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Possibility of Using the By-Product of Fatty Acid Extraction from Fish in Fertilization as an Element of the Circular Economy

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Abstract: The study aimed to compare the effect of urea fatty fraction (UFF) and Pulrea® (urea fertilizer) on plant yield and selected plant and soil parameters determined after the plants were harvested. UFF is a by-product of essential unsaturated fatty acids (UFAs) extraction from fish oil using urea, and Pulrea® is a commercial urea fertilizer. Both products were applied to the soil and the leaves (foliar application). The effect of Pulrea® on plant yield was generally stronger than that of UFF but depended on soil properties and plant species. Both fertilizers, but especially UFF, increased the total N content in the plant and effected nitrate accumulation. The plants used 45–90% of fertilizer nitrogen, with the plants generally using more N from Pulrea® than from UFF. Higher nitrogen production efficiency was achieved using Pulrea® than UFF and when plants were cultivated on medium soil than on light soil. Fertilizers increased the acidity and electrolytic conductivity of both soils but did not induce soil salinization. They increased the content of mineral nitrogen forms in soils, which was generally the case more in soil with Pulrea® application than with UFF application. As a rule, the soil dehydrogenases activity did not change significantly or even decrease after fertilizer application. It was visibly higher in medium soil and after foliar Pulrea® application than after foliar UFF application. This may be due to the content of accompanying substances in UFF that affect nitrogen absorption from this fertilizer. Based on the results, it cannot be clearly stated that one of the tested fertilizers had a better effect on the studied parameters. Generally, the less favorable effects of UFF compared to Pulrea® may indicate the necessity of removing from UFF the accompanying substances that may adversely affect plants and soil microorganisms. This aspect needs to be investigated under controlled conditions in field experiments.

Keywords: Pulrea®; UFF; by-product management; plant yielding; nitrogen content; nitrate accumulation; nitrogen utilization; nitrogen production efficiency

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1. Introduction

Bioeconomy is a sector in which the implementation of circular economy solutions can be promising and relatively simple. One of the elements of the circular economy is the use of by-products (waste) from various branches of the economy to produce innovative fertilizers [1]. Among the essential nutrients, nitrogen is the one that has the greatest impact on the quantity and quality of plant yield. There is a wide range of nitrogen-containing fertilizers on the market. These are single-component and multi-component mineral fertilizers as well as natural and organic fertilizers, available in solid or liquid form. Work is still underway to introduce slow-release fertilizers (SRFs) and controlled-release fertilizers (CRFs) to the market to increase the use of nitrogen by plants, the effectivity of the applied nitrogen, and the production efficiency of the applied nitrogen in order to mitigate N loss [2–5]. There is also research on the possibility of using nitrogen-rich by-products as fertilizers [6]. The Circular Economy program aims to rationally use resources and reduce the negative impact of manufactured products on the environment. These, like materials and raw materials, should remain in the economy as long as possible, and waste generation should be minimized [1,7,8]. Considering the demand for nitrogen as a plant nutrient that determines the yield and quality to the greatest extent, the introduction of a new product to the market, urea fatty fraction (UFF), is justified. UFF is a by-product of the extraction of fatty acids from fish. This process uses the ability of urea to form solid complexes, the so-called urea inclusion compounds (UICs) [9]. The complexes with a hexagonal crystal structure bind molecules with a long hydrocarbon chain. Molecules with double bonds; with a branched, cyclic structure; or with a chain of 6–8 carbon atoms rarely form complexes with urea [10,11]. Thanks to subsequent fractionation stages, it is possible to obtain concentrates of polyunsaturated fatty acids (PUFAs) free from other compounds [12–14]. Under these conditions, it is possible to obtain approximately 97% PUFA concentration, in which the concentration of OMEGA 3 essential unsaturated fatty acids, that is, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) - exogenous fatty acids, reaches 85% [15]. Low-temperature extraction using urea protects fatty acids against oxidation, which is why it has been considered more environmentally friendly and less expensive than previously used methods. UFF is a by-product of this process [16–18]. UFF (known as Danish urea) has been certified in Denmark [19], allowing its use as a fertilizer. UFF fertilizer has a crystalline form and contains approximately 73% urea (which corresponds to 33% N), 22% ethyl esters, and 5% ethanol. According to this certificate, UFF can be sprinkled or used in the form of solutions. It is recommended that UFF be used to supplement the nitrogen content in the soil or in the post-harvest period on the straw left in the field to accelerate its mineralization process.

The aim of the research was to compare the effect of soil and foliar applications of UFF fertilizer with the effect of commercially available urea fertilizer, Pulrea® (Puławy, Poland), on yield and nitrogen content in test plants. Some soil properties were also determined after the plants were harvested. In addition, the use of nitrogen from the applied fertilizers and yield-forming effectiveness of fertilizer nitrogen were assessed.

2. Materials and Methods

2.1. Establishing the Pot Experiment

The research was carried out at the Department of Agricultural and Environmental Chemistry of the University of Agriculture in Krakow in 2022. The pot experiment was conducted in the vegetation hall of the Faculty of Agriculture and Economics of the university, located in Kraków-Mydlniki. The experiment was performed on two soils, one with a sandy clay (light soil) texture and one with a clay silt (medium soil) texture. The properties of the soil material before the experiment was performed are presented in Table 1.

Table 1. Selected properties of the soil material before the experiment was performed.

Parameter	Value	
	Sandy Clay	**Clay Silt**
Soil texture—Fraction, %		
Sand ⌀ 2–0.05 mm	69	30
Silt ⌀ 0.05–0.002 mm	25	67
Clay ⌀ < 0.002 mm	6	3
Agronomic category	light soil	medium soil
pHKCl	5.84	6.16
Maximum water capacity—MWC, %	26.1	30.5
Hydrolytic acidity—Ha, mmol (+) kg^{-1} d.m.	8.67	7.33
Sum of basic cations—BC, mmol (+) kg^{-1} d.m.	371.3	352.7
Cation exchange capacity—CEC, mmol (+) kg^{-1} d.m.	380	360
Saturation coefficient of the sorption complex—V, %	97.8	98.0
Organic carbon—Corg., g kg^{-1} d.m.	12.0	11.8

The experimental design included 5 fertilization treatments on each soil:

- C—Control without fertilization;
- I—Pulrea® applied pre-sowing + top dressing to the soil;
- II—UFF applied pre-sowing + top dressing to the soil;
- III—Pulrea® applied pre-sowing + top dressing to the leaves;
- IV—UFF applied pre-sowing + top dressing to the leaves.

Each treatment was run in 3 replications. The experiment included two series, each with different test plants. Series 1 included maize of the Lokata variety. Series 2 included spinach of the Matador variety and butter lettuce of the Królowa Majowych variety. Both series were grown consecutively. The experiment was established in PVC pots holding 8.0 kg of soil dry matter (Table 2).

Table 2. Scheme of the experiment.

Treatment	Fertilization						
	Series 1			Series 2			
	Pre-Sowing	Top Dressing		Pre-Sowing	Top Dressing	Top Dressing	
	Maize			Spinach		Lettuce	
C	-	-		-	-	-	
I	soil *	4 × soil		soil	2 × soil	3 × soil	
II	soil	4 × soil		soil	2 × soil	3 × soil	
III	soil	1 × soil	3 × leaves **	soil	2 × leaves	1 × soil	2 × leaves
IV	soil	1 × soil	3 × leaves	soil	2 × leaves	1 × soil	2 × leaves

Explanation: Pulrea® or UFF application as top dressing: * to the soil; ** to the leaves.

