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Original Research

Changes in Some Biological and Chemical Properties of an Arable Soil Treated with the Microbial Biofertilizer UGmax

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Abstract

The aim of this study was to determine the effects of the commercial biofertilizer UGmax on soil cellulase (CEL) and dehydrogenase (DH) activities, microbial biomass carbon content (MB-C), and some chemical properties in the humus horizon of an arable field (*Luvisols*) over a three-year period (2005, 2006, 2008) for winter wheat and for winter rapeseed in 2007. Twenty soil samples were taken from the area studied in 2005 (the control year without UGmax treatment), while in the other years ten soil samples were taken after UGmax treatment and ten from the control, always after plants were harvested. No clear effects of UGmax treatment on the studied properties were found. Compared to the control, the application of UGmax increased soil reaction and soil organic carbon (C_{ORG}) concentrations in the entire study period, although for the latter property the changes were not statistically significant in 2007. A significant reduction of CEL activity was noted after the second and third years of UGmax application, while DH activity was significantly higher when UGmax was applied compared to the control only after the first year of treatment.

Keywords: microbial biofertilizers, UGmax, cellulase, dehydrogenase, microbial biomass C

Introduction

One of the methods that can help to maintain and/or to increase the organic matter content and soil fertility in arable soils is the application of microbial fertilizers containing living microorganisms. Biofertilizers have been found to improve soil fertility and enhance plant growth and crop yield [1]. Many different microbial biofertilizers for agricultural use are available on the market. Often the chemical and microbiological composition of these products is not specified in detail, making it difficult for users to evaluate the product and for scientists to prove its effectiveness [2]. One of the most popular techniques used to produce a biofertilizer is the concept of effective microorganisms (EM), which has received a great deal of attention and has been studied often [3-8].

One of the microbial biofertilizers available in Poland is a preparation called UGmax, which is produced by Bogdan Trade-Service Co, Ltd. It is composed of an assorted culture of beneficial fermentative microorganisms such as lactic acid bacteria (*Lactobacillus* spp), yeast (*Saccharomyces* spp), *Pseudomonas* and *Penicillium* bacteria, and actinomycetes and others with some microelements (the chemical and microbiological composition of UGmax is given in the Material and Methods section). UGmax is produced using animal by-products (in accordance with permission No.

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1774/2002/WE of the European Parliament and Committee). Many beneficial effects of UGmax on crop yields and the physico-chemical properties of soil have been documented in numerous field trials, although not many scientific papers have been devoted to this topic to date. It has been stated that UGmax increased the yields of winter wheat, sugar beet, winter rapeseed, and tomato [9, 10]. In a 5-year-long experiment, a gradual increase in the yield of sugar beet roots and in their sugar content was shown, but each year the cultivation was carried out on different fields and after various forecrops [11].

It was shown that UGmax can increase the content of organic matter, soil pH, and available Mg, K, and P concentrations [12-15]. However, it must be stressed that in some research, the farmers concerned did not use P and K fertilization for many years, thus these elements were probably released from the soil because of the application of UGmax [16]. The UGmax biofertilizer influences the physical properties of soil by improving the soil structure, water adsorption, infiltration, and storage [17], accelerating the decomposition rate of the post-harvest residues of corn [18], and increasing the basal soil respiration level with different corn-straw ratios [19]. No further research has been done, however, to document the effects (or lack thereof) of UGmax on the biological activity of soil, especially its enzymatic activity.

Soil enzymes play an important role in the catalysis of some important reactions that are essential for soil microorganisms, decomposition, and the formation of organic matter, and are responsible for nutrient cycling and the decomposition of organic wastes [20, 21], which is of special agricultural significance.

Dehydrogenases are exclusively intracellular enzymes that are not accumulated in soil. Since dehydrogenase enzymes play a significant role in the biological oxidation of soil organic matter [21, 22], their activity is considered to be an indicator of the oxidative metabolism in soil and thus also of microbial activity [21, 23]. The largest parts of soil enzymes are, however, extracellular and are excreted into the soil solution. They are extremely important in the hydrolysis of substrates that are too large or insoluble to be taken up directly by cells [24] like cellulose, which is the most abundant organic compound in the biosphere [25]. The rate of cellulose decomposition is of special importance to agriculture when straw or other plant residues are used in order to improve soil quality. Some studies showed that cellulases in soil may promote straw decomposition by catalyzing cellulose decomposition [26-28]. That is why one of the proposed methods for accelerating straw decomposition is using exogenous cellulase [29]. However, since the application of a cellulolytic preparation in a field system is questionable primarily because of the economic aspects [29], finding an alternative method for increasing the activity of a native cellulase complex seems to be a desirable approach.

