Original Research

Influence of Intensive Vegetable Cultivation in Ground and under Foil Tunnels on the Enzymatic Activity of the Soil

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Abstract

Vegetable cultivation is an example of intensive agricultural production. It is contributing to changes of physico-chemical properties and the biological productivity of the soil. The aim of our examinations was to determine the influence of vegetable cultivation and the intensive fertilizing and soil nurturing associated with it on selected soil physico-chemical properties and the activity of urease and invertase. Findings of conducted examinations indicated the very diversified but always inhibited influence of intensive cultivation of the examined soils on the activity of urease and invertase. Invertase and urease activities strongly positively depended above all on the content of organic substance. Urease activity was the lowest in the soil under foil tunnels, and invertase activity in soils, on which they were growing vegetables in ground, after dismantling foil tunnels.

Keywords: urease, invertase, vegetables, foil tunnel cultivation, ground cultivation

Introduction

Vegetable growing is an example of intensive agricultural production. The profitability of such production is not only conditional on the height of crops, but also on the harvest dates, inducing producers to apply considerable doses of mineral fertilizers and the intensive protection of plants [1, 2]. The income from vegetable growing, on account of great requirements of these plants, must be led on very rich soils, therefore at these targets there are used the best and simultaneously the most resistant to the decline, so as: chernozems. Nevertheless, the long-standing, intense manner of cultivation causes changes of physico-chemical properties and the biological productivity of even resistant ecosystems. Alterations of the activity of soil enzymes, recognized as the good indicator of the general biological soil activity and its fertility, are reflecting directions of changes in the soil environment [3-6].

The aim of conducted examinations was to determine the influence of vegetable cultivation and, associated with it, intensive fertilization and soil nurturing on selected soil physico-chemical properties and the activity of urease and invertase.

Experimental Procedures

Site of Soil Sampling

Research was carried out in 2010-11, in the commune Igolomia-Wawrzenczyce adjoining Kraków (southern Poland) from the eastern side (N50º07', E20º21'). The area of the commune is mostly covered with loess deposits, from which degraded chernozems were formed (*Haplic Chernozems Anthric*) [7]. It is a commune of agricultural character, in which the majority of arable soils are used for intensive vegetable growing in ground and in foil tunnels.

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In the area of the commune, samples of the soil were taken from 12 sites. Five of them included ground-grown vegetable cultivations of: onion (G2), celery (G3), cucumber (G4), cabbages (GT5), and cauliflower (GT6) planted in ground where a foil tunnel was put previously. The ground cultivation was performed in the first growing period after the foil tunnel had been dismantled. These vegetables have been cultivated in the monoculture or in simplified crop rotation with wheat or potatoes and often (every 2 or 3 years) manured in doses of 30-50 $t \cdot ha^{-1}$. Nutritional needs of vegetables have been satisfied with mineral fertilizing in doses of 400-600 $NPK \cdot ha^{-1} \cdot kg^{-1}$, in the ratio 2:1:3. Chemical protection of plants has been applied too, mainly in the form of herbicides (Roundup Max 680 SG, Stomp 330 EC).

Six other soil samples were taken out from sites with vegetable growing in foil tunnels: red, yellow, and green peppers (T7, T8, and T9, respectively); tomato v. 'Bekas' (T10) and v. 'Malinowy Retro' (T11), and cucumber (T12). These vegetables are characterized by very great nutritional requirements, particularly with reference to potassium, nitrogen, and calcium [8]. Vegetables planted in tunnels have been manured every year in the dose of 60-100 $t \cdot ha^{-1}$, and pre-sow with NPK mineral fertilizers, in general in the ratio 2:1:6 in doses of the about 200-300 $kg \cdot ha^{-1}$. Moreover, every 10-14 days foliar nutrition has been applied 0.2-0.5% NPK in a ratio of 1:0.9:2.5. Vegetables cultivated in foil tunnels were fertilized with higher doses of fertilizers compared to those cultivated in grounds in order to shorten time of cultivation and make them ready for market earlier and, in consequences, at a higher price. The cultivation of vegetables in foil tunnel and grounds were performed in the same period of time.

