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Seasonal changes in biochemical and microbiological activity of soil against the background of differentiated irrigation in an apple tree orchard after replantation

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Abstract. The objective of the studies included seasonal changes in the activity of enzymes, emission CO_2 and microbiological activity of soil against the background of differentiated irrigation in an apple tree (*Malus domestica*) orchard after replantation. The most important activity of enzymes and the number of microorganisms were confirmed in the seasons of spring and autumn, while the lowest activity was observed in summer. The highest dehydrogenase activity was found in the last year (from 0.43 to 2.42 $cm^3\ kg^{-1}$) and the lowest the in second year (from 0.19 to 0.58 $cm^3\ kg^{-1}$). A high protease activity was recorded in the last year (from 1.25 to 12.08 mg kg^{-1}) and low in the second year (from 1.65 to 3.47 mg kg^{-1}). The highest urease activity was observed in the first year (from 1.17 to 6.42 μmol g^{-1}) and the lowest in the second year (from 0.74 to 2.82 μmol g^{-1}). High intensity of emission CO_2 was noted in summer. The highest CO_2 emission was found in the last year (from 29.32 to 46.86 mg kg^{-1}) and the lowest in the second year (from 15.35 to 27.95 mg kg^{-1}). The highest number of fungi was found in the soil of the combination without irrigation - W_0. A high number of *Azotobacter,* actinomycetes, proteolytic bacteria, phosphate solubilizing bacteria, enzymes activity and CO_2 emission almost always were observed in the soil in the irrigation combination - W_1 or W_2.

Keywords: soil fatigue, replantation, apple tree orchard, irrigation, enzyme and microbiological activity, CO_2 emission

INTRODUCTION

The replant problem refers to the poor growth of replanted fruit trees on old orchard sites and is caused by biotic and abiotic factors. When the problem is caused by biotic factors, it is referred to as replant disease, caused by fungi, bacteria, actinomycetes, nematodes, and their interactions. Abiotic factors that cause replant problem are phytotoxins, nutrient imbalance, low or high pH, soil structure and drainage, and lack or excess of moisture (Utkhede & Smith, 1994).

Many different aspects of the presence and activities of microorganisms in the soil ecosystem can be measured. However, specific microbial parameters indicate different aspects of soil quality. Together with micro- and macrofauna, microorganisms such as bacteria and fungi are key players in the recycling of carbon decomposing the organic matter, followed by the microbes degrading the carbon compounds. The bulk dimension of this ecosystem process can be measured by soil respiration or organic

matter degradation, which at the same time provides an estimate of microbial activity (Kelting et al., 1998, Winding et al., 2005, Liu et al., 2006).

The objective of the present work was to determine the biochemical activity of soil and the number of microorganisms in different terms depending on the developmental phase and level of irrigation in the soil of an apple tree orchard after replantation.

MATERIALS AND METHODS

Studies were conducted from 2003-2005 in an apple tree orchard after replantation at the Agricultural and Fruit-Growing Experimental Farm in Przybroda of the University of Life Science in Poznań, Poland, on a sandy loamy soil (*Albic Luvisol*). The orchard was established in 1975. In 1988, the first replantation was carried out without any preceding soil preparation. In 1994, a repeated replantation was performed, however, after grubbing up the terrain, a shallow ploughing was applied using 2000 kg CaO/ha. Subsequently, apple trees of Ŝampion cultivar were planted, and as a pollinator Golden Delicious cv. was used on P 60 rootstock in the spacing of 3.5 x 1.5 m (1900 trees/ha). In the experiment, three irrigation levels were used: W_0 - maintenance of soil moisture at the level of atmospheric precipitations, W_1 - maintenance of soil moisture at the level of -0.03MPa of water potential (60% WHC), and W_2 - maintenance of soil moisture at the level of -0.01MPA of water potential (90% WHC). Within each irrigation level, three fertilization combinations were used, i.e. 65 kg N/ha, 65 kg N/ha and 95 kg/ha K_2O and 130 kg N/ha and 190 kg/ha K_2O. Mineral fertilization was applied in the first decade of April, 4-5 weeks before the blooming of orchard trees. Fertilizers were sown in the form of ammonium nitrate and potassium salt (60%). The herbicide program was used during the vegetation season (10 times) from May to September. The following herbicides were applied: Roundup 360 SL (4.0 l ha^{-1}), Chwastox extra (2.0 l ha^{-1}), Basta SL 150 (4.0 l ha^{-1}), Azotop 50 WP (3.0 l ha^{-1}), Agil 100 EC (1.5 l ha^{-1}). Two control samples were introduced: Nowina (apple orchard with Ŝampion cv. Earlier, agricultural plants were grown on this soil) and Virgin Soil (where no plants were previously grown, the so-called fallow). Soil samples for analyses were taken from belts of herbicide fallow from the depth of 0-20 cm, in three terms depending on the developmental phase of apple trees in the orchard: intensive growth of apple trees (June), fruiting/ripening (August) and fruit harvest connected with leaf fall (October). Bacterial and fungal population sizes were determined using the standard soil dilution plate method. Dilutions were used for the determination of the number of culturable cells as colony forming units (CFU) and expressed on dry matter soil. In the studied soil samples, the following microorganisms were estimated: number of actinomycetes on the Pochon medium (Grabińska-Łoniewska, 1999), bacteria from genus *Azotobacter* (Fenglerowa, 1965) and fungi (Martin, 1950). The number of microorganisms were incubated at 24ºC during 3, 4, 5 days. The number of proteolytic bacteria was incubated on the Frazier with gelatine medium at 21ºC for 48 h (Rodina, 1968). The phosphate solubilizing bacteria was estimated on medium soil with tricalcium phosphate during 10 days incubation at 28ºC (Rodina, 1968). The total number of bacteria was developed on 2% agar medium on

