23	-0.38	<b>0.75</b>	0.13

Bold faced loadings are  $\geq 0.70$ . OSR – oilseed rape; CS – cropping sequence; SM – silage maize; PF – principal factor

spring was recorded only in the MS-CS. A slight pH decrease was recorded in autumn compared to spring. The observed changes in K and Mg contents and soil pH within the season can be related to the NH<sub>4</sub><sup>+</sup>-N content increase. The NH<sub>4</sub><sup>+</sup> ions present in the soil solution undergo oxidation, resulting in soil pH decrease, or exchange with cations of the cation exchange complex (Chung and Zasoski 1994).

**Cluster analysis.** The loadings of PFs depending on the date and type of cropping sequence are included in Table 3. Based on PFs computed for OSR-CS, the number of clusters reached 4 in spring and 3 in autumn (Table 4). In spring, the increasing number of samples ( $n$ ) in the respective cluster was negatively correlated with the N<sub>minSt</sub>:  $N_{\min St} = -0.01n + 2.1$  for  $R^2 = 0.74$ ,  $n = 4$ , and  $P < 0.05$ .

This dependency indicates that in OSR fields, soil samples with high N<sub>min</sub> content were an exception rather than the rule. In well N-managed fields, resources of subsoil N are efficiently exploited by seed crops (Haberle et al. 2006).

The impact of the N<sub>minSt</sub> and the PFs on the distance between clusters is presented in Figure 1.

Table 4. Centroids for  $k$ -means clustering depending on the date and cropping sequences

Cropping sequence	Date	Cluster	Number of cases	Variables			
				PF <sub>1</sub>	PF <sub>2</sub>	PF <sub>3</sub>	N <sub>minSt</sub> <sup>*</sup>
OSR-CS	spring	1	99	-0.35	0.19	1.36	0.84
		2	144	-0.17	-0.69	0.03	0.53
		3	140	-0.48	0.29	-0.92	0.74
		4	76	1.67	0.52	-0.12	1.47
	autumn	1	110	-0.04	0.13	1.35	1.27
		2	97	1.37	0.12	-0.41	0.13
		3	252	-0.51	-0.11	-0.43	-0.61
SM-CS	spring	1	33	0.78	1.05	–	0.89
		2	99	-0.79	-0.12	–	-0.66
		3	98	-0.25	0.79	–	-0.40
		4	66	0.37	-0.20	–	0.31
		5	40	-0.16	-2.03	–	-0.31
		6	24	2.46	-0.22	–	2.80
	autumn	1	94	-0.32	-0.24	0.60	0.28
		2	31	2.09	0.16	-0.82	0.91
		3	36	0.41	-0.29	2.05	1.98
		4	97	-0.11	1.06	-0.23	-0.33
		5	102	-0.38	-0.73	-0.81	-0.92

\*standardized values of mineral nitrogen (N<sub>min</sub>); OSR – oilseed rape; CS – cropping sequence; SM – silage maize; PF – principal factor