The study hypotheses were:

(1) the activities of soil enzymes and the concentrations of MB-C and chemical properties would be affected by the application of the UGmax biofertilizer versus the control,
(2) UGmax accelerates straw decomposition, which should be reflected in higher cellulase activity in the UGmax-fertilized field versus the control,
(3) there is a significant relationship between the biological and chemical properties.

In order to verify the above hypotheses, the activities of two soil enzymes and some physico-chemical properties affected by the application of UGmax versus the control soil were assessed. In addition, in the last year of the experiment, the MB-C concentration was determined.

Material and Methods

Study Site and Soil Sampling

The research was carried out on an arable field of winter wheat (2005, 2006, 2008) and winter rapeseed (2007) located in the southern part of Sępopolska Plain near the village of Budniki (54º11'54" N and 20º38'12" E) in northern Poland. A research area of 2 ha was set up for the experiment in 2005 and 20 sampling points were marked using GPS before the first application of UGmax. Soil samples were taken from the soil humus horizon (always after the crop harvest) during the entire experimental period (2005-08). The distance between the sampling points was in the range of 26-37 m×37-49 m. According to the WRB, the soil is eutric, gleic Cambisols composed of 45% sandy clay loam, 35% fine sandy loam, 10% loam, 5% clay loam and 5% clay. In order to determine the surface differentiation of the research area, the first soil samples were collected before the first application of UGmax (2005). The following samplings (2006-08) were always done before the application of the biofertilizer. One-half of the studied area was supplemented with UGmax every year on the stubble after harvest (0.7 l per ha) and as top-dressing in spring (0.3 l per ha), while the other half was the control. The time and the rates of the application of UGmax were followed according to the producer's recommendation. The chemical and microbiological composition of UGmax is given in Table 1. The crop rotation, the type and the rates of the nitrogen fertilizer applied during the experiment are shown in Table 2. No phosphorus or potassium fertilization was applied.

Rainfall and air temperature data were recorded at a weather station located 3 km west of the site of the experiment. The monthly mean values of air temperature and the sum of rainfall in particular years of the study are presented in Fig. 1.

Analysis of Soil Properties

The physico-chemical properties were determined according to standard methods [30]. Every sample was analyzed in triplicate. A particle-size was carried out using Cassagrande'a method as modified by Prószyński; sand fraction content was determined using the sieving method. Total nitrogen (N_{TOT}) in the soil was determined using the Kjeldahl method [31]. Soil organic carbon (C_{ORG}) content

Table 1. Chemical and microbiological composition of the UGmax biofertilizer.

Elements (total values)	(mg·ml^{-1})
N	1800
P	250
K	3000
Mg	120
S	350
Na	350
Mn	7
Bacteria	(CFU·ml^{-1})
Lactic acid bacteria	7.5×10^{2}
Pseudomons spp	1.6×10^{5}
Penicilium	1.8×10^{4}
Actinomycetes spp	3×10^{3}

Table 2. Crop rotation and fertilization used in this study.

Year	Forecrop	Yield (Mg·kg^{-1})	N-fertilization	
			Fertilizer	Dose (kg·ha^{-1})
2005	Winter wheat	6.0	urea	150
2006	Winter wheat	5.2	urea	150
2007	Winter rapeseed	3.2	urea	50
			ammonium nitrate	200
			ammonium sulfate	200
2008	Winter wheat	6.7	urea	200

was determined using the dichromate oxidation procedure, while soil pH (1 M KCl) was measured using the potentiometric method in 1:2.5 soil: solution.

A fumigation-extraction method was used to estimate microbial biomass C (MB-C) with extractable C converted to microbial C using a standard factor [32]. Soil was fumigated with ethanol-free chloroform for 24 h. Fumigated and unfumigated soil samples were then extracted with 0.5 M K_2SO_4 for 30 min. Sub-samples of filtrates from both fumigated and unfumigated soils were analyzed for extractable C [32].

Dehydrogenase activity (DH) was determined according to the method described by Thalmann [33]. Weight 1 g of field-moist soil was placed into test tubes and mixed with a 1% TTC (triphenyltetrazolium chloride) solution and a Tris-HCl buffer (0.1 M, pH 7.6). The tubes were sealed with rubber stoppers and incubated for 24 h at 30ºC. After incubation, acetone was added to each tube, and the tubes were shaken thoroughly and further incubated at room temperature for 2 h in the dark. The soil suspension was later filtered and the optical density of the clear supernatant was measured against the blank at 546 nm.