On account of the fact that in tunnels vegetables are often planted for a dozen or so years without crop rotation and at high temperature and humidity (which support the development of illnesses and pests), chemical protection of plants has been kept in the form of herbicides (Roundup Max 680 SG, Stomp 330 EC), fungicides (Mythos 300 SC, Sumilex 500 SC, Grevit 200 SL), and insecticides (Decis 2.5 EC, Mospilan 20 SP).

In order to determine changes of soil properties going on as a result of their intensive agricultural use (vegetable growing), for comparison, a soil sample was picked up from the site located in the commune, on unfertilized and only occasionally mown meadow, described in the paper as wasteland (ZN1).

Methods

Individual soil samples were taken from three places on every site (the field or the tunnel) of the layer of 0-25 cm and in the case of ground cultivations and wasteland from the depth of 0-25 cm and 25-40 cm. Soil samples for enzyme activity determinations were taken twice, in May and October, on account of seasonal fluctuations of the enzymatic activity [9]. Presented results are mean values from spring and autumn determinations.

Physico-chemical soil properties were determined with the following methods:

- soil texture by densimetric-sieve method, according to Polish Norms [10]
- pH values in 1:2.5 solutions of H_2O potentiometrically [11]
- level of total nitrogen, total and inorganic carbon with the use of an automatic analyzer: TOC-TN 1200 Thermo Euroglas apparatus. The level of organic carbon was calculated as a difference between total and inorganic carbon levels
- urease activity was established after 2-hour sample incubation at 37ºC with the urea solution used as a substrate and the estimation of the amount of ammonia that is left after urea hydrolysis, through the distillation of ammonia nitrogen from the sample extract with the Kjeltec apparatus [12]
- invertase activity, established colorimetrically after 24 hours of incubation of samples in 37ºC with the saccharose solution used as a substrate according to the Frankenberger and Johanson method [13]. The intensity of coloration was measured with Beckman DU 600 spectrophotometer at a wavelength of 540 nm

Statistical analysis of results was carried out with the ANOVA unifactor variation analysis method in the random arrangement. To estimate the significance of differences between mean values, homogeneous groups were appointed using the test *a posteriori* of Fisher. Coefficients of variation (V%) and standard deviations (SD) were calculated. For selected parameters a value of the Pearson's linear correlation coefficient (r) was enumerated [14]. Equations of the stepwise progressive regression, describing relations between physical-chemical properties of the soil and their enzymatic activity were also drawn up. All calculations were performed using the Statistica package v. 10 PL.

Results

The examined soils were ranked among chernozems. They were derived from loess and therefore had a silt texture. Soil of wasteland (ZN1) was characterized by a lightly acid soil reaction in both tested layers of 0-25 cm and 25-40 cm. Reaction of the soil on which they were growing vegetables in ground (G2-GT6) ranged in values pH=6.0-7.1 in surface layers and 5.6-7.4 in subsurface layers, while pH of the soil under tunnels (T7-T12) was clearly lower and amounted to 4.8-6.4 (Table 1). Such a scattering of values shows that the pH value was shaped by an applied liming. Larger doses of mineral fertilizers applied in cultivations of tunnel vegetables than in ground-grown ones lowered the soil pH values.

In the soil under the vegetable growing, as a rule a much lower content of organic carbon was indicated than in unfertilized wasteland, and these differences were statistically significant (Table 1). Especially low contents of this element appeared in the soil, on which at present the vegetable growing is being conducted in tunnels (T7-T11) or such a cultivation was led by a lot of years and at present tunnels were dismantled and vegetables have been planted in the ground (GT5, GT6). An exception was the soil under

Table 1. Selected physico-chemical properties and enzymatic activity of studied soils (arithmetic mean of 3 individual trials ± standard deviation).

