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The Influence of Calcium Sulfate and Different Doses of Potassium on the Soil Enzyme Activity and the Yield of the Sward with a Mixture of Alfalfa and Grasses

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Abstract: Between 2012 and 2015, a field experiment was conducted at the Brody Experimental Farm, Poznan University of Life Sciences, Poland. The following two experimental factors were used in duplicate: calcium sulfate ($CaSO_4$) fertilization—two levels (0 and 500 kg ha^{-1}); and potassium (K) fertilization—four levels (0, 30, 60, and 120 kg ha^{-1}). The soil pH (in H_2O) and enzyme activity (dehydrogenase, acid phosphatase, alkaline phosphatase) were determined. The potassium fertilizer had a significant influence only on the dehydrogenase activity, which increased with the dose of potassium. The research showed that the fertilization of the sward mixture of alfalfa and grasses with potassium doses of 60 and 120 kg ha^{-1} in the K 60 and K 120 combinations resulted in higher yields of fresh matter than in the combination without the potassium fertilizer (K 0). In the last year of the research, the additional $CaSO_4$ fertilization resulted in the highest increase in the yield of the mixture of alfalfa and grasses, as compared with the variant without this fertilizer. The application of this fertilizer in the first years of the research also increased the yield of fresh matter.

Keywords: REA-gypsum; lucerne; dehydrogenase; acid phosphatase; alkaline phosphatase

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Citation: Zielewicz, W.; Swędrzyńska, D.; Swędrzyński, A.; Grzebisz, W.; Goliński, P. The Influence of Calcium Sulfate and Different Doses of Potassium on the Soil Enzyme Activity and the Yield of the Sward with a Mixture of Alfalfa and Grasses. *Agriculture* **2022**, *12*, 475. https://doi.org/10.3390/agriculture12040475

Academic Editors: Mariola Staniak and Anna Kocira

Received: 23 February 2022
Accepted: 25 March 2022
Published: 28 March 2022

1. Introduction

The negative influence of agriculture on the natural environment, and even on the climate, is increasingly often emphasized. One of the most important current challenges is to limit this influence [1,2]. The cultivation of grass and legume mixtures based on natural biological processes is a relatively small burden for the environment. These mixtures help to maintain soil fertility and biological potential, also in the context of carbon sequestration. They have minimal requirements for nitrogen fertilization, because they form efficient symbiotic systems with diazotrophic bacteria [3–7]. The optimization of the fertilization of grass and legume mixtures with other macroelements, including potassium, calcium, and sulfur, remains an important problem.

Potassium is one of the most important macroelements in plant production due to the multifunctional effect of this nutrient on the physiological processes occurring in plants, and in consequence, on their yield. Potassium not only controls the water management, but also the nitrogen management in the plant, and it significantly minimizes abiotic and biotic stresses [8–11]. If the amount of potassium fertilizer exceeds the metabolic capacity of plants, their yield will not increase. Instead, the yield quality may be adversely affected, including the uptake of other macronutrients such as calcium and magnesium [12], which are particularly important for forage crops.

Sulfur is a nutrient with important structural and metabolic functions in plants. It is a component of proteins, fats, and amino acids, and it participates in their synthesis. Sulfur can be found in such amino acids as methionine, cysteine, and cystine, and in ferredoxin and vitamins (biotin, thiamine) [13]. Additionally, sulfur is involved in redox processes and stabilizes the protein structure [14]. If there is sulfur deficiency, the yield volume and quality decrease, e.g., the content of non-protein nitrogen in plants increases [15]. Soil sulfur deficiency reduces the yield-generating efficiency of nitrogen [16]. Sulfur deficiency disrupts the growth of root nodules and the process of atmospheric nitrogen fixation in alfalfa and other legumes due to lower nitrogenase activity [17]. Sulfur affects the process of atmospheric nitrogen fixation and thus determines the yield-generating effect in legumes [18]. Moderate doses of sulfur are the best for plants because then they exhibit the highest photosynthetic activity [19].

Currently, agricultural soils tend to be increasingly deficient in sulfur. One of the reasons for this situation is the effective desulfurization of flue gases from coal combustion. Some forms of sulfur deposited in soil are difficult to access for plants. Most of the agricultural soils in Poland have a low content of sulfate sulfur [20]. The decreasing content of sulfur in soils is a major problem of modern agriculture [21,22]. The issue of fertilization of crops with sulfur has been discussed in various studies (e.g., [23–25]). In order to optimize sulfur fertilization, it is necessary to select the right chemical formula and the time of application [26,27].

