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Assessing the Effects of Whey Hydrogel on Nutrient Stability in Soil and Yield of *Leucosinapis alba* and *Hordeum vulgare*

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Abstract: Agriculture and related crop production are highly dependent on climate and economic factors, and agricultural intensification is associated with a dramatic increase in the consumption of fertilizers. A significant amount of the elements from the most commonly used fertilizers is degraded and lost due to climatic and environmental factors. The soil application of novel whey-based hydrogel represents an innovative approach toward efficient fertilizing and soil water balance that resonates with the concepts of sustainable agriculture and circular economy of waste products. Results of previous research show the positive effect of whey-based hydrogel on water retention after the various levels of hydrogel have been applied into artificial soil. With a view to verifying the effect of the whey hydrogel on soil quality and related crop cultivation in real conditions, the pot experiment compared two different doses of whey hydrogel with control soil, with the conventional NPK treatment of soil and with a mixing strategy combining the conventional NPK treatment with hydrogel application. The controlled pot experiment was conducted with haplic Cambisol, with white mustard (*Leucosinapis alba*) and spring barley (*Hordeum vulgare*) as the testing crops. Soil pH, organic carbon (C), total nitrogen (N), available forms of the essential macronutrients (P, K, Ca, and Mg), and the cation exchange capacity (CEC) were determined in Cambisol samples before and after the experiment. The crop yields of barley and mustard were compared among the various treatments of experimental soils. Results demonstrated that the amendment of whey-based hydrogel increased the bioavailable nutrients' concentrations, which persisted even after the harvest. The nutritional quick boost after the whey-based (co)-application significantly increased the experimental crop yield.

Keywords: hydrogel; whey; agricultural production; soil quality; yield; white mustard; spring barley

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1. Introduction

The input of fertilizer, together with new technologies for nutrient release, generated great plant production and economic development [1]. However, the environmental impacts are huge, and excessive use of fertilizers has led to soil and air quality degradation, (ground) water pollution, and the accrual of fertilizer prices, raising pressure on sustainability in agricultural development [2–4].

Interest in sustainability in agriculture comprises environmentally friendly thinking and agricultural practices that lead toward a productive, economical, and socially needed agriculture [5–7].

Sustainable agriculture should seek management techniques that enhance biomass yield and stability without degrading current or future soil health [8]. Since the yield of plant production can be affected by many environmental factors [9], the ideal fertilizer is one with flexible utilization that addresses minor deficiencies or has immediate stimulative effects while keeping economic benefits and avoiding adverse environmental effects [10].

Hydrogels are produced by the chemical modification of water-receptive polymers and transformed to the tridimensional form [11]. Hydrogels have a considerable ability to imbibe moisture [12–14] and their use in the field of agriculture offers several benefits for soil, improving hydrophysical parameters and reducing the leaching of soil nutrients and dehydration in crops [15,16]. However, frequently used acrylate-based products are characterized by low biodegradability and recondite impact on the soil environment [17].

A number of studies have been published concerning environmentally friendly hydrogels based on chitosan [18,19], starch [20], cellulose [21,22], lignin [23], tragacanth gum [24], kernel gum [25], and others [26,27]. These materials offer multiple advantages over synthetic polymers [28,29].

Most studies focus on the water absorption capacity of soil. Results of practical soil testing were presented by Demitri [22] and Montesano [11], who proved the increase in the water absorption capacity of experimental soil after the application of cellulose-based hydrogel. Nowadays, research also focuses on the multiple advantages of hydrogels in agriculture, as they have the abilities to hold water and release fertilizing nutrients slowly [30,31]. Due to the heterogenous structure of the soil, irregular irrigation followed by low fertilizer retention was noted [32,33]. A composite hydrogel based on gum arabic was presented as an alternative phosphorus source in nutrient-deficient agricultural soils [34]. Similarly, a cellulose-based nanocomposite hydrogel with encapsulated nitrogen fertilizer has lately been introduced to improve the continuous release of nitrogen [35]. Various natural/synthetic polymeric materials are combined with fertilizers, via immobilization, coating, or encapsulation, into controlled-release fertilizer systems to allow a slow nutrient release under various soil conditions [36,37]. A modified hydrogel based on gum arabic was adapted for the slow release of potassium, phosphate, and ammonia [38].

Among natural materials, the high nutrient content in acid whey, a dairy industry by-product, gives it the potential to be repurposed as an efficient fertilizer [39]. Direct whey valorization has been long-term investigated as a fertilizer [40–43] to find a sustainable way for its use [44], due to worldwide production of 115 million metric tons annually [45].

A few studies are available that consider the soil application of whey-based hydrogel and the fertilizing potential created by its composition. In a previous study [46,47], acid whey was applied as a matter mixed with carboxymethylcellulose sodium salt and hydroxyethyl-cellulose, crosslinked with lemon acid to evolve a novel biodegradable hydrogel, with the intention of obtaining an efficient soil treatment with a high water-holding capacity that would enable optimal conditions to improve soil quality in terms of water conservation and nutrients provided. The experimental results with artificial soil clearly showed its effect on improving the water holding capacity with no unfavorable secondary effects for chemical and physical soil properties. At present, the main focus and concern of our research activities is to prove the appropriateness of whey-based hydrogel for improving natural soil quality and the related yield of selected crops in experimental field conditions with natural soil, and hence confirm the usability of whey hydrogel for exploitation in agriculture practices.

2. Materials and Methods

2.1. Sampling of Soil and Analysis

The soil under the long-term agricultural pilot area (VÚMOP, v.v.i., Prague, Czech Republic) was sampled for the experiment. According to the World Reference Base for Soil Resources, IUSS Working Group WRB, 2015 [48], the soil is classified as "haplic Cambisol". Cambisols are among the most common soil types found in the croplands of the Czech Republic. After the field work, the obtained soil was transported to the central laboratory of the Research Institute for Soil and Water Conservation and subjected to chemical analyses before the actual establishment of the pot experiment.