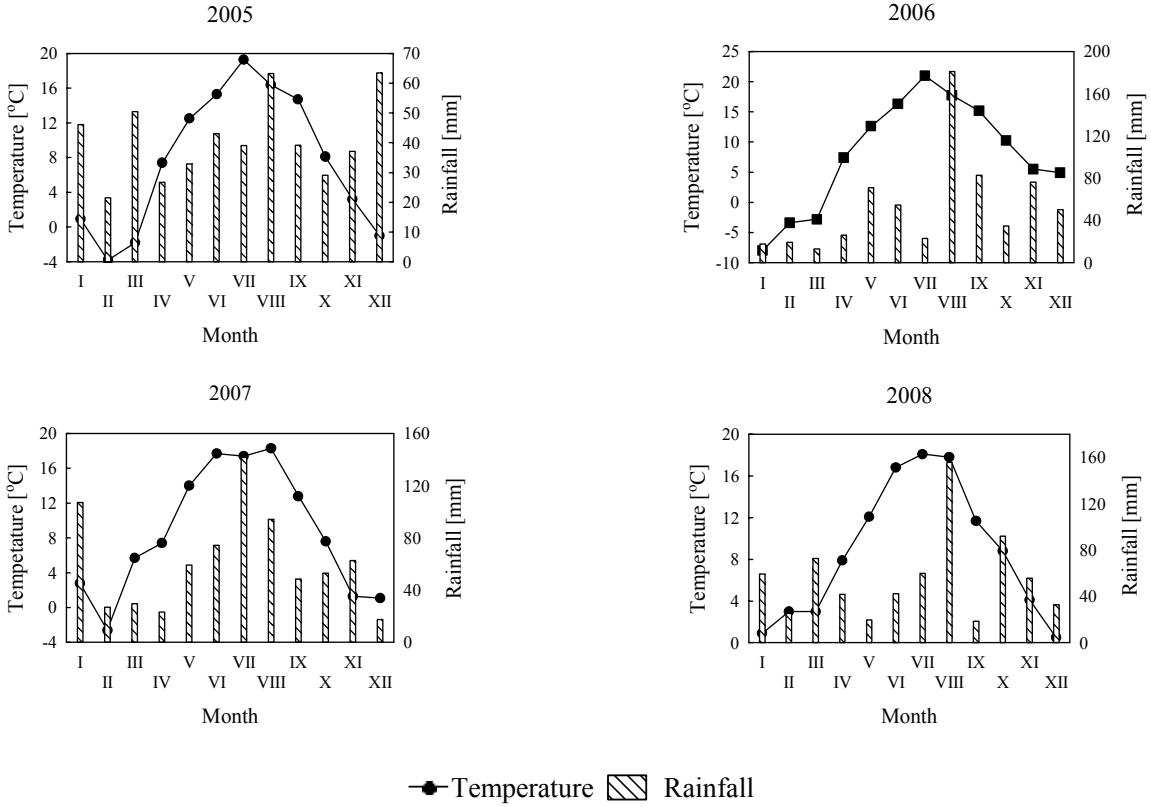

Fig. 1. Temperatures (ºC) and rainfall (mm) at the experimental site from 2005 to 2008.

Cellulase activity (CEL) was assayed as reported by Schinner and von Mersi [34]. Using this method, sugars and products of low molecular weight resulting from the enzyme degradation by carboxymethylcellulose (CMC), for 24 hours at 50ºC and pH 5.5 were determined. Reducing sugar caused a reduction of potassium hexacyanoferrate (III) in an alkaline solution. Reduced potassium hexacyanoferrate (II) reacts with ferric ammonium sulphate in an acid solution to form a complex of ferric hexacyanoferrate (II) (Prussian blue), which was determined spectrophotometrically at 690 nm.

The assays of enzyme activities and MB-C concentration were performed on fresh, moist sieved (< 2 mm) soils and were calculated based on the oven-dry (105ºC) weight of the soil. Control tests with autoclaved soils were carried out in order to evaluate the spontaneous or abiotic transformation of the enzyme substrates. All of the analyses were performed in triplicate. Results of dehydrogenase and cellulase activities were expressed as mM TPF kg^{-1}·d.m·24 h^{-1} and mM glucose·g^{-1}·24 h^{-1}, respectively.