The metabolic functions of calcium are also important for the normal function of plants [28]. This element is essential for the division of cells in the growth buds of the roots and shoots. Calcium deficiency inhibits plant growth [29]. Alfalfa is a plant species with a high demand for calcium [30].

Calcium sulfate ($CaSO_4$) is a calcium fertilizer used in agriculture. It is natural gypsum or a product of SO_2 neutralization in combined heat and power plants [31]. When calcium sulfate is applied to soil, Ca^{2+} ions in the form of chemically neutral salt are available to plants, especially to the species with a high demand for calcium, such as clover and alfalfa [32,33]. Calcium sulfate is also a rich source of sulfur that plants can easily access. It has positive influence on the volume and quality of the yield of plants with a high demand for this element, e.g., rape and alfalfa [34].

Calcium sulfate affects plant growth and yield quality [35–39]. When used as a fertilizer, it directly supplies sulfur and calcium to plants. Calcium sulfate introduced into soil as a chemically neutral salt does not form concentrated solutions and its pH is almost neutral. As a result, it does not damage leaves and it can be safely used for top dressing, even as a dust fertilizer [30]. Calcium sulfate fertilization has positive effects on heavy clay soils because it increases the rate of water infiltration and reduces the crust on the surface of these soils [40,41].

The content of aluminum, which is toxic to plants, increases in acidic soils and disturbs the growth of plants' roots [36,42]. Calcium sulfate only slightly modifies the soil pH, but it limits the toxic effect of aluminum in soil [43,44]. When calcium sulfate is applied in the soil solution, complex $AlSO_4$ ions or molecular $Al(OH)SO_4$ compounds are formed, which reduce the harmful effect of aluminum on plants [45,46].

The soil bioactivity is also manifested by the activity soil enzymes, which is a derivative of the metabolism of plants and soil organisms, especially the soil microbiome. The enzymatic activity of soil is an image and indicator of its biological condition and fertility [47,48]. Dehydrogenases, and acid and alkaline phosphatases, are considered the best indicators of the general population of soil microorganisms and soil microbial activity [49–51].

Dehydrogenases can be found in all living microbial cells, where they reflect the redox processes. They do not accumulate in the extracellular space, which prevents, for example, plant dehydrogenases from entering the soil. Therefore, the activity of soil dehydrogenases is almost exclusively related with the abundance and activity of soil microorganisms [52]. The activity of acid and alkaline phosphatases, which can be observed in living and dead plant cells and soil microorganisms, reflects the activity of enzymes related with soil colloids and humic substances [53,54]. Researchers studying the soil environment usually assess the activity of phosphomonoesterases (phosphoric monoester hydrolases), which are largely responsible for the decomposition of organic phosphorus compounds into inorganic forms—they catalyze the decomposition of organic phosphorus ester and phosphoric acid anhydrides [55,56]. Their activity plays an important role in the phosphorus cycle in nature. By participating in the hydrolysis of various phosphate compounds they stimulate the transformation of organic phosphorus compounds into inorganic phosphates, which are directly available to plants and soil microorganisms [57–59].

The soil enzyme activity is influenced by various general environmental factors (humidity, temperature, oxygen availability, pH, the presence of soil organic matter) and factors specific to a particular habitat (pesticides, heavy metals, farming methods, etc.). Organic and mineral fertilization is one of the most important factors in agrocenoses [60–62].

The aim of this study was to assess how different doses of potassium and calcium sulfate fertilizers applied to a mixture of alfalfa and grasses influenced the yield of the sward and changes in the soil enzyme activity.

2. Materials and Methods

2.1. Research Site

The field experiment was conducted at the Brody Experimental Farm, Poznań University of Life Sciences, Poland (52°44′ N, 16°28′ E) on soil belonging to the typical lessive subtype, the glacial till type, and the type of light and strong loamy sands. The humus depth was greater than 30 cm. The soil was classified as Albic Luvisols according to the WRB, and as Typic Hapludalfs according to Soil Taxonomy. In terms of the grain size, it was classified as loamy sand underlined by loam [63]. The soil was characterized by the following physicochemical parameters: humus content—1.28%, slightly acidic soil pH (pH_{KCl} = 6.5), content of macronutrients: P—82 mg, K—124 mg, and Mg—56 mg kg^{-1} soil.

2.2. Experimental Design

The split-plot field experiment was started in the autumn of 2011. A mixture of alfalfa and DSV Country 2056 grasses (alfalfa—80%, meadow fescue—15%, timothy—5%) was sown in plots sized 5.4 m × 5.0 m (27.0 m^2) at an amount of 25 kg of seeds per 1 ha. Winter oilseed rape was used as a forecrop.