On the day the experiment was established, all pots were fertilized with basic phosphorus and potassium at doses of 0.45 g of P and 2.25 g of K per pot for maize and 0.70 g of P and 2.50 g of K per pot for leafy vegetables. Phosphorus and potassium were applied in the form of solutions of chemically pure salts: KH_2PO_4 and KCl. In treatments involving fertilization with nitrogen, this element was used at a dose of 2.4 g of N per pot in the form of Pulrea® (containing 46% N; producer: Grupa Azoty, Zakłady Azotowe Puławy S.A.) or UFF (the delivered batch contained 33.065% N; supplier: Combineering A/S Denmark; distributor in Poland: Ontrade.pl (Krakow, Poland)). In both series, 1/3 of the N dose was applied before sowing and 2/3 of the N dose was used as top dressing.

2.2. Nitrogen Application

A total of 2.4 g of N in the form of Pulrea® or UFF was applied to each pot of both series in treatments with nitrogen fertilization. Out of this dose, 0.8 g of N was applied in the pre-sowing phase to the soil.

In the treatments of Series 1, with maize, the remaining dose of N was applied as top dressing, divided into four doses of 0.4 g of N each. In treatments I and II, nitrogen was applied four times to the soil, and in treatments III and IV, it was applied once to the soil and three times to the leaves. Nitrogen was used in the form of a 5% Pulrea® solution or a 6.962% aqueous UFF emulsion with an equivalent concentration of urea. The amount of 17.38 cm^3 of the solution was applied to the soil and 19.99 cm^3 of the solution was applied to the leaves.

In the treatments of Series 2, with spinach and lettuce, the remaining dose of N was applied as top dressing, divided into five doses: one dose of 0.4 g of N and four doses of 0.3 g of N each. In treatments I and II, nitrogen was applied five times to the soil, and in treatments III and IV, it was applied once to the soil and foliar application was carried out four times. Top dressing was applied twice to the spinach (0.3 g of N each), once after planting the lettuce (0.4 g of N each), and twice to the lettuce (0.3 g of N each). Nitrogen was applied in the form of Pulrea® solution or UFF aqueous emulsion, as in Series 1. The amount of 17.38 cm^3 of the solution was applied to the soil once, and 13.04 cm^3 of the solution was applied to the soil four times or 15.0 cm^3 of the solution was applied to the leaves.

The foliar dose of nitrogen was increased by 15%, assuming that not all of the spray would settle on the leaves during spraying. On the first and second days after each spraying with nitrogen fertilizer, the plants were sprayed with distilled water.

2.3. The Course of the Experiment

The experiments were established on 31 March 2022. On this day, 60 pre-germinated spinach seeds of the Matador variety were sown in each pot of Series 2. After the seeds had sprouted, the number of plants was reduced to 22 pieces per pot. Top dressing was applied on 2 and 19 May 2022. The above-ground parts of the spinach were harvested on 26 May 2022, and on the same day, three seedlings of lettuce of the Królowa Majowych variety were planted in the pots. Top dressing was applied on 26 May, 28 June, and 5 July 2022. The lettuce was harvested on 19 July 2022. On 11 April 2022, 12 maize grains of the Lokata variety were sown in each pot of Series 1. After they had sprouted, the number of plants was reduced to 5 pieces per pot. Top dressing with nitrogen was applied on 27 and 30 May and 6 and 23 June 2022. On 9 June 2022, burns on the leaves of some maize plants were reported after the foliar application of fertilizers, generally more severe in the case of smaller than larger plants. This unfavorable effect of urea fertilizer on plant growth is due to the release of ammonia as a result of the hydrolysis of urea by urease, which can be eliminated by adding a small amount of urease inhibitor to the fertilizer [20].

During the experiment, soil moisture was maintained by watering with deionized water, initially at 40% of the maximum water capacity (MWC). As the plants grew, the humidity was increased to 45% and 50% of the MWC. Water losses were compensated by watering each pot 1–2 times a day to a constant weight (including the weight of the pot, soil, and plants).

After harvesting the lettuce and maize plants, soil samples were taken from each pot. The collected above-ground parts of the test plants were dried in an air-flow dryer at 65 °C and weighed to determine the dry matter yield, and then the plant material was ground and prepared for chemical analyses. Samples of soil material were air dried, sieved through a sieve with a mesh diameter of 1 mm, and prepared for laboratory analysis.

2.4. Methods for Analyzing Soil and Plant Materials

Before the experiment was established, the following parameters of the soil were determined: the texture according to hydrometric Bouyoucos–Casagrande method [21,22] and the agronomic category [23]; the pH value (pH_{KCl}) by the potentiometric method in a soil suspension in a KCl solution with a concentration of 1 mol·dm^{-3} (1:2.5, m/v) [24], using the CPC-502 multifunction device (Elmetron); hydrolytic acidity (Ha) and the sum of basic cations (BC) using the Kappen method; the maximum water capacity (MWC) by the weight method; and the organic carbon (Corg) content using the Tiurin method [25]. The cationic exchange capacity (CEC) of the sorption complex was calculated according to the formula CEC = Ha + BC. The saturation coefficient of the sorption complex (V%) was calculated according to the formula V% = (BC·100)/CEC (results are presented in Table 1).

The following parameters were determined in the soil after the experiment: the pH value using the potentiometric method in a soil suspension in a KCl solution with a concentration of 1 mol dm^{-3} (1:2.5, m/v) [24], using the CPC-502 multifunction device (Elmetron); the value of electrolytic conductivity (EC), determined using the conductometric method [26]; the ammonium and nitrate nitrogen content [27]; and the soil biological activity based on the activity of dehydrogenases (DHA), by transforming the colorless, water-soluble 2,3,5-triphenyltetrazolium chloride (TTC) into water-insoluble 1,3,5-triphenylformazan (TPF) [28,29]. The soil material was incubated with 1.0% TTC, prepared in a tis(hydroxymethyl)aminomethane hydrochloride (TRIS-HCl) buffer. After incubation (1:1 m/v, 96 h, 30 °C), the obtained TPF was extracted with methyl alcohol and quantified colorimetrically at 485 nm on a DU 640 UV/vis spectrophotometer (Beckman Instruments, Inc., Fullerton, CA, USA).

The total nitrogen content in the plant material was determined according to the Kjeldahl method (using a Kjeltec auto 1030 analyzer with automatic titration) [30] and the nitrate nitrogen content using the colorimetric method using sulfosalicylic acid ($C_7H_6O_6S{\cdot}2H_2O$) [31].

Based on the obtained results, three indicators were calculated: the nitrogen uptake (Nu) by the plant, the utilization of nitrogen from the applied fertilizer (W_N), and the production efficiency of the applied nitrogen (Ep) [32–34].

The nitrogen uptake by the plant was calculated as the product of the biomass yield and its nitrogen content according to Formula (1):

$$Nu = (Y \times Nc)/1000, \quad (1)$$

where Nu stands for nitrogen uptake (g N pot^{-1}), Y stands for the yield of biomass (g pot^{-1}), and Nc refers to the nitrogen content in the plant (g N kg^{-1}).