Statistical Analysis

The results were evaluated using the classical statistical methods (STATISTICA v. 9.0 Software) for calculating arithmetic means, standard deviation, and coefficient of variation (CV). The results were analyzed using Tukey's tests ($p<0.05$) to evaluate any significant differences between the means obtained for the fields treated and untreated with UGmax. Simple linear regressions were calculated between all of the properties measured. A classification scheme was used for identifying the extent of variability for soil properties based on their CV (%) values, in which values of 0-15%, 16-35%, and > 36% indicate low, moderate, or high variability, respectively [35].

Results

Soil Chemical Properties

There were no significant differences in the clay, silt and sand contents between the areas reserved for the application of UGmax and control (2005) (Fig. 2).

The soil reaction was from acid to neutral (pH_{KCl} ranged from 4.5 to 6.8). Over the entire period of the investigation, the pH in KCl was always higher in soil samples taken from the field with UGmax application than in the control. The greatest increase of soil reaction in the field where UGmax was applied in comparison with the control was noted in 2008 (Fig. 3).

Prior to UGmax application (2005), there was no significant difference in the C_{ORG} content between the field with and without the fertilizer, while in 2006, 2007, and 2008 the application of UGmax increased the C_{ORG} concentration by 10-16% as compared to the control, although in 2007 the differences were statistically insignificant (Tables 3, 4, Fig. 4). A statistically significant increase of N_{TOT} in the field with UGmax as compared to the control field (0.26 g·kg^{-1})

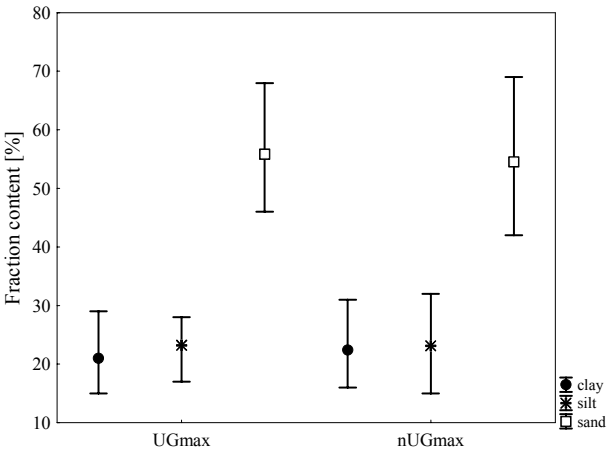

Fig. 2. Fractions content (%) as influenced by UGmax treatment versus control; mean values and the range of data in 2005.

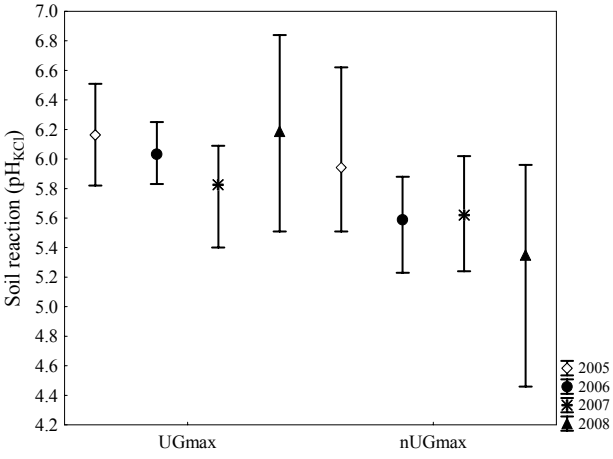

Fig. 3. Soil reaction (pH_{KCl}) as influenced by UGmax treatment versus control; mean values and the range of data in succeeding years.

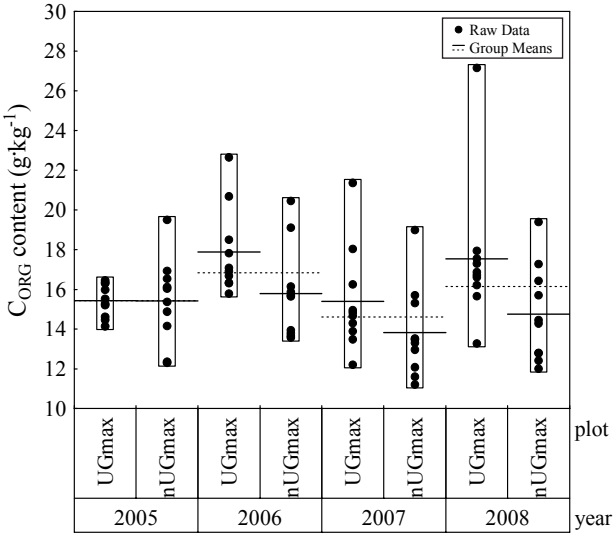

Fig. 4. Organic carbon concentration (C_{ORG}) as influenced by UGmax treatment; — mean value for treatment (UGmax vs. control); ···· mean values for both UGmax and control in succeeding years.