Soil	Layer [cm]	pH H_2O	C [g·kg^{-1}]	N [g·kg^{-1}]	C:N	Urease [μg N-NH_4·2h^{-1}·g^{-1}]	Invertase [mg of inverted sugar]
ZN1*	0-25	6.6	$31.0^{k**}\pm0.25$	$2.57^{i}\pm0.065$	12.1	$4.77^{h}\pm0.10$	$35.3^{l}\pm0.3$
	25-40	6.7	$21.8^{i}\pm0.90$	$2.63^{j}\pm0.05$	8.3	$3.14^{f}\pm0.10$	$8.9^{h}\pm0.1$
Ground vegetable cultivation							
G2	0-25	7.0	$18.7^{g}\pm0.30$	$1.51^{b}\pm0.02$	12.4	$2.59^{d}\pm0.06$	$1.7^{d}\pm0.1$
	25-40	6.4	$16.7^{e}\pm0.65$	$1.48^{ab}\pm0.03$	11.3	$1.34^{a}\pm0.14$	$0.6^{ab}\pm0.1$
G3	0-25	7.1	$22.8^{j}\pm0.60$	$1.47^{ab}\pm0.07$	15.5	$2.23^{c}\pm0.10$	$9.2^{h}\pm0.1$
	25-40	7.4	$20.5^{h}\pm0.50$	$1.91^{g}\pm0.02$	10.7	$1.94^{b}\pm0.04$	$3.2^{f}\pm0.5$
G4	0-25	6.2	$18.8^{g}\pm0.40$	$1.74^{ef}\pm0.04$	10.8	$2.21^{c}\pm0.04$	$1.4^{d}\pm0.0$
	25-40	5.7	$12.9^{c}\pm0.60$	$1.43^{a}\pm0.05$	9.0	$1.93^{b}\pm0.04$	$0.5^{a}\pm0.1$
GT5	0-25	6.0	$11.9^{ab}\pm0.30$	$1.6^{c}\pm0.05$	7.4	$3.38^{g}\pm0.07$	$1.0^{c}\pm0.1$
	25-40	5.6	$12.3^{bc}\pm0.30$	$1.7^{de}\pm0.02$	7.2	$1.85^{b}\pm0.06$	$0.4^{a}\pm0.1$
GT6	0-25	7.0	$11.3^{a}\pm0.30$	$1.5^{b}\pm0.02$	7.5	$3.03^{f}\pm0.05$	$0.9^{bc}\pm0.1$
	25-40	6.0	$11.9^{ab}\pm0.30$	$1.6^{c}\pm0.03$	7.4	$1.81^{b}\pm0.06$	$0.3^{a}\pm0.1$
Foil tunnel vegetable cultivation							
T7	0-25	4.8	$16.1^{e}\pm0.45$	$1.63^{cd}\pm0.07$	9.8	$1.87^{b}\pm0.07$	$4.0^{g}\pm0.5$
T8	0-25	5.7	$11.7^{ab}\pm0.70$	$1.50^{b}\pm0.03$	7.8	$1.29^{a}\pm0.05$	$10.2^{j}\pm0.1$
T9	0-25	6.3	$11.4^{a}\pm0.40$	$1.50^{b}\pm0.01$	7.6	$2.80^{e}\pm0.10$	$9.6^{i}\pm0.1$
T10	0-25	6.1	$17.8^{f}\pm0.50$	$1.76^{ef}\pm0.04$	10.1	$2.20^{c}\pm0.10$	$3.1^{f}\pm0.0$
T11	0-25	6.4	$13.9^{d}\pm0.30$	$1.80^{f}\pm0.03$	7.7	$1.96^{b}\pm0.16$	$2.3^{e}\pm0.1$
T12	0-25	6.2	$34.1^{l}\pm0.60$	$2.42^{h}\pm0.02$	14.1	$5.57^{i}\pm0.17$	$22.2^{k}\pm0.4$