The utilization of nitrogen from the applied fertilizer (W_N) was calculated according to Formula (2):

$$W_N = [(P_N - P_0)/N] \times 100\%, \quad (2)$$

where W_N refers to the nitrogen utilization coefficient (%), P_N stands for nitrogen uptake by the crop in the fertilized treatment (g N pot^{-1}), P_0 stands for nitrogen uptake by the crop in the control object (g N pot^{-1}), and N refers to the dose of the nitrogen (g N pot^{-1}).

The yield-forming effect of a fertilizer is the production efficiency of the applied nitrogen (Ep), which was calculated according to Formula (3):

$$Ep = (Y_N - Y_0)/N, \quad (3)$$

where Ep is the production efficiency (yield in g per 1 g of N applied), Y_N stands for the yield in the fertilized treatment (g pot^{-1}), Y_0 refers to the yield in the control object (g pot^{-1}), and N is the nitrogen dose (g N pot^{-1}).

2.5. Statistical Analysis of Data

The obtained results were subjected to statistical analysis using the Dell Statistica program, version 13 (Dell Inc. (Round Rock, TX, USA)). One-way analysis of variance was performed, and the significance of differences between arithmetic means was determined using Duncan's test ($\alpha \leq 0.05$). Pearson's simple correlation coefficients between the examined parameters were calculated, and their level of significance was assessed using the Student's *t*-test.

3. Results and Discussion

3.1. Dry Matter Yield of the Plant

The dry matter yields of the above-ground parts of test plants are given in Table 3.

Table 3. Yields of the dry matter of the above-ground parts of plants (g pot^{-1}).

Treatment	Maize		Spinach		Lettuce	
	Light Soil	Medium Soil	Light Soil	Medium Soil	Light Soil	Medium Soil
C	21.43 ^{a} *	36.63 ^{a}	7.02 ^{a}	12.06 ^{a}	5.00 ^{a}	5.15 ^{a}
I	72.70 ^{d}	101.60 ^{bc}	25.50 ^{b}	38.09 ^{b}	14.17 ^{c}	17.53 ^{c}
II	67.13 ^{c}	82.53 ^{b}	30.43 ^{c}	43.94 ^{c}	13.33 ^{bc}	16.33 ^{bc}
III	65.03 ^{c}	106.27 ^{c}	23.98 ^{b}	38.55 ^{b}	13.73 ^{bc}	21.17 ^{d}
IV	60.07 ^{b}	83.03 ^{b}	28.88 ^{c}	36.98 ^{b}	11.83 ^{b}	14.37 ^{b}

* Values in columns described with the same letter do not differ statistically significantly at $\alpha \leq 0.05$.

The dry matter yield of the above-ground parts of the maize grown on the light soil was the highest when the entire dose of nitrogen in the form of Pulrea® was applied to the soil and the lowest in the control treatment, approximately 3–3.5 times lower than that in the remaining treatments. Soil fertilization with UFF and the foliar application of Pulrea® gave similar yields, which were significantly higher than the yields after the foliar application of UFF. The foliar application of both fertilizers resulted in significantly lower yields (by 10 and 11%, respectively) than those obtained after their soil application.

The yield of the maize grown on the medium soil was higher than that of the maize grown on the light soil (Table 3). The highest yields were recorded after the application of Pulrea®, both to the soil and the foliar application. The maize yields from all fertilized objects were significantly higher than those obtained from the control treatment. There was no statistical difference between the yields when the soil was treated with UFF or Pulrea®. However, the yield of the above-ground parts of this plant was significantly lower after the foliar application of UFF than after the foliar application of Pulrea®.

The maize yield after the UFF treatments was lower than that after the application of Pulrea®. However, when the medium soil was treated with UFF, the difference was insignificant compared to the analogous treatment where Pulrea® was used.

The lowest spinach yield was obtained from the control treatment, and the highest amount of spinach was harvested after UFF was applied to the soil (Table 3). The yield from all fertilized treatments was significantly higher than that from the control treatment. The application of UFF to the medium soil had a significant impact on the spinach yield in relation to its foliar fertilization. The yield from the remaining treatment did not depend significantly on the method of nitrogen fertilizer application.

The dry matter yield of lettuce was lower than the dry matter yields of maize and spinach (Table 3). The lowest yield on both soils was found in the control treatment, which was significantly lower than the yields from all nitrogen-fertilized treatments. The method of application of either fertilizer did not lead to any significant differences in plant yields except in the case of the foliar application of Pulrea®, which gave significantly higher lettuce yields than the yields after soil application of this fertilizer and UFF fertilization.

Generally, the highest yield of spinach on both soils and lettuce on light soil was obtained after soil application of UFF and the highest yield of lettuce on medium soil was obtained after the foliar application of Pulrea®.

The obtained research results confirmed those achieved by other authors, who examined the impact of urea-containing fertilizers when applied in the pre-sowing phase or the pre-sowing and top dressing phase to the soil or the foliage on the yield of various plant species [35–39].

3.2. Content of Total Nitrogen and Nitrate Nitrogen in Plants

The total nitrogen content and the nitrate nitrogen content in the dry matter of maize, spinach, and lettuce grown on both soils are given in Table 4.

Table 4. Nitrogen content in the biomass of plants grown on light and medium soils (g kg^{-1} d.m.).

Treatment	Maize				Spinach				Lettuce			
	Light Soil		Medium Soil		Light Soil		Medium Soil		Light Soil		Medium Soil	
	N_{total}	NO_3-N	N_{total}	NO_3-N	N_{total}	NO_3-N	N_{total}	NO_3-N	N_{total}	NO_3-N	N_{total}	NO_3-N
C	5.98 a *	1.40 c	5.39 a	1.52 b	11.01 a	1.40 a	16.4 a	3.50 b	12.47 a	1.04 ab	15.84 a	4.49 c
I	18.65 b	0.89 a	15.87 c	1.50 b	38.39 b	3.65 b	38.78 b	2.38 a	31.24 b	1.52 b	36.51 c	2.42 a
II	19.65 b	1.16 b	17.43 c	1.38 ab	34.77 b	3.17 b	36.35 b	2.42 a	28.03 b	1.61 b	35.32 c	2.98 b
III	35.25 c	1.21 b	12.18 b	1.59 b	37.62 b	1.80 a	33.04 b	2.76 a	31.50 b	0.88 a	16.94 a	2.39 a
IV	35.37 c	1.36 c	17.62 c	1.20 a	35.68 b	1.14 a	43.77 b	3.02 b	27.93 b	0.56 a	20.10 b	2.75 b

* Values in columns described with the same letter do not differ statistically significantly at $\alpha \leq 0.05$.

The lowest total nitrogen content was found in maize plants from the control treatments in both soils. There were no significant differences in the nitrogen content in plants from analogous treatments grown on light soil fertilized with both fertilizers and involving both application methods. Plants from treatments involving the foliar application of Pulrea®

or UFF contained significantly more nitrogen than the plants from treatments involving soil fertilization.

The method of applying fertilizers on medium soil had a different impact. When the soil was fertilized with either Pulrea® or UFF, there was no significant difference in the total nitrogen content in plants, but maize from treatments involving foliar UFF fertilization contained significantly more nitrogen than the maize from treatments involving the foliar application of Pulrea®. A significantly higher nitrogen content was found in plants when the soil was fertilized with Pulrea® than after its foliar application, and both UFF application methods had a similar effect on the total nitrogen content in maize.