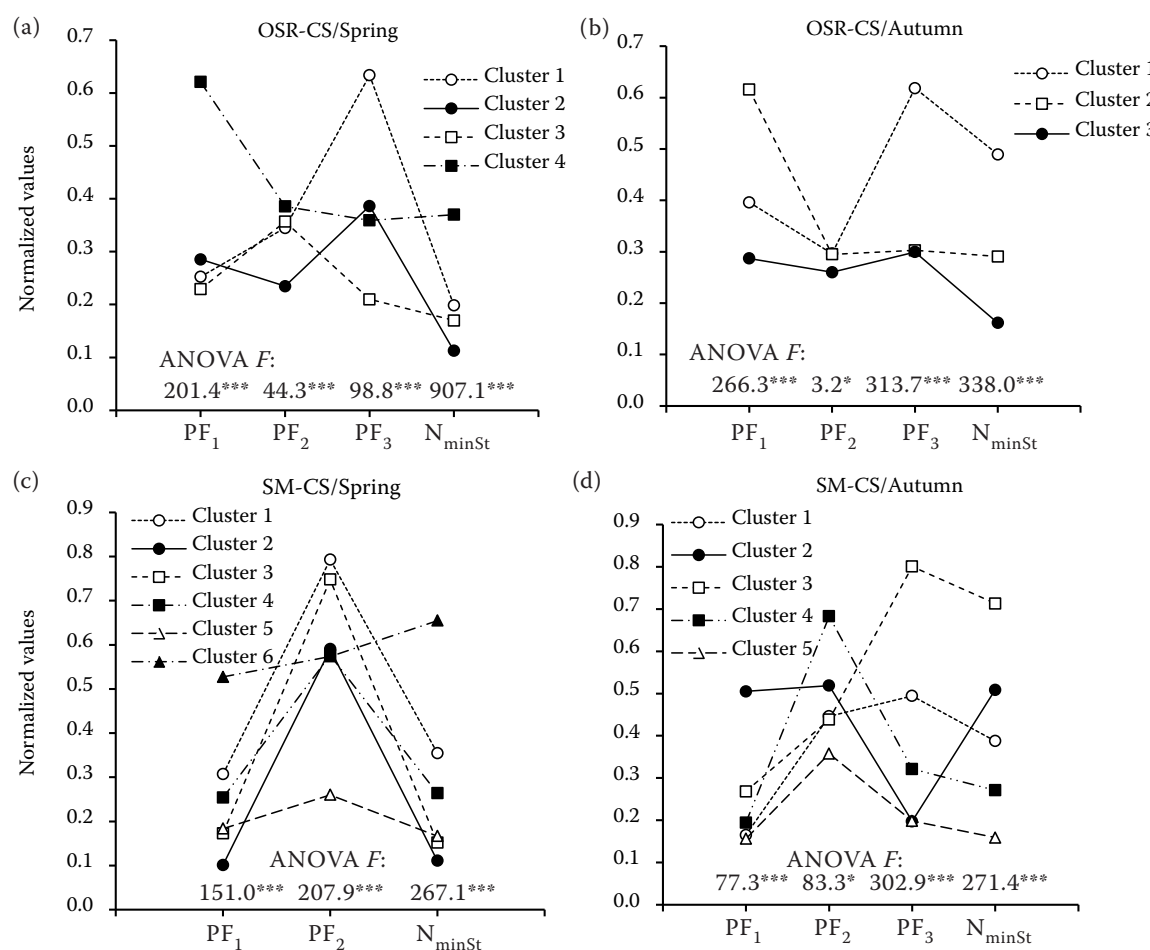


Figure 1. Graphs of means for continuous variables represented principal factors (PFs) and mineral nitrogen content (N<sub>minSt</sub>); OSR – oilseed rape; CS – cropping sequence; SM – silage maize; \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$

The key question of this analysis is to indicate the particular PF showing the highest impact on sample grouping in relation to the N<sub>minSt</sub> as a single criterion. As resulting from the  $F$ -values of ANOVA (Figure 1a), the order of PFs in OSR-CSs was:

$$PF_1 > PF_3 > PF_2.$$

The PF<sub>1</sub> factor, associated with P showed a positive sign for cluster 4 and a negative sign for the other three. For clusters with a negative PF<sub>1</sub> sign, any increase in soil-P content resulted in N<sub>minSt</sub> decrease. This phenomenon can be explained by the presence of plants, leading to N exhaustion.

In autumn, the decisive factor for the cluster arrangement was the PF<sub>3</sub>, associated with NH<sub>4</sub><sup>+</sup>-N (Figure 1b). In general, any increase in PF<sub>3</sub> value resulted in the N<sub>minSt</sub> increase. The lowest and negative N<sub>minSt</sub> was recorded in cluster 3 with the highest number of samples. The observed

relationships indicate that the potential threat of N<sub>min</sub> excess was an attribute of cluster 1, with a small size. The applied procedure resulted in