The following two experimental factors were used in 2 duplicates:

- calcium sulfate fertilization—2 levels (0 and 500 kg ha^{-1} of $CaSO_4$),
- potassium fertilization—4 levels (0, 30, 60, and 120 kg ha^{-1} of K).

These fertilizer combinations are hereinafter referred to as: $CaSO_4$ 0 and $CaSO_4$ 500, and K 0, K 30, K 60, and K 120, respectively.

Calcium sulfate in the form of REA-gypsum ($CaSO_4 \cdot 2H_2O$) with 21.3% Ca and 17% sulfur content was applied in early spring. Potassium, in the form of potassium salt containing 60% K_2O, was applied before the beginning of the growing season. Additionally, in spring, before the beginning of the growing season, two weeks after the application of calcium sulfate, uniform phosphorus fertilization at a dose of 60 kg ha^{-1} of P (triple superphosphate 46% P_2O_5) was applied in all combinations. At the beginning of the experiment, nitrogen fertilizer in the form of 34% ammonium nitrate was applied once at a starting dose of 40 kg ha^{-1} N in all experimental combinations.

2.3. Field and Laboratory Analyses

All analyses were conducted between 2012 and 2015. The sward was harvested three times at the full budding phase of alfalfa. The sward harvested from each plot in the fertilizer combinations was weighed and the yield of fresh weight per hectare was calculated. The total yield from the three harvests was analyzed.

Each time immediately before harvesting, the sward soil samples were collected with a soil sampling probe from the soil profile layer of 0–15 cm. Two pooled samples (10 soil punctures in each) were collected from each experimental combination. The soil pH (in H_2O) and enzyme activity (dehydrogenase, acid phosphatase, alkaline phosphatase) were determined. The dehydrogenase activity (EC 1.1.1. DHA) was determined spectrophotometrically with 1% TTC (2,3,5-triphenyl tetrazolium chloride) as a substrate, after 24 h incubation at 30 °C, pH 7.4. Triphenylformazan (TPF) was produced, extracted with 96% ethanol, and measured spectrophotometrically at 485 nm. The enzyme activity was expressed as µmol TPF kg^{-1} DM of soil 24 h^{-1} [64]. The activity of alkaline phosphatase (AlP) (EC3.1.3.1) and acid phosphatase (AcP) (EC 3.1.3.2) was determined with the method developed by Tabatabei et al. [65], with para-nitrophenyl phosphate (pNPP) used as a substrate, after a one-hour incubation at 37 °C, at a wavelength of 400 nm. It was expressed as µmol pNP kg^{-1} h^{-1} DM of soil.

2.4. Statistical Analysis

The three-way ANOVA was used, in which Year, $CaSO_4$, and K fertilization were the main factors (all the factors were considered fixed). This analysis was carried out according to the linear model given by the formula:

$$y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + e_{ijkl}$$

where: μ is the general mean; α_i is the year effect; β_j is the $CaSO_4$ fertilization effect; γ_k is the K fertilization effect; $(\alpha\beta)_{ij}$ is the second order effect; $(\alpha\gamma)_{ik}$ is the second order effect; $(\beta\gamma)_{jk}$ is the second order effect; $(\alpha\beta\gamma)_{ijk}$ is the third order effect; e_{ijkl} is a random error.

The confidence intervals for the means were determined and the differences between the means were verified with Tukey's HSD test at a significance of p = 0.05. Statistica and MS Excel software were used for calculations and graphical presentation of the results.

2.5. Weather Conditions

The course of weather conditions in the growing seasons between 2012 and 2015 is shown in Table 1. In 2012 there were favorable weather conditions for the growth and development of plants in the mixture of alfalfa with grasses. The average annual air temperature was moderate, i.e., 8.9 °C. The rainfall was fairly high for the Wielkopolska region, i.e., 811.5 mm. In 2013, the annual rainfall was 295 mm lower than in the previous year. July was a particularly dry month, with a total rainfall of 67.3 mm. In 2014, the total rainfall was 632.5 mm. The rainfall in May was similar to that in previous years, but in June there was a significant water shortage and an unfavorable distribution of rainfall, which negatively influenced the development of the plants. In the last year of the experiment, the total rainfall was the lowest, i.e., only 471.0 mm (Table 1). Rainfall deficits, which occurred during the growing season in the second and last years of the study, had quite often been observed in this part of the Wielkopolska region before [66].