Table 3. Analysis of variance (ANOVA) of properties studied.

Year	Properties studied			
	C_{ORG}	N_{TOT}	DH	CEL
2005	n.s.	n.s.	n.s.	0.0311
2006	0.0407*	n.s.	0.0126	n.s.*
2007	n.s.	n.s.	n.s.	0.0106
2008	0.0402	0.0360	n.s.	0.0110
2005-06	0.0194	0.0298	0.0182	0.0006
2005-07	n.s.	n.s.	n.s.	0.0000
2005-08	0.0241	0.0168	n.s.	0.0140

*Kruskal-Wallis nonparametric test (significance level), n.s. – not significant, C_{ORG} – organic carbon content ($g \cdot kg^{-1}$), N_{TOT} – total nitrogen content ($g \cdot kg^{-1}$), DH – dehydrogenase activity (mM TPF$\cdot kg^{-1} \cdot 24\ h^{-1}$), CEL – cellulase activity (mM glucose$\cdot g^{-1} \cdot 24h^{-1}$)

was noted only in the last year of the investigation (2008) (Table 4). Trends of N_{TOT} content differed in specific years of the investigation and were similar to the changes in C_{ORG} concentration. Lower and similar values of both properties (C_{ORG}, N_{TOT}) were noted in 2005 and 2007, while they were higher in 2006 and 2008 (Table 4, Figs. 4, 5). The most considerable dispersion of the results around the mean was noted for both C_{ORG} and N_{TOT} concentrations in the soil samples with UGmax in 2008 (Figs. 4, 5). Organic C and N_{TOT} concentrations in 2007 and 2008 were the most varied with the CV values ranging from 15.2 to 20.7%, while in 2005 and 2006 their content was distributed more homogeneously across the area studied with CV values below 15% (Table 5).

Soil Enzymatic Activity and Microbial Biomass Carbon

Prior to the experiment (2005), cellulase activity was significantly higher in the samples taken from the sites where UGmax was to be used in 2006-08 compared to the control soil (Table 4, Figs. 6 and 7). In the three-year experiment period (2006-07), cellulase activity was always higher in the field without UGmax treatment than in the field with the biofertilizer, although in 2006 the difference was not statistically significant. Comparing the initial year of the investigations (2005) with the following years (2006-08), a gradual decrease of CEL activity was observed in the area with the UGmax treatment (Table 4, Fig. 6). As compared to the control, the highest decrease in cellulase activity (43%) in the field with UGmax was noted in 2007, while in 2008 the activity decreased by 36.5%. The total reduction (2005-2008) of the enzyme activity studied after the use of UGmax reached 0.55 mM glucose$\cdot g^{-1} \cdot 24\ h^{-1}$, while the cellulase activity determined in the soil samples taken from the control plot changed only negligibly. With the exception of 2008, the results of CEL activity varied more

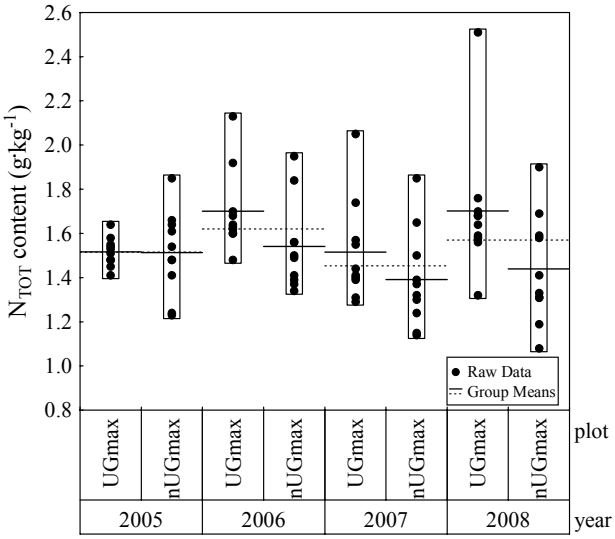

Fig. 5. Total nitrogen concentration (N_{TOT}) as influenced by UGmax treatment; — mean value for treatment (UGmax vs. control); ···· mean values for both UGmax and control in succeeding years.