Soil pH was analyzed by a traditional method [49] for the routine analysis of pH by using a glass electrode in a 1:5 (solid fraction) suspended matter of soil in water (pH_{H2O}); the amount of oxidizable carbon was found by sulfochromic oxidation in agreement with ISO 14235 [50] and total nitrogen by the modified Kjeldahl method in agreement with ISO 11261 [51]. The available nutrient status of the soil was examined using the bioavailable forms of the basic macronutrients (P, K, Ca, and Mg) after traditional extraction by Mehlich III solution [52]. Mehlich III is a weak acid material extraction commonly used to extract bioavailable nutrients in different materials; it is standardized as a conjunction of five chemicals at an attenuation rate of 1:10 (0.2 mol.L^{-1} glacial acetic acid, 0.25 mol.L^{-1} ammonium nitrate, 0.015 mol.L^{-1} ammonium fluoride, 0.013 mol.L^{-1} ethanoic acid, and 0.001 mol.L^{-1} ethene diamine four acetic acid). Inductively coupled plasma optical emission spectrometry (ICP-OES) was used to analyze the extracts from the soil samples. As an additional property of the soil for the potential of availability of nutrients, the cation exchange capacity (CEC) was determined by using barium chloride solution buffered at pH = 8.1, using triethanolamine [53]. Table 1 lists the mentioned methods.

Table 1. Overview of the analyzed properties and used methods.

	Method	Reference	Accuracy (% rel.)
pH	determination of pH	ISO 10390 [49]	4–5
C	oxidimetric method	ISO 14235 [50]	10–15
N	modified Kjeldahl method	ISO 11261 [51]	15–20
P	Mehlich III solution	Mehlich (1984) [52]	20
K	Mehlich III solution	Mehlich (1984) [52]	20
Ca	Mehlich III solution	Mehlich (1984) [52]	20
Mg	Mehlich III solution	Mehlich (1984) [52]	20
CEC	barium chloride solution	ISO 13536 [53]	20

2.2. Adjustment of Hydrogel and Analysis

Carboxymethyl cellulose natrium salt (Sigma Aldrich No.C5013, St. Louis, MO, USA) and 2-hydroxyethyl cellulose (Sigma Aldrich No. 434973) (3%) with a mass ratio of 3:1 were resolved in acid whey (a sideline product of the dairy industry) to acquire the material for hydrogel preparation (Figure 1). The lemon acid in dehydrated form (Lach-Ner, Neratovice, Czech Republic) was supplemented at a dosage compared to 10% by weight as a cross-linking element. The whey hydrogel samples were prepared in accordance with the methodology presented by Durpekova et al. [47]. The hydrogel samples were analyzed by the standard methods listed above (Table 1) in the central laboratory of the Research Institute for Soil and Water Conservation.

2.3. Pot Trial

The outside pot trial was conducted in 2022 at the Research Institute for Soil and Water Conservation, Czech Republic. The plants were sown in May and harvested in September. The square pots, which were 0.30 m in depth, with five 1.0 cm diameter holes at the base, were filled with 4 kg of material each. Haplic Cambisol with no treatment was used as control soil K1. For other variants, Cambisol was mixed with 1% of whey hydrogel (H1), 2% of whey hydrogel (H2), 1% of whey hydrogel with NPK (HN1), and finally with NPK only (N1) (Figures 1 and 2). The pot experiment was conducted in an outdoor setting in triplicate repetition with randomized location of pots to minimize the effects of factors not under direct experimental control.

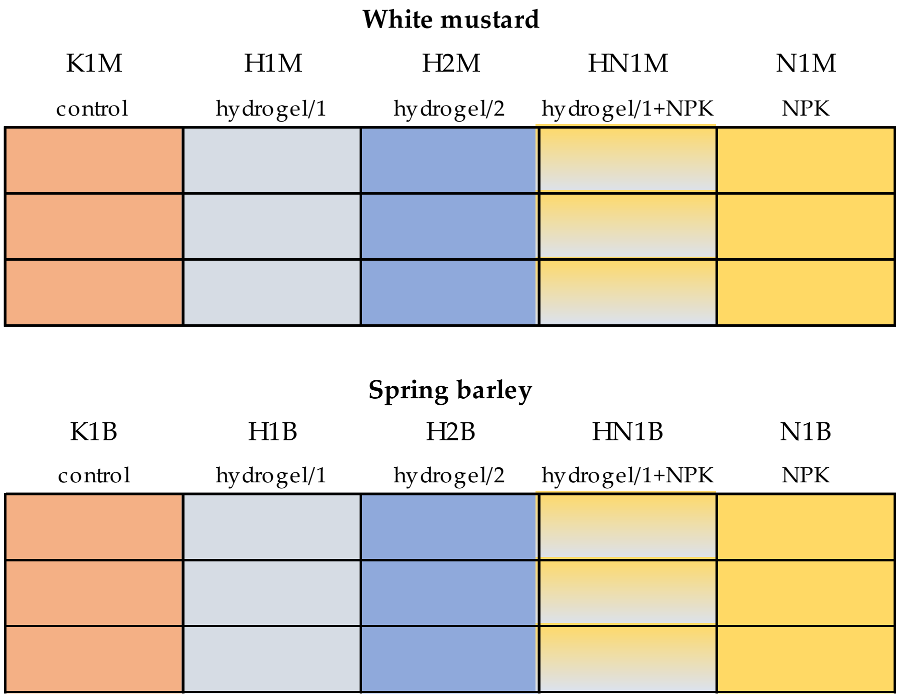

Figure 1. Pot experiment variants.