The biomass of the maize grown on light soil contained less nitrate nitrogen than that on medium soil (Table 4). When light soil was used, the lowest concentration of nitrate nitrogen was found in the above-ground parts of maize when the soil was fertilized with Pulrea®. The highest yield of plants was obtained in this treatment, so there may have been an effect of diluting nitrates where the crop yield was higher. Plants from the remaining treatments contained significantly more nitrates, and the highest concentration was found in the control treatment and after the foliar application of UFF. Foliar fertilization with both fertilizers resulted in a significantly higher accumulation of nitrates than their application to the soil.

Different relationships were recorded on medium soil. There was no statistically significant difference between the nitrate nitrogen contents of the plants from the control treatment and the plants where the soil was fertilized with either of the two fertilizers or foliar fertilization was carried out. Only maize that was given foliar UFF treatment contained significantly less nitrate nitrogen than plants from the other treatments.

The share of NO_3-N in the total nitrogen content was the highest in plants grown on light soil in the control treatment (23.41%). Maize from the remaining treatments had a 4–6 times lower content of nitrates than that observed in plants from the control treatment, as follows: 4.77% when Pulrea® was applied to the soil and 5.90% when UFF was applied to the soil, 3.85% in the foliar application of UFF, and 3.43% (the lowest) when the treatment involved the foliar application of Pulrea®.

On medium soil, the share of NO_3-N in the total nitrogen content was also the highest in plants from the control treatment (28.20%). In the maize biomass from the remaining treatments, the share of nitrates in the total nitrogen content was also 2–4 times lower compared to that in plants from the control treatment, as follows: 13.05% when the treatment involved the foliar application of Pulrea® and 5.90% when the treatment involved the soil application of Pulrea®, 7.92% when UFF was applied to the soil, and 6.81% (the lowest) when the treatment involved UFF foliar application.

The total nitrogen content in spinach and lettuce grown in the control treatment on light soil was significantly lower than in plants from nitrogen-fertilized treatments (Table 4). Spinach plants fertilized with Pulrea® or UFF had a total nitrogen content 3–3.5 times higher than those collected from the control treatment. No significant differences were found in the content of this element in plants from analogous treatments involving Pulrea® or UFF as the fertilizer (both soil and foliar application).

Spinach plants grown on medium soil in the control treatment had a significantly lower total nitrogen content than those harvested from nitrogen-fertilized ones, in which the total nitrogen content was 2 to 3.5 times higher (Table 4). Similarly to spinach grown on light soil, no significant differences were found in the total content of this element in plants from treatments where Pulrea® or UFF was applied (both soil and foliar application).

The content of nitrate nitrogen in spinach grown on light soil was significantly higher after soil and foliar fertilization with Pulrea® than in plants from the control treatment or plants given soil and foliar UFF treatment (Table 4). The method of application of either Pulrea® or UFF led to no significant differences in the N-nitrate contents in the fertilized plants. The content of nitrate nitrogen in the spinach grown on medium soil was significantly lower after the foliar application of both fertilizers than after their application to the soil. The lowest nitrate content was observed in plants subjected to the foliar application of UFF, and the highest (3 times more) was observed in plants after UFF application to the soil.

The total nitrogen content in the dry matter of lettuce grown on light soil as a successor plant to spinach when Pulrea® or UFF was used was significantly higher than that in the dry matter of plants from the control treatment (Table 4). Plants fertilized with nitrogen contained 2 to over 2.5 times more nitrogen than those grown in the control treatment. The method of application of either Pulrea® or UFF did not lead to any significant differences between the total nitrogen contents of the fertilized plants.

The total nitrogen content in the dry matter of lettuce grown on medium soil was less diverse than in spinach (Table 4). Lettuce when Pulrea® and UFF were used to fertilize the soil contained significantly more nitrogen (approximately 1.5 to 2 times more) than plants from the control treatments and plants involving the foliar application of both fertilizers. Significantly more nitrogen was recorded in plants after the foliar application of UFF than from analogous treatment where Pulrea® was used as the fertilizer.

The lettuce biomass collected from the control plants grown on the light soil and after the foliar application of UFF contained significantly more nitrate nitrogen than that grown using other fertilized treatments (Table 4). The lowest amount of nitrates was accumulated by plants where both fertilizers were applied to the soil, but their accumulation did not differ significantly from those recorded in spinach after the foliar application of Pulrea®. Lettuce grown on medium soil in the control contained significantly more NO_3-N than plants collected from the other treatments. The nitrate content in plants was significantly lower after soil or foliar application of Pulrea® compared to plants from analogous treatments where UFF was applied. However, there was no significant effect of the method of application of either fertilizer on the accumulation of nitrates in the lettuce biomass.

The share of NO_3-N in the total nitrogen content was the highest in spinach and lettuce grown on light soil in the control treatment (12.72 and 21.34%, respectively). A lower share of nitrates was recorded in spinach involving the application of Pulrea® (9.51%) and UFF (9.12%) to the soil and more than twice as much after the foliar application of Pulrea® (4.78%) and UFF (3.20%). An inverse relationship was noted in the case of lettuce, in which the share of nitrates in the total nitrogen content was higher after the foliar application of Pulrea® (8.35%) and UFF (6.90%) than after their soil application (6.14 and 6.66%, respectively).

The share of NO_3-N in the total nitrogen content in spinach and lettuce grown on medium soil was also the highest in plants from the control treatments (8.34 and 28.35%, respectively). In the spinach biomass from the remaining treatments, the share of nitrates in the total nitrogen content was lower in the treatments with soil application of UFF (5.74%) and Pulrea® (4.87%) and the lowest after the foliar application of Pulrea® (2.79%) and UFF (2.01%). Similarly to light soil, a higher share of nitrates in the lettuce biomass was recorded after the foliar application of Pulrea® (14.11%) and UFF (13.68%) than after their soil application. It was higher after UFF fertilization (8.44%) than after Pulrea® application (6.63%).

The lowest total nitrogen content was found in all test plants from control treatments. There was no such clear relationship in the case of nitrate accumulation in the plant biomass. In general, compared with plants grown on light soil, more nitrates were accumulated by plants grown on medium soil in control treatments and after foliar nitrogen application. A similar relationship was also noted for maize and lettuce after the soil application of both fertilizers. A higher yield because of nitrogen fertilization may result in lower N-nitrate accumulation as an effect of the so-called dilution.

The highest share of NO_3-N in the total nitrogen content was recorded in plants from control treatments: it was 23.4 and 28.2% in maize, 12.7 and 8.32% in spinach, and 21.3 and 28.3% in lettuce on light soil and medium soil, respectively. On light soil, more nitrates were accumulated in maize fertilized with UFF, and on medium soil, more nitrates were accumulated in those fertilized with Pulrea®, regardless of the method of application. A lower share of NO_3-N in total N was recorded in spinach and lettuce after the foliar application of both fertilizers than when they were applied to the soil.

The results of our own research have confirmed the influence of urea application, especially localized fertilization, on the total content of nitrogen in biomass and nitrate accumulation in plant tissue observed by other authors [37,40,41], and, in consequence, the percentage of NO_3-N in the total N content.

3.3. Nitrogen Uptake with Harvested Yields of Plants

The amount of nitrogen removed with plant yield (nitrogen uptake) can be used to explain the observed relationships.

Nitrogen absorption with yields of all plants is presented in Table 5.

Table 5. Nitrogen absorption with harvested yields of plants.