Table 4. Effect of UGmax treatment on the soil properties studied ±SD.

Years	C_{ORG}		N_{TOT}		DH		CEL	
	UGmax	Control	UGmax	Control	UGmax	Control	UGmax	Control
2005	15.43a±0.8	15.4a±2.2	1.52a±0.07	1.51a±0.19	1.09a±0.23	1.19a±0.18	0.88a±0.09	0.70b±0.25
2006	17.9a±2.2	15.9b±2.3	1.70a±0.19	1.54a±0.20	1.31a±0.19	1.14b±0,17)	0.74a±0.12	0.82a±0,.22
2007	15.4a±2.6	13.8b±2.3	1.51a±0.23	1.39a±0.22	2.68a±0.69	2.57a±0.40	0.45a±0.17	0.79b±0.34
2008	17.5a±3.6	14.7b±2.4	1.70a±0.31	1.44b±0.25	2.00a±0.34	1.85a±0.21	0.33a±0.22	0.52b±0.18
2005-06	2.45a±2.0	0.37b±1,6	0.18a±0.15	0.03b±0.14	0.21a±0.27	-0.06b±0.19	-0.14a±0.11	0.12b±0.17
2005-07	0.03a±2.2	1.6a±1.7	0.00a±0.17	-0.12a±0.15	1.59a±0.68	1.37a±0.44	-0.42a±0.16	0.09b±0.28
2005-08	2.1a±3.3	0.67b±1.3	0.18a±0.15	-0.08b±0.16	0.90a±0.35	0.66a±0.26	-0.55a±0.20	0.17b±0.20

Differing letters in columns indicate significant differences between years (Tukey test, $p<0.05$); C_{ORG} – organic carbon content ($g \cdot kg^{-1}$), N_{TOT} – total nitrogen content ($g \cdot kg^{-1}$), DH – dehydrogenase activity (mM TPF$\cdot kg^{-1} \cdot 24\ h^{-1}$), CEL – cellulase activity (mM glucose$\cdot g^{-1} \cdot 24h^{-1}$).

Table 5. Coefficients of variation [CV%] of properties studied.

Years	C_{ORG}		N_{TOT}		DH		CEL	
	UGmax	Control	UGmax	Control	UGmax	Control	UGmax	Control
2005	5.4	14.0	4.4	12.6	20.8	15.3	10.1	35.6
2006	12.2	14.9	11.0	13.2	14.6	15.7	15.9	27.3
2007	17.0	16.8	15.2	16.0	25.7	11.4	38.4	42.6
2008	20.7	16.4	18.1	17.2	16.8	14.6	65.2	34.5

C_{ORG} – organic carbon content (g·kg^{-1}), N_{TOT} – total nitrogen content (g·kg^{-1}), DH – dehydrogenase activity (mM TPF·kg^{-1}·24 h^{-1}), CEL – cellulase activity (mM glucose·g^{-1}·24 h^{-1}).

in the control plot compared to the UGmax treated area (Table 5, Fig. 6).

UGmax treatment significantly decreased DH activity only in 2006, while in the other years there were no statistically significant differences in the DH activity data between the field treated with UGmax and the control (Table 3). Much higher DH activity than in 2005 and 2006 (in both the treated and untreated fields) was noted in the two last years of the experiment. Compared to the initial year of the experiment (2005), the greatest in DH activity was obtained in 2007, giving an average values of 54% and 57% for the UGmax treated site and the control, respectively (Table 4). A more significant dispersion of DH activity results around the mean was obtained in 2007 and 2008 than in the first two years of the investigation (Fig. 7). With the exception of 2006, the application of UGmax caused a more considerable variability of the results expressed by the coefficient of variation (CV) compared to the control (Table 5).

After 3 years of UGmax treatment, a statistically significant increase of MB-C versus the control was found (Fig. 8). In contrast to CEL activity, a higher dispersion of MB-C concentration data was noted in the area treated with UGmax than in the control.