Table 1. Weather conditions during the vegetation period in RGD Brody in the years 2012–2015.

Month	Average Air Temperature (°C)				Total Rainfall (mm)			
	2012	2013	2014	2015	2012	2013	2014	2015
IV	8.8	8.0	10.5	10.4	22.9	15.4	46.3	46.4
V	14.9	14.4	13.1	13.0	77.2	69.8	73.5	25.6
VI	16.0	17.3	16.1	15.5	163.0	125.3	42.0	85.3
VII	19.2	20.1	21.5	19.2	197.6	67.3	83.1	84.9
VIII	18.7	19.1	17.3	22.1	60.1	51.5	137.2	15.1
IX	14.3	12.9	15.4	14.7	30.0	33.7	64.8	40.6
X	8.2	10.3	10.9	7.9	10.9	10.9	39.8	21.7
IV–X average temperature	14.3	14.6	15.0	14.7	-	-	-	-
IV–X rainfall	-	-	-	-	561.7	373.9	486.7	319.6
Annual average temperature	8.9	8.8	10.1	10.2	-	-	-	-
Annual rainfall	-	-	-	-	811.5	516.5	632.5	471.0

3. Results

The analysis of variance (Table 2) indicated a significant effect of $CaSO_4$ fertilization on the yield of the sward, the soil pH, and the activity of dehydrogenases and alkaline phosphatase. The potassium fertilizer significantly influenced the yield of the fresh matter of the alfalfa and grass mixture and the dehydrogenase activity. The interaction between the $CaSO_4$ and K fertilization was observed only for the yield. The main effect of the year was significant for all five variables (fresh matter yield, soil pH, dehydrogenase, acid phosphatase, and alkaline phosphatase activities). The year × $CaSO_4$ fertilization interaction was statistically significant for the yield and alkaline phosphatase activity.

Table 2. Three-way ANOVA for analyzed depended variables.

Source of Variation		Yield		pH		Dehydrogenase		Acid Phosphatase		Alkaline Phosphatase	
	df	SS	F	SS	F	SS	F	SS	F	SS	F
Year	3	7741.3	1066.30 ***	1.116	21.444 ***	356.04	44.105 ***	0.02096	10.743 ***	0.0716	90.688 ***
$CaS0_4$	1	180.4	74.54 ***	0.817	47.093 ***	34.59	12.854 ***	0.00031	0.470	0.0086	32.804 ***
K	3	684.7	94.32 ***	0.025	0.486	74.60	9.241 ***	0.00030	0.156	0.0005	0.686
Year × $CaS0_4$	3	145.3	20.02 ***	0.060	1.151	6.82	0.844	0.00053	0.270	0.0039	4.890 **
Year × K	9	319.2	14.66 ***	0.067	0.432	13.87	0.573	0.00049	0.083	0.0011	0.470
$CaS0_4$ × K	3	76.8	10.57 ***	0.010	0.196	1.36	0.169	0.00019	0.099	0.0001	0.109
Year × $CaS0_4$ × K	9	185.5	8.52 ***	0.327	2.095 *	8.62	0.356	0.00031	0.052	0.0004	0.176
Error	160 (32) [1]	77.4		2.775		430.54		0.10403		0.0421	
Total	191 (63) [1]	9410.7		5.198		926.43		0.12711		0.1283	

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. [1]—number of degrees of freedom for yield analysis.

During the experiment, the average yield of the mixture of alfalfa and grasses (Table 3) ranged from 30.26 to 67.92 t of fresh matter per hectare in the combination without the $CaSO_4$ fertilizer, and from 36.48 to 70.82 t per hectare in the combinations with the $CaSO_4$ fertilizer. The highest yields of fresh matter were noted in 2013, although it was a rather dry year. The yield of the sward in the combination with the $CaSO_4$ fertilizer and the full dose of potassium (120 kg ha^{-1}) was over 70 t of fresh matter (FM) per ha. The lowest yields were noted in the last year of the experiment.

Table 3. Effect of $CaSO_4$ application and varying doses of potassium fertilization on sward yields of alfalfa-grass mixtures (t ha^{-1} FM).