Treatment	Maize		Spinach		Lettuce		Spinach + Lettuce	
	Light Soil	Medium Soil	Light Soil	Medium Soil	Light Soil	Medium Soil	Light Soil	Medium Soil
	g N pot^{-1}							
C	0.128	0.197	0.077	0.150	0.082	0.082	0.159	0.232
I	1.356	1.612	0.979	1.190	0.550	0.640	1.528	1.830
II	1.319	1.438	1.058	1.232	0.485	0.577	1.543	1.808
III	2.288	1.295	0.902	1.214	0.459	0.359	1.361	1.573
IV	2.126	1.462	1.030	1.033	0.518	0.289	1.548	1.322

All test plants from control treatments took up the lowest amounts of nitrogen. Maize absorbed 0.128–2.288 g N pot^{-1} when grown on light soil and 0.197–1.612 g N pot^{-1} when grown on medium soil. The highest amounts of nitrogen were taken up by plants after the foliar application of both fertilizers when they were grown on light soil, about 17 times more than that taken up by plants from the soil of the control treatment. Maize absorbed 8.2 and 7.4 times more nitrogen from medium soil after soil fertilization with Pulrea® and after the soil and foliar application of UFF, respectively, than from the control treatment.

Spinach absorbed 0.077–1.030 g N pot^{-1} on light soil and 0.150–1.232 g N pot^{-1} on medium soil. The highest amounts of nitrogen were taken up by plants after the foliar application of UFF when the plants were grown on light soil (17 times more than that taken up by plants from the control treatment) and after soil and foliar fertilization with Pulrea® when they were grown on medium soil (about 8 times more than that taken up by plants from the control treatment). On light soil, the use of both fertilizers led to similar effects but lower amounts of nitrogen were absorbed by plants fertilized with Pulrea®. In medium soil, nitrogen uptake depended on the method of application of both fertilizers, and higher amounts of nitrogen were absorbed by spinach when UFF was applied to the soil or after the foliar application of Pulrea®.

Lettuce absorbed 0.082–0.550 g N pot^{-1} on light soil and 0.082–0.640 g N pot^{-1} on medium soil. In light soil, nitrogen uptake after all fertilized treatments was little different and 5–7 times higher than the nitrogen uptake in the control treatment. The highest amounts of N were absorbed by plants after soil fertilization with Pulrea® and the foliar application of UFF. When either of the two fertilizers was applied to the medium soil, the plants took up 7–8 times more N than the control plants. After foliar application of fertilizers, lettuce absorbed approximately 50% less nitrogen than after soil application of fertilizers.

In total, spinach and lettuce took up 0.159–1.548 g N pot^{-1} from light soil and 0.232–1.830 g N pot^{-1} from medium soil. The spinach and lettuce in the control treatments, all treatments fertilized with Pulrea®, and the treatments involving the application of UFF to both soils absorbed more nitrogen in total than maize. After foliar application of these fertilizers, maize removed approximately 1.5 times more N from light soil, maize's absorption from medium soil was lower after the foliar application of Pulrea®, and 10% more N was taken up by this plant after the foliar application of UFF.

3.4. Utilization of Nitrogen from Applied Fertilizers and the Yield-Forming Efficiency of the Nitrogen

An important assessment of the effect of the applied fertilizer is the utilization of nitrogen (W_N) and so is the assessment of the yield-forming effect of a fertilizer as the production efficiency of the applied nitrogen (Ep) [33,34,42,43].

The utilization of nitrogen from the applied fertilizers (W_N) and the production efficiency of the applied nitrogen (Ep) are presented in Table 6.

Table 6. Utilization of nitrogen from applied fertilizers (W_N) and the production efficiency of the applied nitrogen (Ep).

Treatment	W_N				Ep			
	Maize		Spinach + Lettuce		Maize		Spinach + Lettuce	
	Light Soil	Medium Soil	Light Soil	Medium Soil	Light Soil	Medium Soil	Light Soil	Medium Soil
	%							
I	51.2	59.0	57.0	66.6	21.4	27.1	11.5	16.0
II	49.6	51.7	57.6	65.7	19.0	19.1	13.2	17.9
III	90.0	45.7	50.1	55.9	18.2	29.0	10.7	17.7
IV	83.2	52.7	57.9	45.4	16.1	19.3	11.9	14.2

The use of nitrogen by maize grown on light soil from both fertilizers was approximately 70% higher after their foliar application than after their soil fertilization (Table 6). Maize used more nitrogen from Pulrea® than from UFF. The use of nitrogen from Pulrea® applied to medium soil was 23% higher than that after foliar application, and the use of nitrogen from UFF was similar in both application methods. Spinach and lettuce used 22% more nitrogen from Pulrea® fertilizer when it was applied to light soil than after its foliar application. These plants absorbed more nitrogen from UFF than from Pulrea®, regardless of the method of application. The use of nitrogen by these plants from medium soil was higher after both fertilizers were applied to the soil than after their foliar application. Spinach and lettuce used more nitrogen from Pulrea® than from UFF.

The production efficiency of the applied nitrogen (Ep) in the maize biomass yield on light soil was the highest after Pulrea® was applied to the soil, and both fertilizers were more effective when they were applied to the soil than when they were applied as a foliar spray (Table 6). On medium soil, Pulrea® was the most effective fertilizer as a foliar application and slightly less effective when applied to the soil. The efficiency of nitrogen used as UFF was approximately 30% lower. In the case of spinach and lettuce, the use of UFF led to a better yield effectiveness than the use of Pulrea®. On medium soil, Pulrea® applied as a foliar spray was more effective and UFF was more effective when applied to the soil.

Trawczyński [2] found that the amount of yield obtained and the yield-forming efficiency when nitrogen is applied in the form of fertilizers containing the amide form is slightly lower than that reported in the case of a slow-release fertilizer but more effective than fertilizers containing the ammonium or nitrate form of nitrogen [2]. In his research, nitrogen productivity also depended on the timing of fertilizer application. Furthermore, he reported significantly higher nitrogen efficiency when the total dose of this element was applied before sowing than when it was applied in a divided dose, especially when using slower-acting fertilizers.

3.5. Soil Properties

3.5.1. The pH and Electrolytic Conductivity Values of the Soil after the Plants Are Harvested

The pH and electrolytic conductivity (EC) values of the soil after the harvest of the maize and lettuce grown on both soils are given in Table 7.

Table 7. The pH and electrolytic conductivity (EC) values of the soil after the harvest of maize and lettuce.

Treatment	pH_{KCl}				EC ($\mu S\ cm^{-1}$)			
	Maize		Lettuce		Maize		Lettuce	
	Light Soil	Medium Soil	Light Soil	Medium Soil	Light Soil	Medium Soil	Light Soil	Medium Soil
C	5.64 ^{b} *	5.78 ^{d}	5.78 ^{c}	6.03 ^{c}	191 ^{a}	200 ^{b}	201 ^{a}	515 ^{a}
I	5.07 ^{a}	5.14 ^{b}	5.11 ^{a}	5.18 ^{a}	211 ^{ab}	179 ^{a}	1125 ^{c}	863 ^{b}
II	5.01 ^{a}	4.91 ^{a}	5.10 ^{a}	5.44 ^{b}	201 ^{ab}	168 ^{a}	699 ^{b}	577 ^{a}
III	5.08 ^{a}	5.29 ^{c}	5.27 ^{b}	5.42 ^{b}	273 ^{b}	221 ^{c}	695 ^{b}	1157 ^{c}
IV	5.19 ^{a}	5.39 ^{c}	5.30 ^{b}	5.46 ^{b}	225 ^{ab}	225 ^{c}	804 ^{b}	882 ^{b}

* Values in columns described with the same letter do not differ statistically significantly at $\alpha \leq 0.05$.