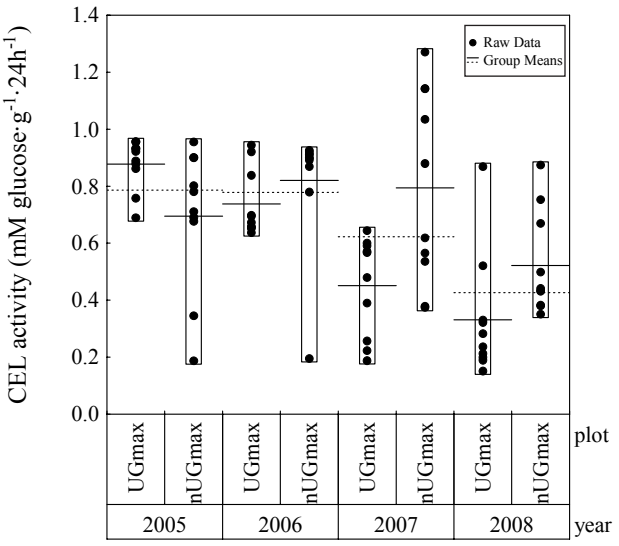

Fig. 6. Cellulase activity (CEL) as influenced by UGmax treatment; — mean value for treatment (UGmax vs. control); ···· mean values for both UGmax and control in succeeding years.

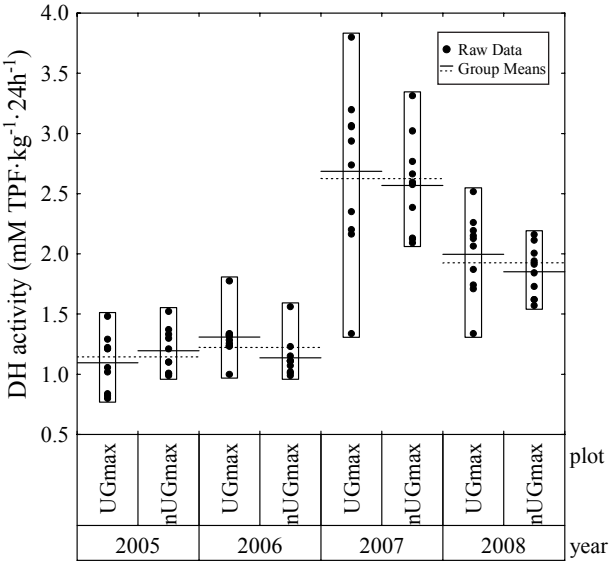

Fig. 7. Dehydrogenase activity (DH) as influenced by UGmax treatment; — mean value for treatment (UGmax vs. control); ···· mean values for both UGmax and control in succeeding years.

Analysis of the correlation did not show any significant relationships among the properties studied or between rainfall and air temperature data and other properties considered (results not shown).

Discussion

The initial effects of biofertilizers containing affective microorganisms, such as UGmax or 'Effective Microorganisms' (EM), might first be indicated by a change in the microbial properties of the soil since they are generally considered early and sensitive indicators of both natural and anthropogenic factors [36-39]. In general, it can be expected that the inoculated microorganisms establish and stimulate indigenous microorganisms and processes in the soil after repeated applications [8]. This kind of treatment also should improve soil fertility by increasing biological activity in the soil, which in turn should reduce the need for fertilizers. Of course, this result is very desirable from both economic and ecological points of view.

In this study, we attempted to optimize the conditions for soil microbes in order to accelerate the decomposition of the residues of winter wheat and winter rapeseed and to convert them into soil humus through the application UGmax biofertilizer. The results showed that treatment with UGmax clearly influenced cellulase activity, the group of enzymes that take part in cellulose decomposition. A study of Han and He [29] showed that exogenous cellulase application promoted cellulose decomposition of wheat and rice straw residues. Since cellulose is reported to be an important limiting factor of straw decomposition [26, 27], every factor that increases cellulase activity can be a potential means to accelerate the decomposition of straw and thus increase soil fertility. The results of our study showed that the microbiological fertilizer UGmax probably accelerated the initial phase of the decomposition of post-harvest residues, which was confirmed by a significant decrease in cellulase activity in the soil samples taken from the field where UGmax was applied as compared with the control field. One possible explanation is that the cellulase activity increased directly after UGmax treatment, and therefore the post-harvest residues decomposed faster than in the control field. As a result of this fact, 5 months after the second UGmax application (soil samples were always taken shortly before the autumn UGmax treatment), the post-harvest residue content was probably lower than in the control soil, simultaneously decreasing cellulase activity. Results presented by Chen et al. [40] suggested that the two agricultural biostimulants they used significantly augmented cellulase activity, which was measured as the rate of filter paper weight loss, systematically increasing up to the end of the incubation (56 days). Boopathy et al. [41] showed that the cellulase activity of soil treated with sugarcane crop residue with the addition of molasses increased over a 200-day period with a simultaneous decrease in the concentration of cellulose. The authors suggested that a technology that would accelerate straw decomposition in soil could be a possible alternative to the current practice of open air burning of sugarcane residue.