Figure 2. Experimental area, Research Institute for Soil and Water Conservation, part of experiment.

Fertilizers that contain three major nutrients, NPK, are known by a variety of terms. In our experiment, we used the common fertilizer NPK 11-7-7, composed of 11% N + 7% P_2O_5 + 7% K_2O, intended for basic fertilization. The fertilizer is suitable for treating slightly acidic and neutral soils. NPK fertilizer was added at the rate of 70 $g.m^{-1}$.

Soils were sampled before the experimental plant sowing and basic chemical properties were determined. Two agricultural plants were used in this study: white mustard (*Leucosinapis alba*) and spring barley (*Hordeum vulgare*). The plants were sown using a seed rate of 5 × 2 seeds per pot. Pots received irrigation after sowing, and optimal equable irrigation was assured by a special cultivation method using a seed bed with a lockable bottom (Figure 2). The barley germinated three days after sowing and the mustard after five days. The germination capacity was more than 90%. The seedlings were thinned to achieve five seedlings per pot. After the vegetation, the plants were harvested and laboratory treated to determine dry matter and biomass yield. Drying was carried out in the Memmert UF160 drier at 75 °C for 36 h.

2.4. Data Analysis

Soil and plant samples were assayed in three repetitions for each variant. The obtained data were submitted to a normal distribution analysis using the Shapiro–Wilk normality test. Single-way ANOVA was used to examine the significance of differences in the soil measurements using Statistica software (Version 10 for Windows; StatSoft, Inc., Tulsa, OK, USA). Statistical significance was considered to be at $p < 0.05$ unless otherwise stated.

3. Results and Discussion

3.1. Hydrogel and Soil Characterization

The characterization of prepared whey-based hydrogel was determined before the experiment was set up; the measured pH_{H2O} was 4.17 and the analysis of nutrient amounts indicated high content and availability of three macronutrients (potassium, phosphorus and calcium) and relatively low amounts of total nitrogen (Table 2). Whey-based hydrogel is characterized by a high level of cation exchangeable capacity, which gives it the potential to increase the availability of nutrients in the soil. The control soil is characterized as Haplic Cambisol with sabulous-loam texture and its basic characteristics are given in Table 3.

Table 2. Whey hydrogel characteristics.

Properties	Hydrogel Characteristics	
pH_{H2O}		4.17
C	%	40.1
N	%	1.26
P	$mg \cdot kg^{-1}$	6683
K	$mg \cdot kg^{-1}$	13.059
Ca	$mg \cdot kg^{-1}$	6093
Mg	$mg \cdot kg^{-1}$	806
CEC	$mmol \cdot 100\ g^{-1}$	82.2

Table 3. Haplic Cambisol (control) characteristics.

Soil Properties	Haplic Cambisol Characteristics	
pH_{H2O}		6.80
C	%	0.66
N	%	0.10
P	$mg \cdot kg^{-1}$	113
K	$mg \cdot kg^{-1}$	164
Ca	$mg \cdot kg^{-1}$	2370
Mg	$mg \cdot kg^{-1}$	204
CEC	$mmol \cdot 100\ g^{-1}$	14.8

Control soil is characterized by a weakly acidic soil reaction, a very low content of organic matter, and nutrient contents (N, P, K, Ca, and Mg) at the average level of agricultural soils in the Czech Republic; these pedological properties cover the most common soil conditions in the Czech Republic.

3.2. Soil Quality after Whey Hydrogel and NPK Addition

The addition of 1% whey hydrogel (H1), 2% whey hydrogel (H2), 1% whey hydrogel and NPK (HN1), and NPK (N1) showed a slight effect on soil acidity. Except for NPK, the additions significantly decreased soil pH_{H2O}. Soil pH dropped after whey hydrogel application from 6.83 (control) to at least 6.24. The lowest soil pH was determined after the introduction of 2% whey hydrogel, followed by 1% whey hydrogel mixed with NPK (Figure 3).

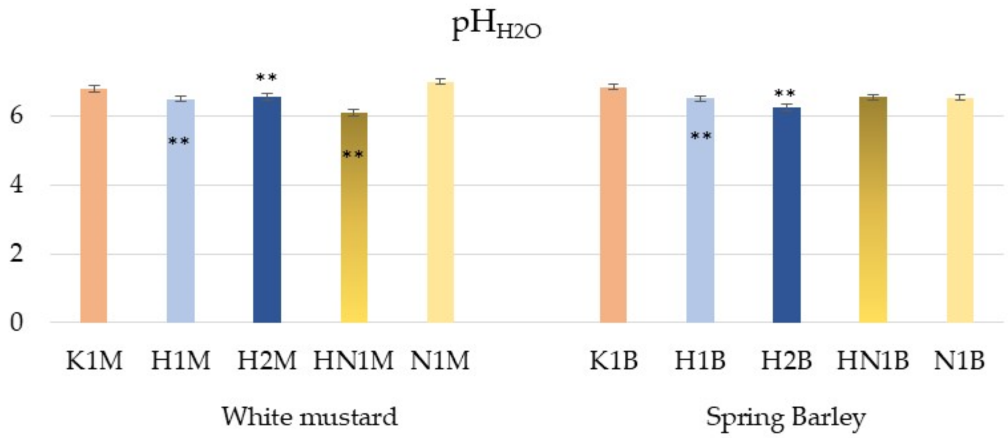

Figure 3. Soil pH_{H2O} in control soil (K1) and after addition of: 1% whey hydrogel (H1), 2% whey hydrogel (H2), 1% whey hydrogel and NPK (HN1) and NPK (N1). ** mean levels significantly divergent at $p = 0.05$ in comparison with control.