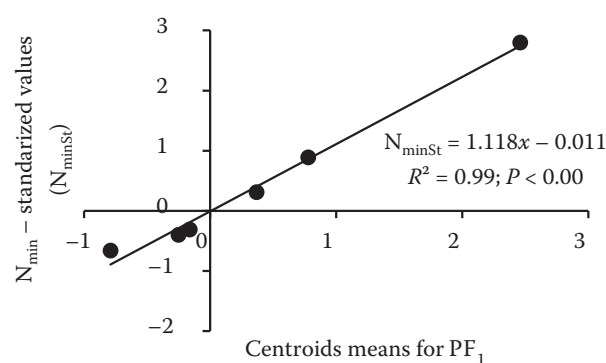


Figure 2. Relationships between principal factors (PFs) scores for SM-CS in spring and mineral nitrogen content (N<sub>minSt</sub>) values. CS – cropping sequence; SM – silage maize

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Table 5. Nutrient content ranges in separated clusters ranked by increasing content of mineral nitrogen ( $N_{\min}$ ) in soil

No. of cluster	$N_{\min}$	$NO_3^-$ -N	$NH_4^+$ -N	P	K	Mg	pH
(mg/kg)							
<b>OSR-CS/spring</b>							
2	3.0–7.5	1.6–5.1	0.4–3.5	0.8–11.6	7.2–12.9	8.3–13.6	6.9–7.5
3	3.9–10.8	1.8–6.0	0.9–6.0	0.0–9.6	4.7–8.8	5.8–10.5	6.4–7.3
1	4.8–12.0	2.2–5.5	1.5–7.6	0.0–9.2	6.6–15.2	12.7–20.1	6.7–7.3
4	9.3–20.1	6.2–14.8	0.8–7.6	8.9–27.3	7.8–14.3	8.0–13.6	6.3–7.2
<b>OSR-CS/autumn</b>							
3	5.7–12.0	1.6–4.8	2.7–8.6	0.2–8.1	3.6–16.2	6.3–14.0	5.9–7.2
2	9.7–19.2	4.6–12.0	2.7–9.6	8.7–21.2	6.2–16.2	7.2–12.9	6.4–7.4
1	15.7–30.4	2.0–7.1	11.9–25.2	0.4–13.1	4.7–16.7	9.1–18.8	6.2–7.1
<b>SM-CS/spring</b>							
2	2.4–5.4	0.9–3.8	0.5–2.6	1.9–13.8	2.9–7.8	7.4–11.9	6.6–7.1
5	3.0–8.6	1.1–6.1	1.0–3.3	4.2–28.0	4.1–8.6	4.4–8.6	5.3–6.3
3	3.4–7.1	1.1–4.3	0.9–4.2	1.4–13.1	4.7–12.3	10.9–17.7	6.7–7.3
4	6.4–11.6	3.8–9.3	0.8–4.1	5.5–25.0	4.6–12.6	7.6–14.8	6.4–7.1
1	9.0–15.1	4.3–10.4	2.1–7.4	0.0–12.6	4.9–18.1	10.4–18.9	6.8–7.3
6	17.6–26.5	12.0–21.4	2.2–8.5	0.0–52.7	7.2–17.4	7.9–14.1	6.5–7.2
<b>SM-CS/autumn</b>							
5	5.0–10.4	1.5–6.1	2.0–5.7	0.0–11.2	2.8–8.6	6.7–14.1	5.4–7.0
4	8.5–14.2	2.6–7.3	4.0–8.8	0.1–10.5	8.2–19.1	11.8–22.3	6.7–7.6
1	12.2–18.2	1.7–6.6	9.0–13.0	0.7–10.3	3.2–12.1	6.8–14.6	6.0–7.4
2	15.0–23.4	12.3–19.3	0.6–6.2	6.0–31.4	7.3–15.7	10.1–18.3	5.6–6.8
3	21.8–30.1	1.9–11.8	15.2–22.5	2.3–16.6	3.8–13.4	7.1–15.0	5.9–6.9

OSR – oilseed rape; CS – cropping sequence; SM – silage maize

discriminating soil samples with a potential threat of N leaching.