*ZN1 – wasteland, G2 – onion ground cultivation, G3 – celery ground cultivation, G4 – cucumber ground cultivation, GT5 – cabbage ground cultivation (after dismantling foil tunnel), GT6 – cauliflower ground cultivation (after dismantling foil tunnel), T7, T8, and T9 – pepper foil tunnel cultivation, T10 and T11 – tomato foil tunnel cultivation, T12 – cucumber foil tunnel cultivation.
**the same letters indicate homogenous groups, $\alpha<0.05$

cucumber cultivation in the tunnel (G12), in which content of organic carbon (34.1 g·kg^{-1}) exceeded the content of this element, indicated in the unused agriculturally soil (31.0 g·kg^{-1}). The content of nitrogen in examined cultivated soils placed in rather narrow range, from 1.43 (layer 25-40 cm, G4) to 1.91 g·kg^{-1} (layer 25-40 cm, G3), even though there were statistically significant differences between these values. The highest contents of nitrogen (about 2.5 g·kg^{-1}) were marked only in the soil on which the cucumber has been planted in the tunnel (T12) and in the unused agricultural soil (ZN1). In surface layers the ratio of carbon to nitrogen was placed in studied soils in a quite wide range of values: from 7.4 to 15.5. Especially narrow values of the discussed ratio were calculated for soils after taking tunnels and some soils under vegetable cultivation in tunnels. In the layer of 25-40 cm the value of the C:N ratio ranged 7.2-11.3 in G5 and G2, respectively.

Urease activity in soils from the ground-grown and tunnel cultivation of vegetables in the layer of 0-25 cm was slightly diversified and oscillated in major cases about 2 μg N-NH_4·2h^{-1}·g^{-1} of the soil (2.59 μg N-NH_4·2h^{-1}·g^{-1} was noted in G2). A much higher and statistically significant different value of the activity of this enzyme, exceeding 3 μg N-NH_4·2h^{-1}·g^{-1}, was determined in the soil under the vegetable growing in ground after taking the tunnel (GT5 and GT6). Urease activity at the level of 4.77 μg N-NH_4·2h^{-1}·g^{-1} was indicated in the wasteland soil in the surface layer, and the highest value in the soil in under tunnel cultivation of cucumber (T12 – 5.57 μg N-NH_4·2h^{-1}·g^{-1}). In the layer of 25-40 cm urease activity was clearly lower than in the surface layer and as a rule fluctuated around 2 μg N-NH_4·2h^{-1}·g^{-1} soil.

Invertase activity indicated in studied soils was very much diversified, and these differences were statistically significant. The lowest activity of this enzyme (0.3-0.5 mg of the inverted sugar) was indicated in deeper layers of the soil from ground-grown cultivations of cucumber (G4),

Table 2. Simple correlation coefficients of Pearson (r) describing relations between enzyme activity and studied soil properties.

	pH	C	N	C:N	Urease activity
Urease activity	0.11	0.72***	0.66***	0.35**	1
Inwertase activity	0.00	0.77***	0.69***	0.43**	0.74***

N=54, ***$\alpha < 0.001$, ** $\alpha < 0.01$

cabbage (GT5), and of cauliflower (GT6), and highest: 35.26 mg of inverted sugar in the wasteland in layer of 0-25 cm. Quite high invertase activities were determined in soils from tunnel cultivations of: peppers (T8 and T9) and cucumber (T12). The activity of this enzyme in the discussed soils amounted to appropriately: 10.2, 9.6, 22.3 mg of inverted sugar. High invertase activity was marked also in both examined layers of the soil from ground-grown cultivation of celery (G3), which in the layer of 0-25 cm amounted to 9.2 and in the layer of 25-40 cm to 3.2 mg of inverted sugar (Table 1).

Discussion of Results

Agro-technological treatments belong to anthropogenic factors exerting the significant influence on physico-chemical and biological properties of soils and hence to their ability to function in the ecosystem and the biological productivity [15]. Studied chernozems, located in the same physio-graphic conditions, derived from an identical, loess parent rock, are characterized by similar, natural properties and therefore constitute good material for observation of alterations of soil features caused by anthropogenic factors. Chernozems contain a large amount of organic matter and, in consequence, are characterized by a considerable resistance to changes of properties by outside factors [16]. However the examined soils were exploited intensively. Long-term vegetable growing, many times in the monoculture, frequent aerating of the soil, and large doses of mineral fertilizers (besides manuring) contributed to a big diversifying of the properties of soils under vegetable growing compared to features of wasteland soil of this area. It proves values of coefficients of variation of examined soil properties, such as pH, organic carbon, and total nitrogen contents amounting to appropriately: 10.5, 37.0, and 21.6%.