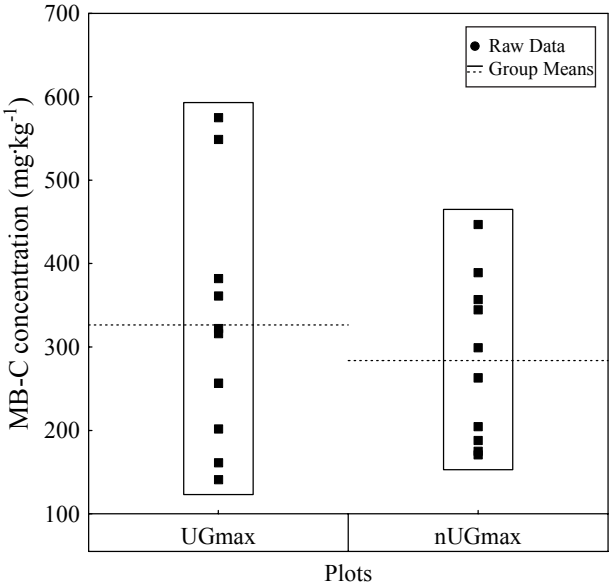

Fig. 8. Microbial biomass carbon (MB-C) concentration as influenced by UGmax treatment; — mean value for treatment (UGmax vs. control); ···· mean values for both UGmax and control in 2008 year.

The application of UGmax did not increase the activity of dehydrogenases, except in 2006, while the MB-C concentration in 2008 was significantly higher in the UGmax area than in the control field. Therefore, the results suggest that UGmax treatment increased the content of microbial biomass but did not influence microbial activity. Earlier in a laboratory experiment with UGmax and different corn straw rates [19], it was shown that UGmax caused a significant increase in CO_2 accumulation compared with control soil over a 42-day-long incubation. The intensity of CO_2 accumulation is, along with DH activity, one of the most often studied indicators of soil microbial activity as well as an indicator of the decomposition rate of organic matter [24, 42-44].

The lack of significant differences in DH activity between the UGmax-treated site and the control was probably because the soil samples were analyzed a few months after the second application of UGmax and the activity was aligned until then. Similarly, in the study of Mayer et al. [8], the dehydrogenase activity determined in March 2006 did not differ significantly among treatments combined with two forms of EM (namely EMA and Bokashi) or their combination with cattle manure applied in November 2005. As stated by Chen et al. [40], DH activity was the highest in the first 3 days of incubation with two soil biostimulants (described as Z93 and W91) and decreased steadily thereafter for up to 8 weeks.

An increase in soil carbon content that was found during the entire period of the application of UGmax compared to the control (although differences in 2007 were statistically insignificant) indicated humification of straw and post-harvest residue by soil microorganisms. As found by Boopathy et al. [41], decomposition of lignocellulosic materials can bring back the fertility of top-soil that had been lost due to improper agricultural practices. The significant increase in the MB-C concentration that was found in this study is contradictory to the results obtained by Schenck et al. [2], who found no effect of EM on the microbial biomass of C and N in soil without amendments and with the application of wheat straw.

Conclusions

The application of UGmax in an arable field in the temperate climate of northern Poland over a three-year period caused no clear effects on the biological and physico-chemical properties of soil, with the exception of cellulase activity and C_{ORG} content. When compared with the control, the microbial biofertiliser, UGmax, clearly decreased the activity of cellulase in soil samples taken from the field where the preparation was applied (although in 2006 the changes

were not statistically significant). This suggests that UGmax is probably a medium that determines the decomposition rate of post-harvest residues and that the activity of cellulase was a distinct indicator of soil changes after the application of UGmax. No clear tendency in changes in dehydrogenase activity in soil samples taken from the UGmax field and the control was noted. This indicates the limited usefulness of enzymes of this type in evaluation of the long-term impact of UGmax on soil. A higher concentration of C_{ORG} was noted after UGmax treatment in each subsequent year, although in 2006 the differences were not statistically significant. Although the MB-C concentration was significantly higher in the field treated with UGmax compared to control, a one-year research study is not enough to draw any conclusions.

Further extensive studies to investigate a wide range of soil properties are required in order to evaluate the effectiveness of UGmax on different types of soil, cultivation, and plant residues. Moreover, these studies should include sampling several times a year in order to monitor any seasonal dynamics in the different properties as influenced by treatment with UGmax, which is of special importance in the case of microbial biomass content and activity.

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