Year	$CaSO_4$ Fertilization	Potassium Fertilization			
		K 0	K 30	K 60	K 120
2012	0	46.33 ^{c}	52.35 ^{bc}	53.19 ^{b}	55.84 ^{ab}
	500	50.90 ^{bc}	55.41 ^{ab}	56.57 ^{ab}	59.92 ^{a}
2013	0	62.21 ^{c}	63.83 ^{bc}	67.70 ^{abc}	67.92 ^{abc}
	500	65.34 ^{abc}	67.75 ^{abc}	69.94 ^{ab}	70.82 ^{a}
2014	0	45.49 ^{b}	45.51 ^{b}	50.37 ^{ab}	51.53 ^{ab}
	500	47.26 ^{ab}	46.07 ^{b}	54.96 ^{ab}	56.09 ^{a}
2015	0	30.26 ^{d}	30.96 ^{cd}	32.56 ^{cd}	36.52 ^{bc}
	500	36.48 ^{bc}	36.87 ^{abc}	42.11 ^{ab}	44.98 ^{a}

Within each year, values followed by the same letter are not statistically different at $p < 0.05$.

The analysis of the effect of the applied doses of the potassium fertilizer on the average yield of the sward mixture in individual combinations and years of the study showed that, in the variant without the $CaSO_4$ fertilizer, the difference in the total yields between the K 0 and K 120 combinations was 27.52 t of fresh matter per hectare (14.93%). In the variant with the $CaSO_4$ fertilizer, the difference between these combinations in the total yield obtained during the years of the study amounted to 31.83 t of fresh matter per hectare (15.91%) (Table 3).

The analysis of the effect of the $CaSO_4$ fertilization showed that, in the subsequent years of the study, the yields increased in all the K combinations. In comparison with the K 0 combination, the application of potassium at the doses of 30, 60, and 120 kg ha^{-1} in the variants without $CaSO_4$ increased the yield of fresh matter by 8.36 t ha^{-1} (4.5%), 19.53 t ha^{-1} (10.6%), and 27.52 t ha^{-1} (14.9%), respectively. In comparison with the K 0 combination, the application of potassium at the doses of 30, 60, and 120 kg ha^{-1} in the variants with $CaSO_4$ increased the yield of fresh matter by 6.22 t ha^{-1} (3.11%), 23.7 t ha^{-1} (11.8%), and 31.93 t ha^{-1} (15.9%), respectively.

The statistical analysis of the yields revealed a significant difference between the variants with and without the $CaSO_4$ fertilizer. The potassium fertilizer also significantly increased the yields. The fertilization levels of 60 and 120 kg K ha^{-1} increased the yields considerably, both in the variants with and without $CaSO_4$. It turned out that the dose of 60 kg K ha^{-1} was as effective as the full dose of 120 kg K ha^{-1}, despite the lower amount of potassium applied to the soil.

The effect of $CaSO_4$ application on the efficiency of potassium fertilization in relation to sward yield increase is shown in Figure 1. In each year, the application of $CaSO_4$ significantly increased the yields of fresh matter.

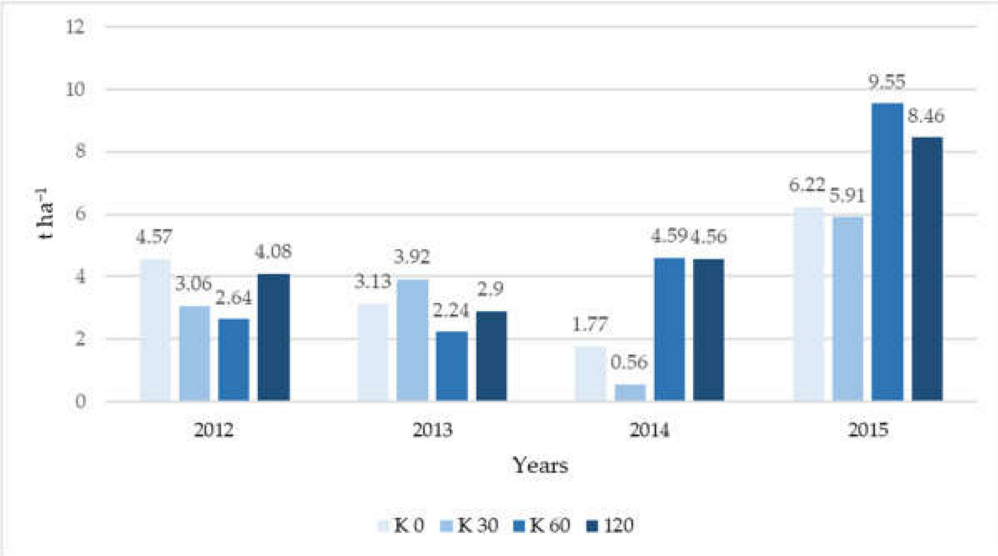

Figure 1. Increase sward yield in individual combinations of potassium fertilization under the influence of $CaSO_4$ (t ha^{-1} FM).