The number of clusters in the SM-CS was 6 in spring and 5 in autumn. In spring, the key variable affecting the number and size of a particular cluster was the  $N_{\min St}$ , followed by  $PF_2$  (Figure 1c). The  $PF_2$  cluster averages did not show any significant relationship with  $N_{\min St}$ . It was observed that  $PF_1$ , associated with  $NO_3^-$ -N, exerted a highly specific effect on soil sample distribution in spring. The  $N_{\min St}$  responded linearly to  $PF_1$  averages (Figure 2). This dependence implicitly corroborated the opinion that in maize or CSs with maize, the  $N_{\min}$  assessment can be based only on  $NO_3^-$ -N measurement (Luce et al. 2011).

In autumn, the strongest impact on sample partitioning between clusters was exerted by the  $PF_3$ , followed by  $N_{\min St}$  (Figure 1d). The  $NH_4^+$ -N was solely the key determinant of  $PF_3$ . The equation, based on averages, except for cluster 2, was as follows:

$$N_{\min St} = 1.0 PF_3 - 0.17 \text{ for } n = 4, R^2 = 0.99 \text{ and } P < 0.01.$$

This equation clearly indicates that in autumn in soil cropped with maize the increase in  $NH_4^+$ -N content was not an exception, but a rule.

**Nutrient rating.** In spring, for the OSR-CS, the  $N_{\min}$  content in relevant classes showed a high resemblance only with the  $NO_3^-$ -N rating (Table 5). This relationship was much weaker for  $NH_4^+$ -N because of the elevated differences in the highest  $N_{\min}$  classes. For P, this discrepancy refers to medium  $N_{\min}$  classes. In each class, except for the highest one, P content was below the respective  $N_{\min}$  values, indirectly indicating its shortage. Potassium and Mg showed a different course. The observed patterns indicate that the K and Mg contents were above those recorded for N, except for the high class. The question remains if these two nutrients were antagonistic with each other, taking into account the considerably higher content of extractable Mg with respect to K. The

$N_{\min}$  rating in autumn followed not only  $NH_4^+$ -N but also Mg.

In the SM-CS, the  $N_{\min}$  rating in spring, to some extent, followed the order of classes observed for  $NO_3^-$ -N and K. The resemblance of  $N_{\min}$  classes with  $NO_3^-$ -N suggests an opportunity to determine  $N_{\min}$  based only on this N form (Luce et al. 2011). The ranges of K were broader, except clusters 5 and 6, than the corresponding ranges of  $N_{\min}$ . The order of P classes was quite distinct from that observed for  $N_{\min}$ . Its content in classes showed higher variability of  $N_{\min}$ , indicating a potential shortage or excess. The Mg rating showed an elevated variability, but it was higher compared to the contents of both N forms and K, except for the top  $N_{\min}$  class (Table 5).

In autumn, the rating of  $N_{\min}$  did not show any resemblance with other nutrients or with soil pH. The content of  $NO_3^-$ -N and P reached the largest ranges for  $N_{\min}$  class 4, whereas  $NH_4^+$ -N reached the widest ranges for the 3<sup>rd</sup> one. The P ranges, depicted for classes 1, 2, and 5 were below those observed for the respective  $N_{\min}$  classes. It indicates a high probability of soil-P shortage. The orders of classes for K and Mg in comparison to  $N_{\min}$  were quite distinct. For both nutrients, the widest classes coincide with the 2<sup>nd</sup>  $N_{\min}$  class. In general, K content was narrower, but for Mg, it was much wider compared to  $N_{\min}$  (Table 5).

Cluster analysis was revealed as a useful tool to evaluate the impact of  $N_{\min}$  content on contents of  $CaCl_2$  extractable nutrients. In spring, the content of K, irrespective of the CS, was a soil characteristic fully independent on  $N_{\min}$ . The discriminative function of K in spring can be related to the elevated content of  $NH_4^+$ -N in autumn. In soil cropped with maize, the  $N_{\min}$  content can be determined based on  $PF_1$  that was associated with  $NO_3^-$ -N. Cluster analysis implicitly indicated P as the limiting growth factor. In autumn, irrespective of the cropping sequence, the decisive factor in  $N_{\min}$  content was the  $NH_4^+$ -N pool. Cluster

analysis seems to be a useful tool in determining the set of samples with excess of  $N_{\min}$  content in autumn, in turn signalling a potential threat for the environment.

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