Application of acid whey into soil decreases soil pH and its changes can temporarily achieve a value of 2 pH units [54]; the duration period is dependent on the amount of added whey [42,55]. The benefits of whey-released nutrients for crops are demonstrable in soils whose pH is from 7.6 to 8.8. Aboukila [56] found the highest pH in the control soil and the lowest pH in soil amended by the highest whey dosage. Despite the proved acidification effect, the pH in our experiments did not drop below adverse levels and remained suitable for crop sowing and planting.

The nitrogen amount in the control soil at the beginning of the pot trial was 0.1%. Nitrogen concentrations increased after the application of 2% hydrogel, 1% hydrogel with NPK, and NPK [Figure 4]. The highest nitrogen amount in amended soil after the addition was found in the HN1M pot, followed by the 2% whey hydrogel and NPK variants [Figure 4]. Differences between variants were rather low but statistically significant. Soil nitrogen was mostly affected by 2% whey hydrogel and NPK with hydrogel mixture addition, considering its very low amount in whey hydrogel. A comparison of nitrogen fertilization and foliar spraying by whey peptides was presented by Sanatawy et al. [57] and proved the significantly different effects of nitrogen fertilization on all measurements. Our results showed both the prevailing effect of the whey hydrogel dose and the effect of NPK joint application. Despite the low nitrogen amount in primary hydrogel, the indispensable fertilizing effect of 2%whey hydrogel was found.

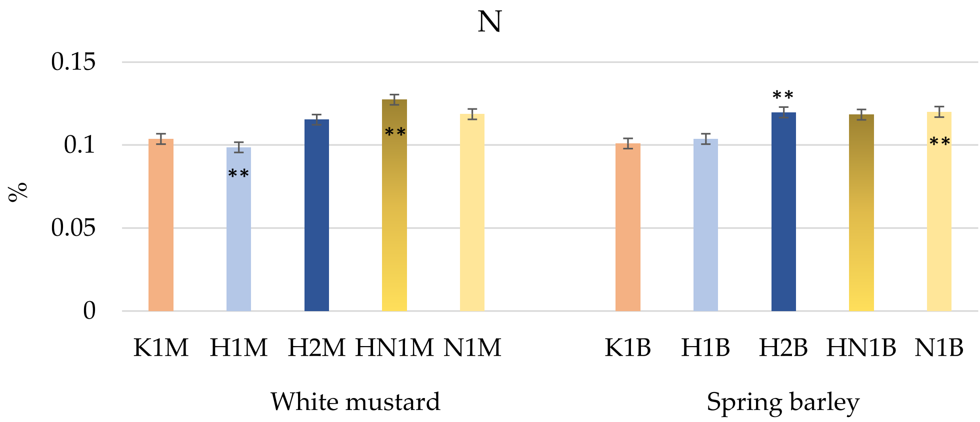

Figure 4. Nitrogen content after addition: 1% of whey hydrogel (H1), 2% of whey hydrogel (H2), 1% of whey hydrogel and NPK (HN1), and NPK (N1). ** mean levels significantly divergent at $p = 0.05$ in comparison with control.

Concentrations of phosphorus increased after the additions of whey hydrogel and NPK. The application of 1% whey led to the lowest increase. The application of 2% whey hydrogel raised available phosphorus by about 30% compared to the control soil, analogous to the increase caused by the application of NPK. Available phosphorus content was strongly affected by the application of whey hydrogel with NPK [Figure 5].

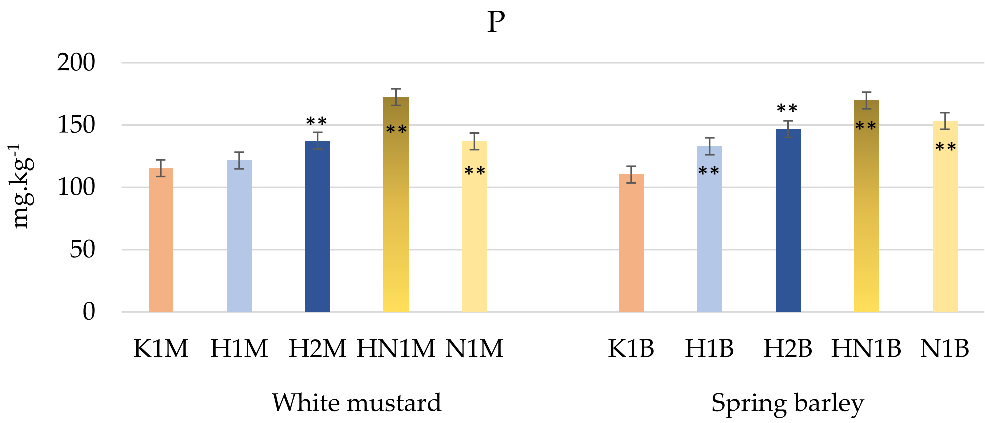

Figure 5. Phosphorus content after addition: 1% whey hydrogel (H1), 2% whey hydrogel (H2), 1% whey hydrogel and NPK (HN1), and NPK (N1). ** mean levels significantly divergent at $p = 0.05$ in comparison with control.

Potassium showed a similar trend to phosphorus and its content increased most after the addition of whey hydrogel with NPK [Figure 6]. The effect of potassium on crops depends on the different forms of soil potassium, whose deficiency may reduce the quality and quantity of crop yield [58]. The effect of fertilizing with potassium on tomato yield was tested by Elwaziri et al. [59], who observed the significant increase of yield with the potassium dose.