The assessment of the effect of the applied fertilizer combinations on the soil pH was the starting point for the interpretation of the soil enzyme activity (Tables 2 and 4). The $CaSO_4$ fertilization decreased the soil pH. The effect was minimal but unambiguous. It exhibited an upward trend in the following years and it was statistically significant. The effect of different doses of potassium fertilization on soil pH was statistically insignificant (Table 4).

All the analyzed factors clearly influenced the activity of dehydrogenases (Table 5). Only the main effects Year, $CaSO_4$ and K fertilization, separately, were significant. The $CaSO_4$ fertilizer reduced the dyhydrogenase activity, whereas the potassium fertilizer clearly increased the activity of this enzyme as the dose of potassium increased. There was no interaction between the independent variables (Table 2).

Table 4. Effect of analyzed factors on pH of soil.

Year	$CaSO_4$ Fertilization	Potassium Fertilization			
		K 0	K 30	K 60	K 120
2012	0	6.61 ^{ab}	6.52 ^{ab}	6.63 ^{a}	6.53 ^{ab}
	500	6.48 ^{b}	6.50 ^{ab}	6.47 ^{b}	6.51 ^{ab}
2013	0	6.50 ^{ab}	6.54 ^{ab}	6.56 ^{a}	6.55 ^{a}
	500	6.47 ^{ab}	6.39 ^{bc}	6.44 ^{abc}	6.31 ^{c}
2014	0	6.50 ^{a}	6.52 ^{a}	6.47 ^{a}	6.32 ^{b}
	500	6.28 ^{b}	6.31 ^{b}	6.32 ^{b}	6.41 ^{ab}
2015	0	6.45 ^{a}	6.43 ^{ab}	6.35 ^{abc}	6.46 ^{a}
	500	6.24 ^{c}	6.28 ^{bc}	6.23 ^{c}	6.21 ^{c}

Within each year, values followed by the same letter are not statistically different at $p < 0.05$.

Table 5. Effect of analyzed factors on dehydrogenases activity (μmol TPF kg^{-1} DM of soil 24 h^{-1}).

Year	2012	2013	2014	2015
	1.528 ^{b}	**4.128 ^{a}**	**4.843 ^{a}**	**2.125 ^{b}**
$CaSO_4$ fertilization	$CaSO_4$ 0 3.570 ^{a}		$CaSO_4$ 500 2.742 ^{b}	
Potassium fertilization	K 0 2.245 ^{c}	K 30 2.952 ^{bc}	K 60 3.452 ^{ab}	K 120 3.974 ^{a}

Within each variable, values followed by the same letter are not statistically different at $p < 0.05$.

The significant differences in acid phosphatase activity were only obtained for Year (Tables 2 and 6). The influence of the experimental variants on the acid phosphatase activity was minimal, inconclusive, and statistically insignificant.

Table 6. Effect of analyzed factors on acid phosphatase activity (μmol pNP g^{-1} DM of soil h^{-1}).

Year	2012	2013	2014	2015
	0.0762 ^{b}	**0.0987 ^{a}**	**0.0728 ^{b}**	**0.0751 ^{b}**
$CaSO_4$ fertilization	$CaSO_4$ 0 0.0794 ^{a}		$CaSO_4$ 500 0.0820 ^{a}	
Potassium fertilization	K 0 0.0801 ^{a}	K 30 0.0790 ^{a}	K 60 0.0817 ^{a}	K 120 0.0821 ^{a}

Within each variable values followed by the same letter are not statistically different at $p < 0.05$.

Unlike acid phosphatase, alkaline phosphatase is mainly of microbial origin. The significant differences in alkaline phosphatase activity were only obtained for Year, $CaSO_4$ and the interaction Year × $CaSO_4$ (Tables 2 and 7). $CaSO_4$ fertilization significantly reduced the activity of this enzyme.

Table 7. Effect of year × $CaSO_4$ fertilization interaction on alkaline phosphatase activity (μmol pNP g^{-1} DM of soil h^{-1}).

Year	$CaSO_4$ Fertilization	
	$CaSO_4$ 0	$CaSO_4$ 500
2012	0.0618 ^{a}	0.0497 ^{b}
2013	0.1141 ^{a}	0.0885 ^{b}
2014	0.0811 ^{a}	0.0657 ^{b}
2015	0.0529 ^{a}	0.0525 a

Within each year, values followed by the same letter are not statistically different at $p < 0.05$.