A good indicator of changes of the soil quality caused by environmental stress related to agro-technological treatments constitutes an enzymatic activity. Literature emphasizes a relationship of the soil urease and invertase activities with the method of soil use [5, 6, 17, 18]. Values of urease and invertase activities in the examined soil were characterized by great variability. Particularly great value of the variation coefficient, amounting to 227.6%, was noted in the case of urease activity. Variation coefficient of invertase activity, though also high, was clearly lower and amounted to 137%. It confirms Wang's et al. observations [17], according to which urease sensitivity to environmental stresses is stronger than invertase. Such a huge variability of the activity of examined enzymes was caused probably by diversified doses of fertilizers applied in the vegetable growing, especially in cultivations under covers, often exceeding needs of plants. An influence of crop protection chemicals on the activity of soil enzymes is also significant. According to literature data, applying pesticides as a rule strongly influences the activity of enzymes, although both harmful and positive influences were noted, depending on administered agents, doses and other environmental factors [19-24]. In the presented study the hampering effect of pesticides may be observed, especially in the case of soils from foil tunnel vegetable cultivation.

Calculated simple correlation coefficients (Table 2) show the strongly significant and positive effects of content of organic carbon and total nitrogen on the activity of examined enzymes. Based on these results, it is possible to regard the activity of both invertase and urease as regulated above all by a content of organic matter in the soil. Occurring relations between the levels of activity of examined enzymes and discussed other properties of the soil were described in the form of equations of the stepwise progressive regression. We took into consideration urease activity, invertase activity, pH, content of organic carbon (C), content of total nitrogen (N), and value of the ratio of the content of organic carbon to total nitrogen (C:N). At the number of observations N=54, these equations have the following forms (in brackets values of standard errors were given for individual factors of equations):

$$\text{Urease activity} = 6.71 - 3.68\,N + 0.49\,C - 0.78\,C{:}N \qquad R=0.86$$
$$(1.81) \quad (1.06) \quad (0.10) \quad (0.18)$$

$$\text{Invertase activity} = 24.18 + 2.88\,C - 3.99\,C{:}N \qquad R=0.85$$
$$(16.87) \quad (0.96) \quad (1.61)$$

Values of multiple correlation coefficients (R) describing these equations are high (0.86 and 0.85), and equations are statistically significant at significance level $\alpha<0.05$. Considering interactions of different soil properties, it is possible to predict that urease activity is strongly suppressed by the content of nitrogen in the soil, but stimulated by a content of carbon. Shi et al. [18] achieved similar results of multiple correlation equations for the activity of discussed enzymes and interpreted this fact via the stimulating effect of organic matter and inhibiting effect of total nitrogen in the soil. Higher urease activity was observed in the soil in which lower mineral fertilizing is applied and higher organic ones with large amounts of vegetable rests left.

Abundance in easily available nitrogen coming from mineral fertilizers hampered the synthesis of urease [18, 25]. Similar prognostic value (R=0.85) has the equation describing relations between invertase activity and different features of studied soils. As we can expect, in this equation a particularly strong influence of the organic carbon content on the activity of discussed enzyme is noticeable. In the equation described above, a negative connection of the invertase activity and C:N values appears. These relationships are a result of the fact that invertase decomposes the sucrose to the glucose and the fructose, and is participating in the decay of plant tissues and transformations of soil organic matter.

Obtained results explicitly are pointing at the hindering influence of agro-technological treatments of vegetables connected with ground-grown and tunnel cultivated vegetables on the activity of urease and invertase. Although the activity of discussed enzymes has always been lower in the soil under vegetable growing than in the unused agriculturally soil, the inhibiting influence of agro-technology on the enzymatic activity was intensified to a different degree, depending on the ability of running the vegetable production in the way limiting the degradation of the soil, which means according to principles of the agricultural good practice.

Conclusions

1. The influence of intensive agricultural use of the examined soils on the activity of urease and invertase was diversified but always hampering.
2. Invertase and urease activities in examined soils above all strongly positively depend on the content of organic matter.
3. Urease activity was the lowest in the soil under foil tunnels, and invertase activity in soils, in which they were grown vegetables in ground, after dismantling foil tunnels.

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