Our results showed an increase of bioavailable elements after the addition of whey hydrogel, NPK, and their mixture into natural Cambisol. As shown in Figure 7, cation exchange capacity (CEC) likewise significantly changed after application. Significant differences in soil CEC between Cambisol from the control pot and from the amended pot were found after application of the 1% whey hydrogel. Significant changes in cation exchange capacity were presented by Rittonga et al. [60] after the addition into soil of hydrogel synthesized by copolymerization of chitosan-TiO_2 composite with polyacrylamide. They proved differences in soil CEC between variants without hydrogel and with hydrogel addition; the highest increase was 38%. Our results show the remarkable influence of whey hydrogel on CEC despite the low application dose.

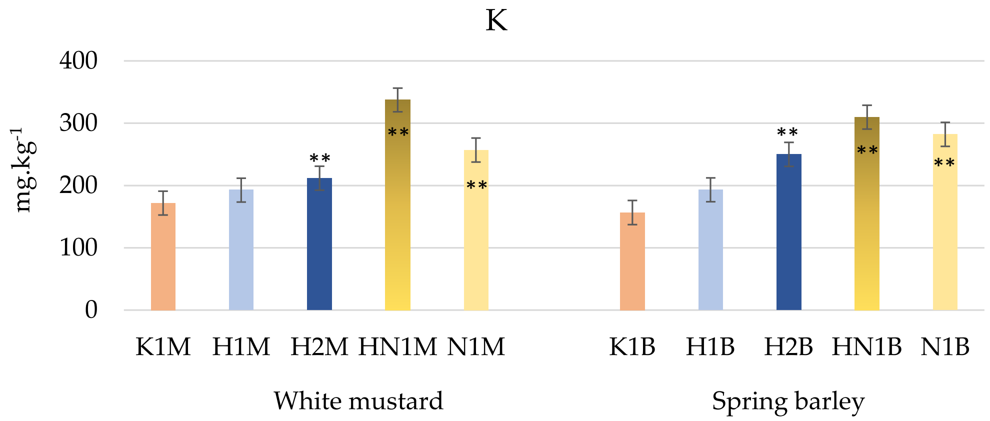

Figure 6. Potassium content after addition: 1% whey hydrogel (H1), 2% whey hydrogel (H2), 1% whey hydrogel and NPK (HN1), and NPK (N1). ** mean levels significantly divergent at p = 0.05 in comparison with control.

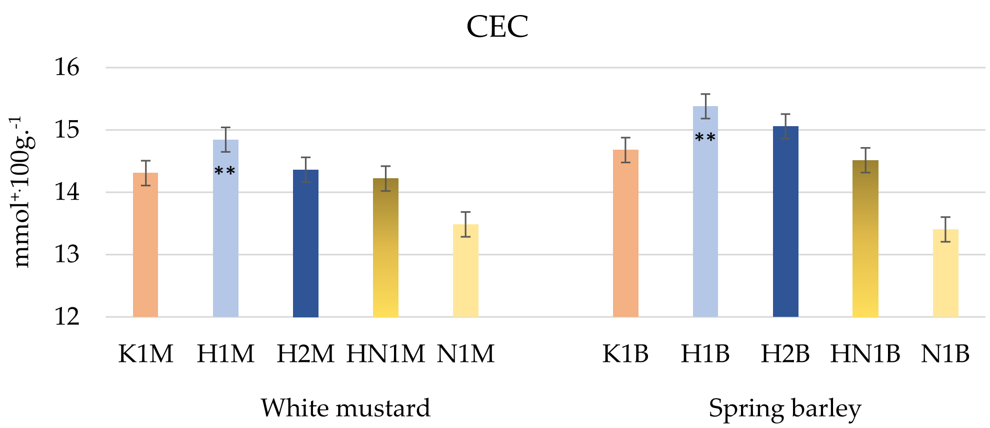

Figure 7. Cation exchangeable capacity after addition: 1% whey hydrogel (H1), 2% whey hydrogel (H2), 1% whey hydrogel and NPK (HN1), and NPK (N1). ** mean levels significantly divergent at p = 0.05 in comparison with control.

3.3. Yield of Dry Biomass

The yield of biomass is significantly affected by fertilizing and agricultural production practices that aim to increase the accumulation of organic carbon, nitrogen, and phosphorus in the soil to supply nutrients for crops. Our results follow from replenishing these nutrients into soil in the form of whey hydrogel, whey hydrogel + NPK, and NPK in selected doses.

The determination of biomass yield showed that all additions significantly increased the yield of white mustard dry biomass as compared to the yield from untreated soil [Table 4], and hence the lowest biomass values were observed in control Cambisol without any addition [Table 4, Figures 8 and 9].

Table 4. Yield of dry biomass (g/pot).

Variant	White Mustard	Spring Barley
control	3.19	1.52
1% whey hydrogel	3.96 **	2.93 **
2% whey hydrogel	5.80 **	7.34 **
1% whey hydrogel+ NPK	8.35 **	10.3 **
NPK	6.74 **	8.36 **