In order to assess the correlations between the variables, the Pearson correlation coefficient was used for analysis (Table 8). The strongest correlations were found between the activity of both phosphatases, the activity of phosphatases and the yield, and between the dehydrogenase activity and the soil pH. The analysis did not reveal any correlation between the dehydrogenase activity and the yield. These correlations confirmed the aforementioned explanations of the research results and observed phenomena.

Table 8. The r-Pearson correlation coefficient between the parameters examined.

	Dehydrogenase	Acid Phosphatase	Alkaline Phosphatase	pH
Yield	0.0560	0.5766 *	0.4112 *	0.0793
pH	0.4434 *	−0.1557	0.2932 *	
Alkaline phosphatase	0.3717 *	0.5698 *		
Acid phosphatase	0.0905			

* $p < 0.01$.

4. Discussion

There are several reasons why the year of the experiment and its interactions influenced the variables under analysis. The most important of these are the differences in the weather conditions in individual years of the experiment, and especially the differences in the amount and distribution of rainfall during the growing season, which determined the supply of water available to plants. It is a well-known fact that water is one of the main factors determining the growth and development of plants, because it influences their ability to take up soil minerals. Although alfalfa, which was the dominant plant in the sward, is relatively resistant to periodic droughts [67,68], it is not indifferent to the influence of drought stress. Grasses react very strongly to periodic water shortages in the soil profile [69]. During the growing seasons in 2012 and 2014, there was a sufficient amount of rainfall for the development of the mixture of alfalfa and grasses (Table 1), but 2013, and especially 2015, were dry. The significant differences between the variables in individual years of the experiment were also caused by changes in the viability of the plants, which resulted from the specificity of their development in the subsequent years. In the first year, the plants fully developed and achieved their full yield potential. In the last years of the experiment this potential decreased due to the plants' ageing [70]. In consequence, the sward became less dense and low weeds appeared.

The doses of potassium fertilizer applied in individual experimental combinations during the years of the study had a significant influence on the plants, both in the variants with and without the $CaSO_4$ fertilizer. Robin et al. [71] conducted a study on white clover and observed that when the plants had a higher supply of potassium during a water deficit, the water potential and stomatal resistance in the leaves decreased, which reduced the loss in the growth of the biomass of this species. This observation was also confirmed by the yields of the plants analyzed in our experiment. In 2015, there was a very interesting reaction of the mixture of alfalfa and grasses to the $CaSO_4$ fertilizer. The last year of the experiment was characterized by a rainfall deficit, which resulted in the lowest yields (Table 3). However, under such unfavorable soil moisture conditions there was an interaction between $CaSO_4$ and potassium applied at the doses of 60 and 120 kg ha^{-1}. The yield of fresh matter from the sward in the K 120 combination increased by 8.46 t ha^{-1}. It was 23.16% higher than the yield from the same combination but without the $CaSO_4$ fertilizer. The dose of potassium reduced by half, i.e., the K 60 combined with the $CaSO_4$ fertilizer had an even better effect. The yield of fresh matter from the sward increased by 9.55 t ha^{-1}, i.e., by 29.3%.

The accumulation of the effects of fertilization in the subsequent years of the experiment was undoubtedly another important reason for the changes in the variables under analysis. This particularly applied to the slow changes in the physicochemical properties of the soil, such as pH. Although the analysis did not reveal any significant effect of the potassium fertilization on the soil pH, the interaction between the year of the experiment, $CaSO_4$ fertilization, and K fertilization had a statistically significant influence on this parameter.

The research conducted by Tirado-Corbalá et al. [72] showed that the application of $CaSO_4$ stimulated the growth of the root system of alfalfa in soils with limited content of water and minerals. It also improved soil fertility and the growth of alfalfa. The application of $CaSO_4$ did not have a measurable effect on the yield of alfalfa, nor did it have a major effect on the content of macronutrients, except for the plants' increased uptake of sulfur. $CaSO_4$ is an excellent source of sulfur, so the fertilization of the soils where the availability of this element is limited improves the production of alfalfa biomass.

Most authors indicate that $CaSO_4$ has either no or a very poor deacidification effect [73,74]. However, there have also been reports, including our study, which documented the slightly acidifying effect of this fertilizer on the soil [75,76]. The effect is caused by the sulfate ions formed after the decomposition of the salt ($CaSO_4$), which react with water to produce sulfuric acid. Potassium fertilization may also reduce the soil pH [73,77].