The applied fertilization significantly decreased the pH value of light and medium soils after maize harvest compared to the soil of the control treatment (Table 7) and the soil determined at the beginning of the experiment, amounting to 5.84 and 6.16, respectively (see Table 1). No significant differences were found in the acidification of light soil as a result of the fertilizers used or their application method compared with that from individual treatments using Pulrea® or UFF.

The pH value of medium soil after maize harvest varied more as a result of nitrogen fertilization than that of light soil. The pH value was significantly lower after UFF was applied to the soil than after Pulrea® was used. The soil when foliar treatments of Pulrea® or UFF were carried out showed significantly higher pH values than after their application to the soil (Table 7).

The pH value of light soil after the harvest of the lettuce grown as a successor plant after spinach was significantly lower in the case of all fertilized treatments than that of the soil of the control treatment. However, these changes were smaller than after maize harvest. Soil application of Pulrea® or UFF reduced the soil pH value to the greatest extent, and this effect was significantly stronger than when the foliar application of these fertilizers was used (Table 7).

The pH value of the medium soil after the harvest of the lettuce grown in all fertilized treatments was significantly lower than that of the soil of the control treatment. No significant differences in pH values were noted under the influence of Pulrea® or UFF fertilization, except for a significant change after the soil application of Pulrea®, which caused the strongest soil acidification (Table 7). The reaction of light and medium soils after the harvest of both maize and lettuce plants changed from slightly acidic in the control treatment to acidic in the remaining treatments as a result of nitrogen fertilization.

The applied nitrogen fertilization increased the acidification of the soil after the plants were harvested, assessed in relation to the pH value of the soil used in the experiment and to the pH value found after the harvest of the plants in the control treatment, without fertilization. In relation to light and medium soil, respectively, the pH value after maize harvest decreased by 0.20 and 0.38 units in relation to the initial pH value and in others treatments by 0.45–0.63 and 0.39–0.87 units compared to the soil from the control treatment. Once the lettuce grown after the spinach was harvested, the pH value of the light soil from the control treatment decreased by 0.06 units and that of the medium soil by 0.13 units in relation to the initial pH values. The pH of the soil from the remaining treatments decreased, respectively, by 0.5–0.7 and 0.6–0.8 units compared to the pH of the soil from the control treatment. The strongest acidification resulted when each fertilizer was applied to the soil.

The main cause of soil acidification after the application of both tested fertilizers containing urea is the ongoing transformation of the amide form, a component of urea, in the soil [44]. The NH_2-N form is initially enzymatically hydrolyzed to NH_3-N, which increases the pH value. NH_3-N, in turn, is oxidized to NO_3-N, causing a pH decrease [45]. These fertilizers are called physiologically acidic. The share of amide fertilizers or other fertilizers containing this form of nitrogen (e.g., UAN series, that is, urea ammonium nitrate solutions) in the total mass of fertilizers used in Poland is relatively high and continues to increase [46,47]. The application of high rates of nitrogen in the form of urea leads to a decrease in the soil pH value [36,48], especially when urea is used over a long period as a fertilizer, but at the same time, it may reduce the rate of urea hydrolysis [49]. By predicting changes in soil pH in response to the use of urea and urine, NH_3 losses can be initially estimated [48]. These losses can be effectively reduced by using slow-release fertilizers [50]. On the other hand, Martikainen [51] found that ammonium sulfate added to forest soil inhibited nitrification and slightly decreased soil pH value but urea application did not inhibit nitrification and had an adverse effect on pH.

The value of electrolytic conductivity (EC) of light soil after the harvest of maize from the control treatment was the lowest but did not differ significantly from the values recorded in the soil fertilized with Pulrea® or UFF (Table 7). This parameter had the highest value in the soil after the foliar application of Pulrea®, and it was significantly higher than in the soil of the control treatment. The electrolytic conductivity of the medium soil after the harvest of the maize from the soil treated with both fertilizers was significantly lower than that of the soil from the control treatment. The electrolytic conductivity of the soil after foliar application of both fertilizers was significantly higher than that of the soil from the control treatment and the soil to which the fertilizers had been applied (Table 7).

The electrolytic conductivity (EC) of the light soil after the harvest of lettuce in the control treatment was 3.5 to 5.5 times lower than that of the light soil treated with fertilizers (Table 7). Fertilization significantly increased the EC value of the soil in all treatments, especially after soil fertilization with Pulrea®. The EC value of the soil from other nitrogen-fertilized objects did not differ significantly. In the control group, after lettuce grown on medium soil was harvested, the lowest value of electrolytic conductivity was found (Table 7). Soil UFF fertilization did not significantly change the EC value. Significantly higher values of this parameter were recorded in the soil after the soil application of Pulrea® and after the foliar application of both fertilizers. The highest value of electrolytic conductivity was recorded after the foliar application of Pulrea®, significantly higher than in the other treatments.

There was little difference in the values of electrolytic conductivity of both soils after maize harvest, and the EC value of the average soil decreased when both fertilizers were applied to the soil. Generally, the EC value of soils from unfertilized control treatments was the lowest, and in both soils it was many times lower after maize harvest than after lettuce harvest. Both soils after maize harvest and the medium soil after lettuce harvest from treatments involving the foliar application of both fertilizers showed higher electrolytic conductivity than after the application of the fertilizers to the soil.

In his research, Martikainen [51] noticed that the electrical conductivity of the soil increases much more as a result of the use of ammonium sulfate than after fertilization with urea, which does not belong to the salt group. However, the inhibition of nitrification because of the application of a salt does not result in an increase in osmotic pressure and an increase in the EC value. Much lower values of soil electrolytic conductivity, 129–245 $\mu S\ cm^{-1}$, were found by Lisowska et al. [52] in soil with sulfur addition, regardless of the liming applied. Kiełbasa et al. [53] compared the differences in soil electrolytic conductivity based on humidity and soil texture and found much lower values, ranging from several to several dozen $\mu S\ cm^{-1}$, 1 or 2 orders of magnitude lower than those recorded in our studies. The authors mentioned above showed higher EC values in the case of light soils and with higher soil moisture. In our research, the humidity of both soils was maintained at the same level throughout the experiment. Therefore, it did not have a significant impact on the EC value. In no case did soil EC reach the value of 4000 $\mu S\ cm^{-1}$, indicating soil salinity [54].

3.5.2. The Contents of Mineral Forms of Nitrogen in the Soil after the Harvest of Plants

The contents of mineral forms of nitrogen, NH_4-N and NO_3-N, in soils after the harvest of plants is presented in Table 8.

Table 8. Contents of ammonium and nitrate nitrogen in the soil after the harvest of maize and lettuce.