** mean levels significantly divergent at p = 0.05 in comparison with control.

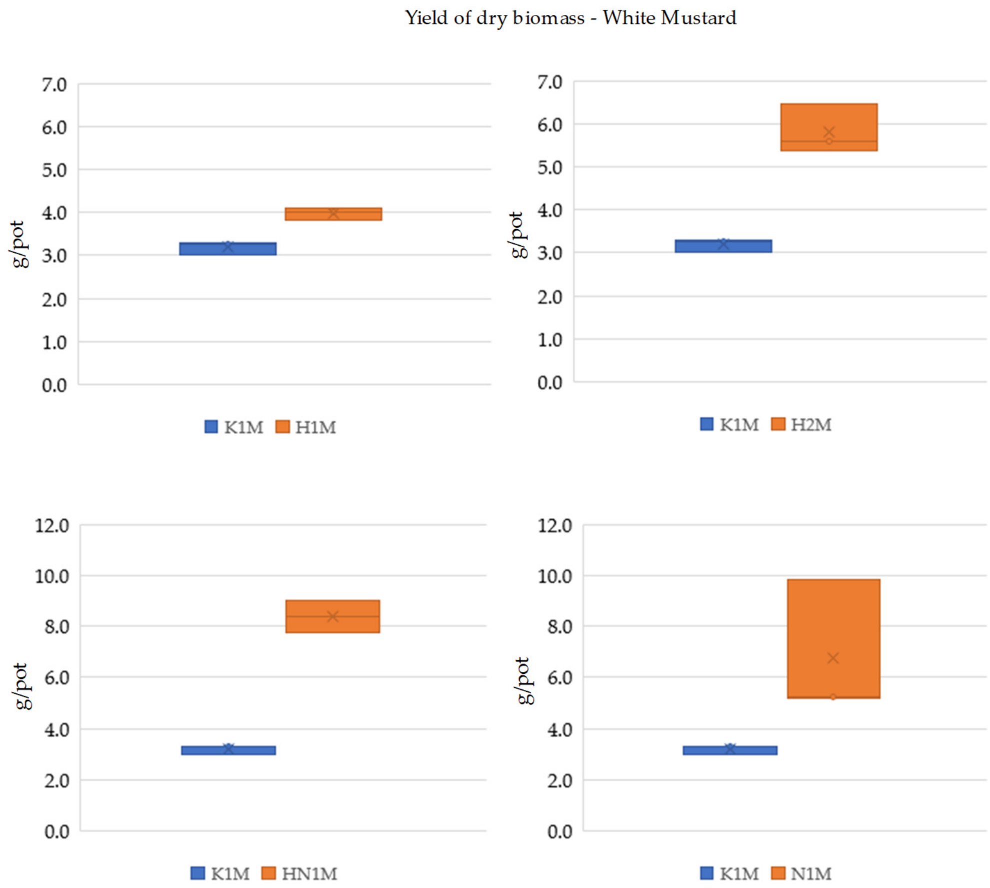

Figure 8. Yield of White Mustard dry biomass (g/pot) harvested from pot experiment (g/pot): control soil (KM) compared to soil with application of 1% whey hydrogel (H1M), 2% whey hydrogel (H2M), 1% whey hydrogel and NPK (HN1M), and NPK (N1M).

Yield biomass values of mustard ranged from 3.19 g/pot (Cambisol without treatment) to 3.96 g/pot (1% whey hydrogel), 5.80 g/pot (2% whey hydrogel), 6.74 g/pot (NPK) and 8.35 g/per pot (1% whey hydrogel with NPK). In agricultural soil, the amendment of 1% whey hydrogel increased biomass yield by 24% compared with control soil without any treatment. The experimental addition of 2% whey hydrogel increased biomass yield by 80%. Amending the control soil with NPK led to an increase of biomass yield of about 111%. The greatest effect on mustard biomass yield was detected after joint application of 1% whey hydrogel together with NPK and led to a 160% increase.

Akay and Sert [43] showed similar results by assessing the effect of the application of whey powder solution on plant growth parameters. The soil treated by the demineralized whey powder solution significantly increased values of plant growth parameters compared to the control. Elwaziri et al. [59] used whey protein hydrolysates by foliar application on tomato plants, which resulted in an increase of yield from 24 ton/ha at the control site up to 32 ton/ha. Motamedi et al. [61] used nanocomposite hydrogel with fertilizers during tomato cultivation and compared their results with NPK treatment. The efficiency of hydrogel was higher than common NPK fertilizer and significantly increased tomato growth.

Similar trends were determined for the biomass yield of spring barley. The biomass increased in the sequence: 1.52 g/pot (Cambisol without treatment), 2.93 g/pot (Cambisol with 1% whey hydrogel), 7.34 g/pot (Cambisol with 2% whey hydrogel), 8.36 g/pot (Cambisol with NPK treatment) and 10.3 g/per pot (Cambisol with joint treatment by 1% whey hydrogel with NPK), in the relative sequence of 90%, 380%, 490%, and 570% of yield from control Cambisol, respectively. The amendment of 1% whey hydrogel mixed with NPK led to an almost sevenfold increase of barley biomass compared to the control soil. Significant results were presented by Talaat et al. [62], who prepared a fertilizing base from starch, urea, zinc, potassium, and phosphorus for experimental testing of the mixture's effects on bean and wheat yield. Moreover, mixing this fertilizer base with hydrogel from starch and acrylonitrile significantly amplified the effect. Similarly, in our experiment, the joint application of whey-based hydrogel with conventional mineral fertilizer showed the highest biomass yield of mustard and barley.