The experimental combinations had a diversified influence on the biological properties of soil in individual years of the study, which was manifested by the differences between the mean values and their significance. In some cases, the lack of statistical significance of the differences was caused by the considerable difference in the results between individual dates of analyses. However, the same trends were observed in each year, as can be seen in the charts (Tables 4–7).

It is most likely that the lower soil pH reduced the dehydrogenase activity in the combinations fertilized with $CaSO_4$. According to Swędrzyńska et al. [78], even slight changes in pH may strongly modify the qualitative and quantitative composition of the soil microbiome, and thus affect the activity of soil enzymes. The negative effect of $CaSO_4$ on the dehydrogenase activity should not be attributed to sulfur alone, because the experiments conducted by Niewiadomska et al. [79] clearly showed that the dehydrogenase activity increased after the application of both elemental sulfur fertilizer and potassium fertilizer.

The lack of the effect of the applied experimental factors on the acid phosphatase activity may be explained by the fact that the activity of this enzyme is largely the plant's response to phosphorus deficiency in soil [80]. In our experiment, the amount of phosphorus in the fertilizer met the nutritional demand of alfalfa and grasses in the sward. The usefulness of acid phosphatase as an indicator of soil bioactivity was also limited by the high pH of the soil in our experiment.

It is noteworthy that the course of the alkaline phosphatase activity, which is mostly affected by the activity of soil microorganisms, was similar to the course of the dehydrogenase activity—it was reduced by the $CaSO_4$ fertilizer. Also in this case, it is most likely that the decreased activity of this enzyme was caused by the lower pH of the soil in the combinations fertilized with $CaSO_4$. Other authors observed a similar dependence between the soil pH and the alkaline phosphatase activity in their studies [76].

Symanowicz et al. [81] conducted a study on barley and observed that balanced nitrogen and potassium fertilization should be applied both to the forecrop and the succeeding crop in order to maintain the optimal activity of soil enzymes. Higher doses of potassium improved the dehydrogenase activity, whereas lower doses improved the activity of acid and alkaline phosphatases. The same authors observed that the fertilization of pea plants with nitrogen at a dose of 20 kg ha^{-1} and potassium at a dose of 166 kg ha^{-1} increased the urease and dehydrogenase activity in the soil [82]. Swędrzyńska et al. [78] found that soil bioconditioners based on calcium carbonate stimulated the activity of dehydrogenases, phosphatases, and urease. As shown in most studies discussing fluctuations in the soil enzyme activity during the growing season, it is most likely that they are influenced by variable weather conditions, which consequently affect the soil temperature and moisture [83].

5. Conclusions

The results of this experiment showed that all the factors under analysis, i.e., potassium fertilization, $CaSO_4$ fertilization, and the weather conditions, significantly influenced the yield of the sward with the mixture of alfalfa and grasses, in addition to the variables determining the soil bioactivity.

1 The calcium sulfate ($CaSO_4$) fertilizer significantly decreased the activity of dehydrogenases and alkaline phosphatase. It is most likely that this effect was caused indirectly by the decrease in the soil pH as a result of the calcium sulfate fertilization. The potassium fertilizer had a significant influence only on the dehydrogenase activity, which increased with the dose of potassium.
2 The research showed that the fertilization of the sward mixture of alfalfa and grasses with potassium doses of 60 and 120 kg ha^{-1} in the K 60 and K 120 combinations resulted in higher yields of fresh matter than in the combination without the potassium fertilizer (K 0).
3 In the last year of the research, the additional $CaSO_4$ fertilization resulted in the highest increase in the yield of the mixture of alfalfa and grasses, compared with the variant without this fertilizer. The application of this fertilizer in the first years of the research also increased the yield of fresh matter.

Author Contributions: Conceptualization, W.Z., D.S., W.G.; methodology, W.Z., D.S., W.G.; software, A.S. validation, W.G. and P.G.; formal analysis, W.Z., D.S.; investigation, W.Z., D.S.; resources, W.Z.; data curation, W.Z., A.S.; writing—original draft preparation, W.Z., D.S., A.S., W.G., P.G.; writing—review and editing, W.Z., D.S., A.S., W.G., P.G. visualization, W.Z., A.S.; supervision, W.Z.; project administration, W.Z.; funding acquisition, W.Z. All authors have read and agreed to the published version of the manuscript.

Funding: Publication was co-financed within the framework of the Polish Ministry of Science and Higher Education's program: "Regional Initiative Excellence" in the years 2019–2022 (No. 005/RID/2018/19).

Data Availability Statement: All data generated or analyzed during this study are included in this published article.

Conflicts of Interest: The authors declare no conflict of interest.

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