Treatment	Maize				Lettuce			
	Light Soil		Medium Soil		Light Soil		Medium Soil	
	NH_4-N	NO_3-N	NH_4-N	NO_3-N	NH_4-N	NO_3-N	NH_4-N	NO_3-N
	mg in 100 g of Soil							
C	0.98 ^{a} *	0.79 ^{a}	5.09 ^{ab}	1.87 ^{a}	4.62 ^{a}	2.52 ^{a}	5.60 ^{ab}	1.35 ^{a}
I	2.45 ^{c}	1.05 ^{ab}	4.34 ^{a}	2.45 ^{ab}	7.84 ^{c}	5.37 ^{b}	7.37 ^{b}	4.83 ^{c}
II	2.15 ^{c}	1.54 ^{b}	6.37 ^{b}	2.29 ^{ab}	7.47 ^{c}	7.35 ^{c}	8.63 ^{c}	4.90 ^{c}
III	1.63 ^{b}	0.64 ^{a}	4.43 ^{a}	2.33 ^{ab}	6.49 ^{b}	4.11 ^{ab}	4.29 ^{a}	2.57 ^{b}
IV	1.63 ^{b}	0.70 ^{a}	7.75 ^{c}	3.69 ^{b}	10.15 ^{d}	7.47 ^{c}	5.37 ^{ab}	7.07 ^{d}

* Values in columns described with the same letter do not differ statistically significantly at $\alpha \leq 0.05$.

The use of both fertilizers resulted in a significantly higher NH_4-N content in the light soil after maize harvest compared to the control treatment, especially after their application to the soil (Table 8). After UFF application to the soil, NO_3-N concentration was significantly higher, and after foliar application of both fertilizers, the nitrate-N content was lower than in the soil of the control treatment. The NH_4-N content in the medium soil after maize harvest in the treatments involving UFF was significantly higher than that in the treatments involving Pulrea® fertilization, especially after foliar application. Pulrea® application resulted in a lower NH_4-N content in the soil than that found in the control treatment. After the foliar application of UFF, the soil contained significantly more NO_3-N than the soil samples from the control treatment and more than the soil from other nitrogen-fertilized treatments.

The highest amounts of NH_4-N and NO_3-N were recorded in the light soil after lettuce harvest as a result of the foliar application of UFF, and the content was significantly higher than in the soil from other treatments. After the foliar application of Pulrea®, the soil contained the lowest amounts of both forms of nitrogen among the nitrogen-fertilized treatments. After lettuce harvest, the medium soil fertilized with UFF contained significantly more NH_4-N than the soil samples collected from the other treatments. Significantly more of this form of nitrogen in the soil was recorded after the application of both fertilizers to the soil than after their foliar application. The soil after the foliar application of Pulrea® contained the lowest amounts of NH_4-N. The application of both fertilizers resulted in soil with a significantly higher content of NO_3-N than the soil of the control treatment, and the content of this form of nitrogen in the soil after the foliar application of UFF was significantly higher than in the soil from the other treatments. After the foliar application of Pulrea®, the soil contained the lowest amounts of NO_3-N among the nitrogen-fertilized treatments.

The total content of mineral nitrogen (N_{min} = NH_4-N + NO_3-N) in both soils after the harvest of maize and lettuce indicated that the lowest amounts of these forms were in the soil of the control treatments (Figure 1).

The light soil contained 4 to 7.5 times less N_{min} after the harvest of maize than after the harvest of lettuce, which confirmed the high nutritional requirements of maize and the depletion of available forms of nitrogen in the soil [55]. The soil and foliar application of urea resulted in lower N_{min} content than the application of UFF fertilizer using analogous methods.

The total content of the mineral forms of nitrogen (N_{min}) in the medium soil after maize harvest indicated that higher amounts of these forms were present in the soil after the soil and foliar application of UFF. Similarly, the total content of the mineral forms of nitrogen (N_{min}) in the medium soil after lettuce harvest indicated that higher amounts of these forms were present in the soil after the application of Pulrea® with both methods and after the foliar application of UFF (Figure 1).

After the harvest of maize and lettuce, the medium soil contained similar amounts of N_{min}, and only after lettuce was harvested from soil to which both fertilizers were applied was this content 1.6–1.8 times higher.

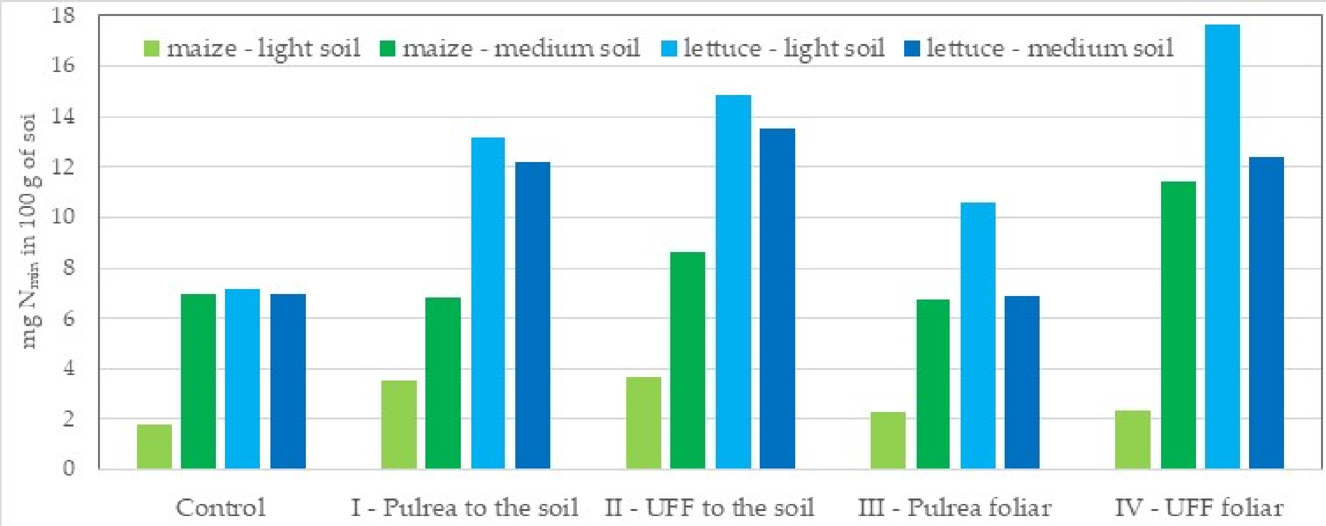

Figure 1. Contents of mineral forms of nitrogen (N_{min}) in soils after the harvest of maize and lettuce.

Nowak and Sowiński [56], examining the effect of nitrogen fertilization on the content of mineral nitrogen in the soil, noticed that the NH_4-N form constitutes on average 64% of the mineral nitrogen in the soil. Our own research confirmed such a relationship. In light soil, after maize cultivation, the NH_4-N form generally dominated, which accounted for 55% of N_{min} in the soil from the control treatment and 58–72% after the application of both nitrogen fertilizers. The share of NH_4-N in N_{min} in the medium soil from the control treatment amounted to 73%, and from all nitrogen-fertilized treatments, it amounted to 64–74%, the highest after the soil application of UFF.

In the light soil from the control treatment after the cultivation of spinach and lettuce, NH_4-N constituted 65% of N_{min}. In the soil from the remaining treatments, the share of NH_4-N in N_{min} was lower and accounted for 50–61%, with a larger share of NH_4-N in the soil after the foliar application of both nitrogen fertilizers. The highest share of NH_4-N in N_{min} was recorded in the medium soil from the control treatment and amounted to 81%. In the soil from the remaining treatments after lettuce harvest, the share of NH_4-N in N_{min} was 43–64% and was the lowest after the foliar application of UFF.