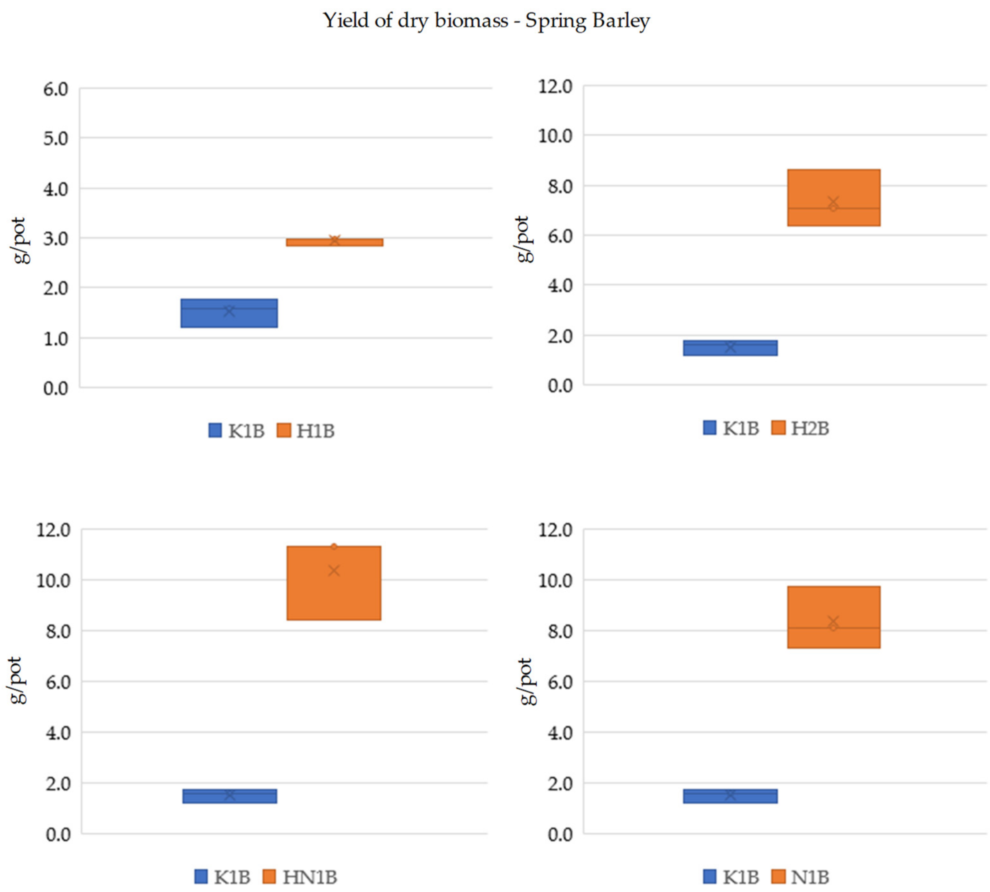

Figure 9. Yield of Spring Barley dry biomass (g/pot) harvested from pot experiment: control soil (KB) compared to soil with application of 1% whey hydrogel (H1B), 2% whey hydrogel (H2B), 1% whey hydrogel and NPK (HN1B), and NPK (N1B).

3.4. Soil Quality after the Harvest

The addition of whey hydrogel at the beginning of the experiment decreased soil pH temporarily. The pH drop had no adverse effects on crop growth and remained in the optimal range for crop production during experimental testing. Soil pH values determined after harvest showed recaptured soil pH at the level of the control soil, except for the application of whey hydrogel with NPK, which gave the lowest drop before sowing. Our results confirmed the findings of Akay and Sert [43], who observed a general increase of nutrient availability for crops with the hydrogel-induced pH drop.

As shown in the chapter above, the application of whey hydrogel, NPK, and hydrogel with NPK resulted in an increase of available nutrients and cation exchangeable capacity. Generally, all amendments had positive effects on soil quality compared with the control Cambisol. After the harvest of mustard and barley, the levels of nitrogen, phosphorus, and potassium decreased to the level of the control soil, indicating that the available nutrients were utilized for yield growth.

The chemical composition of whey represents nutrients available for plants [42]. Results of testing during our study confirmed that nutrients [Figures 10–13] increased during vegetation, and the effect strongly depends on the treatment with various amendments. The nutrient changes caused crop yield changes that hinted at the gradual slow release of nutrient. This slow release was also observed after experimental combinations of cellulose hydrogels with urea release, where the free urea release into water was reduced from 98.7% within 1 day to 50.77% within 1 day [63]. Similarly, a new fertilizer based on chitosan hydrogel with incorporated nutrients was applied in precise agriculture with a minimum loss of nutrients and potential for use in house and greenhouse planting [64]. Cation exchangeable capacity on equivalent or higher values in whey hydrogel amended variants after harvest shows the advantage of whey hydrogel on higher CEC levels (Figure 14).

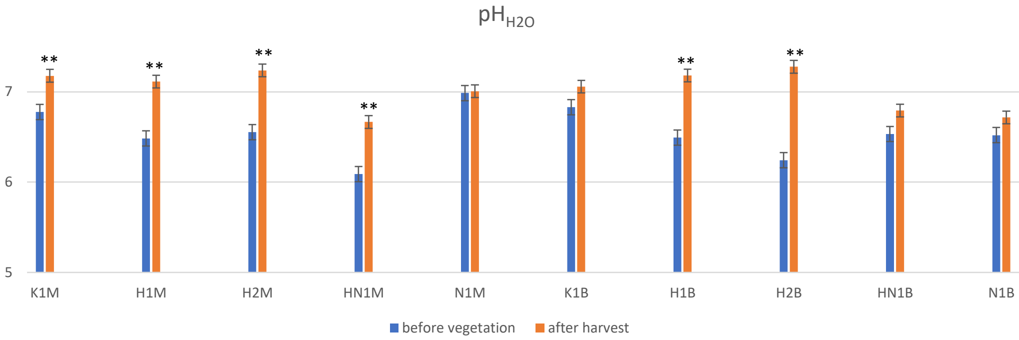

Figure 10. Soil pH$_{H2O}$ before vegetation and after mustard (M) and barley (B) harvest. ** mean levels significantly divergent at p = 0.05.