The content of mineral forms of nitrogen (sum of NH_4-N and NO_3-N) in both soils from the control treatment after lettuce harvest was the lowest, and changes in their content as a result of fertilization were similar to those after maize harvest. The only exception was the soil of the treatment with the foliar application of UFF, in which the N_{min} content after lettuce harvest was almost two times lower in relation to the control treatment than that after maize harvest.

Changes in the content of the mineral forms of nitrogen may be related to the amount of root mass produced by test plants and the mineralization of organic matter [57]. They are closely related to the content of organic matter that is relatively easily mineralized [58]. Bednarek and Tkaczyk [59] showed that only nitrogen fertilization caused significant increases in the content of NH_4-N and NO_3-N in the soil but that their stability in the soil is low.

3.5.3. Activity of Dehydrogenases in the Soil after the Harvest of Plants

The activity of dehydrogenases in the soil after plant harvest is presented in Table 9.

The activity of dehydrogenases is an indicator of the intensity of the respiratory metabolism of microorganisms, primarily bacteria and actinomycetes [60,61], and thus a good indicator of soil biological activity. Determining the activity of these enzymes in soil is a common way to assess factors that adversely affect soil microorganisms. The conducted experiment did not demonstrate a significant adverse effect of the fertilization applied on this soil parameter (Table 9).

Table 9. Activity of dehydrogenases in light and medium soil after the harvest of maize and lettuce.

Treatment	Maize		Lettuce	
	Light Soil	Medium Soil	Light Soil	Medium Soil
	μg TPF g^{-1} h^{-1}			
C	0.874 [ab] *	0.544 [a]	0.671 [ab]	0.346 [ab]
I	0.659 [a]	0.520 [a]	0.509 [a]	0.223 [a]
II	0.781 [a]	0.586 [a]	0.486 [a]	0.270 [a]
III	0.711 [a]	0.570 [a]	0.576 [ab]	0.570 [c]
IV	0.805 [a]	0.561 [a]	0.822 [b]	0.434 [b]

* Values in columns described with the same letter do not differ statistically significantly at $\alpha \leq 0.05$.

The obtained values of the activity of dehydrogenases are similar to the results of a long-term experiment conducted on grasslands in mountain conditions, which were within the range 11.6–20.6 μg TPF g^{-1} d^{-1} [62]. The highest values of this parameter were observed in the soil when balanced NPK fertilization and liming were applied.

In a previous study, it was shown that changing soil oxygenation significantly modifies the activity of dehydrogenases [63]. This was confirmed in the discussed experiment because the activity of dehydrogenases in light soil after maize harvest was 1.25 to 1.61 times higher and after lettuce harvest was 1.01 to 2.28 times higher than in medium soil. The lowest value of this proportion was recorded in the soil after lettuce harvest with the foliar application of Pulrea®. Numerous literature data indicate that the dynamics of processes catalyzed by soil microorganisms and the activity levels of their enzymes are closely related to factors that have a large share in shaping biological parameters in the soil, including agrotechnical treatments, plant rotation, content of organic matter and available forms of carbon and nitrogen, optimal fertilization, irrigation, and the hydrothermal conditions, which determine the favorable properties of the soil [64–66]. The root system of maize leaves more organic matter in the soil than the root systems of spinach and lettuce. Hence, after the harvest of maize, the activity of dehydrogenases in light soil was 1 to 1.61 times higher and in medium soil 1 to 2.33 times higher than after the harvest of lettuce grown after spinach.

The activity of dehydrogenases in soil is a good indicator of biological activity as well as soil quality [67,68]. Enzymes catalyze a number of biochemical reactions, such as the decomposition of organic matter in the soil, the availability of mineral forms of nutrients for plants, and the detoxification of toxic substances. However, they are sensitive to the influence of various environmental factors, such as humidity, temperature, soil pH, and soil salinity, which can explain the changes in dehydrogenase activity as a result of fertilization [66,69,70]. The activity of dehydrogenases is an indicator of the redox system in the soil and a measure of the respiratory activity of soil microorganisms. Therefore, the activity of dehydrogenases indicates the presence of physiologically active microorganisms in the soil, including decomposing organic matter [71].

3.6. Correlation Dependencies

Maize yield on light soil was negatively correlated with soil pH ($r_{0.05} = -0.982$). Lettuce yield was negatively correlated with soil pH (for light soil $r_{0.05} = -0.966$ and for medium soil $r_{0.05} = -0.873$). The total nitrogen content in the biomass of lettuce was positively correlated with the content of mineral forms of nitrogen in the soil ($r_{0.05} = 0.926$). The nitrate-N content in the biomass of maize grown on medium soil was negatively correlated with the N_{min} content in the soil ($r_{0.05} = -0.973$) and positively correlated with the dehydrogenases activity ($r_{0.05} = 0.886$) in the soil and the pH value ($r_{0.05} = 0.884$) of the soil after the harvest of the plant. Nitrogen uptake with the biomass yield of lettuce grown on light soil was negatively correlated with the soil pH value ($r_{0.05} = -0.948$) after the plant was harvested.

In our own research, no correlation was found between the activity of dehydrogenases and soil properties, especially the soil pH value, as noted by Błońska and Januszek [72].

4. Conclusions

The yields of plants fertilized with Pulrea® were generally higher than those fertilized with UFF. However, the differences between the treatments in the yields were not always statistically significant. The effect of UFF on the yield of spinach grown on light soil was more favorable than that of urea, and on medium soil, both fertilizers had a similar effect. Higher lettuce yields were obtained after the use of Pulrea® than after the use of UFF. Fertilization increased the total N content in the plant. At the same time, the nitrate content decreased in maize and lettuce and its accumulation increased in spinach, especially after UFF application. The utilization of nitrogen from fertilizers was 45–90% and, in general, more nitrogen was absorbed by plants when Pulrea® was used compared with UFF. The production efficiency of 1 kg of nitrogen applied was higher after the use of Pulrea® than after the use of UFF and higher in medium than in light soil. The use of both fertilizers resulted in the acidification of both soils. Pulrea® and UFF fertilization increased the electrolytic conductivity of the soil, but the EC value did not indicate soil salinity. The content of mineral forms of nitrogen generally increased after the application of both fertilizers. Soil fertilization with urea had a more pronounced effect in this direction than soil fertilization with UFF. The use of UFF increased the total content of the mineral forms of nitrogen in the soil, and Pulrea® fertilization had a smaller effect. The activity of dehydrogenases in both soils generally did not change significantly or decrease after the application of either fertilizer. Significantly higher dehydrogenase activity was recorded in medium soil after the foliar application of urea and to a lesser extent after the foliar application of UFF. This may indicate a disruption of the absorption of nitrogen from this fertilizer in relation to the absorption of nitrogen from Pulrea®, perhaps due to the accompanying substances that it contained. Based on the research carried out, it was not possible to clearly state which one of the tested fertilizers had a better effect on plant yields and parameters characterizing plant and soil properties. These relationships are conditioned by the plant species and the properties of the soil used in the experiment. Generally, less favorable effects of UFF on plant yields were observed, and the assessed parameters may indicate the necessity to remove the accompanying substances whose action may have adverse effects on plants and soil microorganisms. The impact of these substances on plant yields may be a factor limiting the use of UFF as a source of nitrogen in fertilization and the management of this by-product in a circular economy. This aspect needs to be investigated under controlled conditions in field experiments.

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