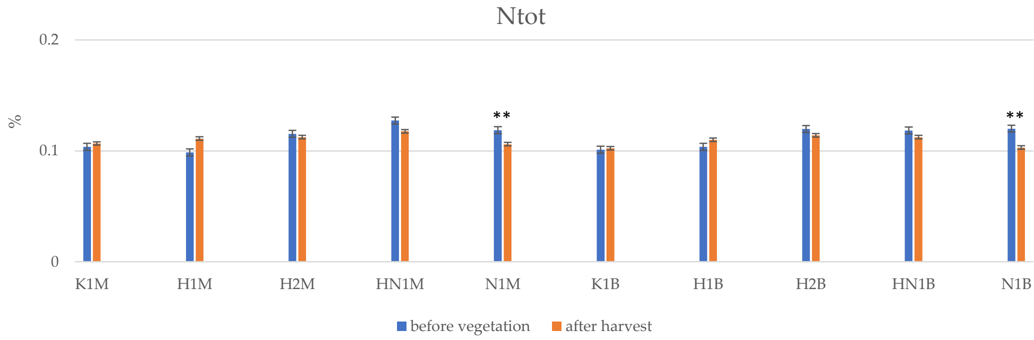

Figure 11. The nitrogen content before vegetation and after mustard (M) and barley (B) harvest. ** mean levels significantly divergent at p = 0.05.

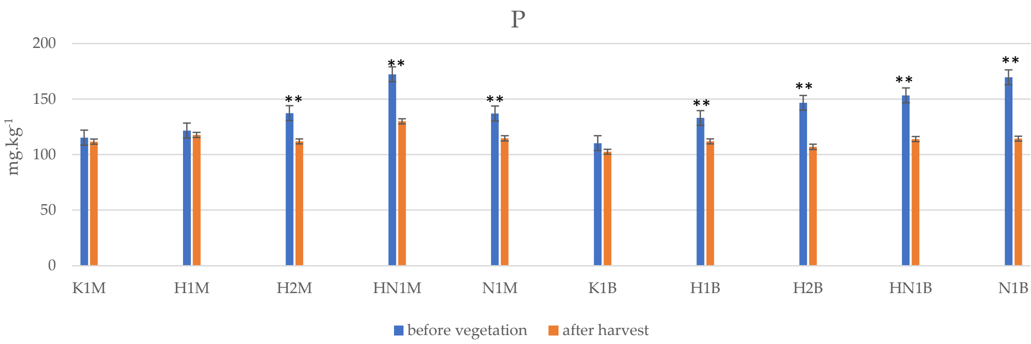

Figure 12. The phosphorus content before vegetation and after mustard (M) and barley (B) harvest. ** mean levels significantly divergent at p = 0.05.

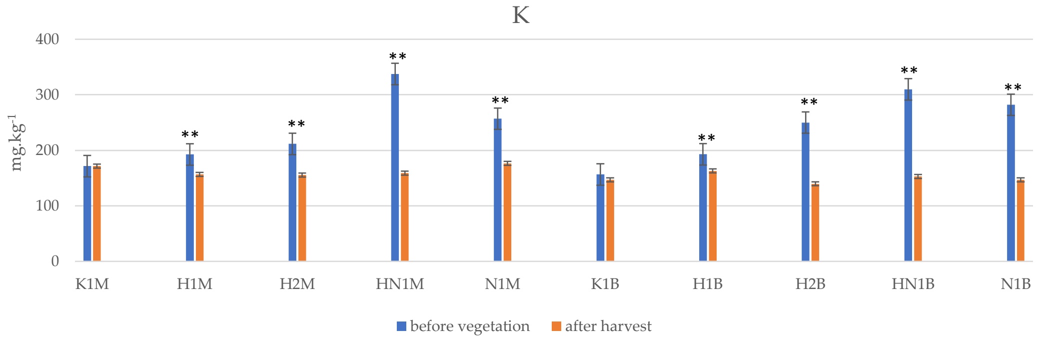

Figure 13. The potassium content before vegetation and after mustard (M) and barley (B) harvest. ** mean levels significantly divergent at p = 0.05.

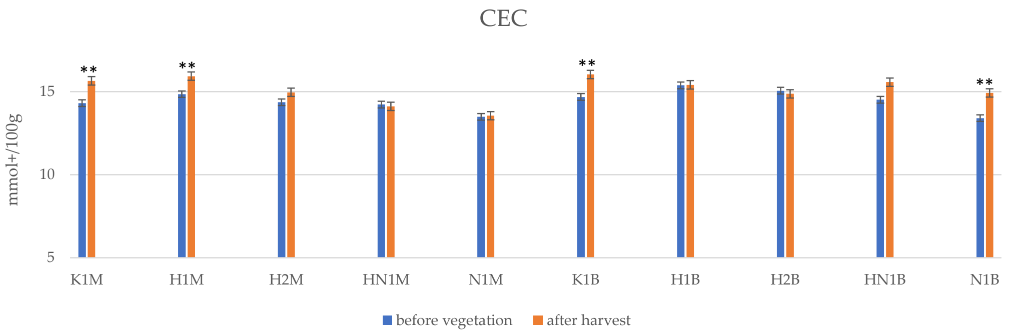

Figure 14. Cation exchangeable capacity before vegetation and after mustard (M) and barley (B) harvest. ** mean levels significantly divergent at p = 0.05.

4. Conclusions

This study provides an advanced approach to the use of environmentally friendly hydrogels in agriculture. Our results indicate that whey-based hydrogels are a prospective option for sustainable agriculture and fulfill environmental and production benefits. Acid whey, as a component of hydrogel substance, gives flexible utilization potential, addressing both minor deficiencies and immediate stimulation effects by replenishing the pools of available nutrients while keeping the primary effect of hydrogel to hold soil moisture and improve the capacity of the soil to hold water. The suitability of whey-based hydrogel for the improvement of natural soil quality and the related increase in yield of agricultural crops in real conditions was proved by experimental testing. Our results showed that the novel whey-based hydrogel combines fertilizer and hydrogel into one system.

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