soil extract with K_2HPO_4 during 14 days incubation at 28ºC (Löchnis, 1920). Dehydrogenase activity in soils was assayed by reduction of triphenyltetrazolium chloride (TTC) to triphenyl formazone (TPF) and expressed as cm^3 H_2 24 h^{-1} kg^{-1} d.m. soil (Thalmann, 1968). Protease activity was assayed by production tyrosine from casein sodium salt and expressed as mg tyrosine h^{-1} kg^{-1} d.m. soil (Ladd & Butler, 1972). Urease activity was measured by released NH_4 and CO_2 from urea and expressed as μmol urea h^{-1} g^{-1} d.m. soil (Hoffmann & Teicher, 1961). The soil emission CO_2 was measured by absorption method and expressed as mg CO_2 48 h^{-1} kg^{-1}f.m. soil (Gołębiowska & Pędziwilk, 1984), Soil pH's were developed from soil-water (1:2 v/v) suspensions by potentiometer method. The total organic carbon (TOC) was analysed on TOC 5050A equipment produced by Shimadzu, Japan. Obtained results were subject to analysis of variance and differences between mean values were estimated by *Tukey's* test. The mean temperatures and total rainfall data during the period of vegetation of the apple orchard are presented in Table 1.

Table 1. Weather characteristics.

Months	2003		2004		2005	
	Temperature (ºC)	Precipitation (mm)	Temperature (ºC)	Precipitation (mm)	Temperature (ºC)	Precipitation (mm)
April	8.9	24.5	10.0	14.9	9.6	14.2
May	16.2	14.6	13.2	46.9	14.3	68.0
June	20.0	24.6	16.7	63.8	17.5	11.5
July	20.8	85.7	18.7	41.7	20.4	96.6
August	20.9	14.5	21.0	41.8	17.6	52.8
September	15.2	19.8	14.9	33.3	16.7	52.7
October	5.6	27.9	10.4	40.6	10.5	5.6
Mean	15.3	30.2	14.9	40.4	15.2	43.0
Sum of precipitation	-	211.6	-	283.0	-	301.4

Mean temperature and the sum of rainfall in the vegetation period of 2003 (Apr.-Oct.) amounted to 15.3°C and 211.6 mm respectively. In the second year of sample-taking (2004), the mean temperature was 14.9°C and the sum of rainfalls was 283.0 mm. In the year 2005, the respective values were: 15.2°C and 301.4 mm.

RESULTS AND DISCUSSION

The date in Tables 1, 2 clearly show the highest number of microorganisms in autumn and in spring, while in summer, the number was low. The number of fungi in the following years ranged from 33.58-238.85 cfu kg^{-1} in 2003, from 19.48-182.22 cfu kg^{-1} in the second year and from 16.53-260.01 cfu kg^{-1} in the last year. The number of actinomycetes in the following years ranged from 378.57 to 1490.37 cfu kg^{-1} in 2003, from 132.16 to 1696.59 cfu kg^{-1} in the second year and from 386.41 to 1662.72 cfu kg^{-1} in the last year. The number of *Azotobacter* in the following years ranged from 0 to 9 cfu kg^{-1} in 2003, from 0 to 5 cfu kg^{-1} in the second year and from 2 to 50 cfu kg^{-1} in the

last year. The number of proteolytic bacteria in the following years ranged from 15.17 to 266.12 cfu kg^{-1} in 2003, from 27.25 to 211.72 cfu kg^{-1} in the second year and from 70.33 to 184.49 cfu kg^{-1} in the last year. The number of phosphate solubilizing bacteria in the following years ranged from 224.20 to 985.56 cfu kg^{-1} in 2003, from 297.18 to 1857.55 cfu kg^{-1} in the second year and from 28.45 to 1406.40 cfu kg^{-1} in the last year. The total number of bacteria in the following years ranged from 2810.03 to 4539.35 cfu kg^{-1} in 2003, from 668.45 to 3541.15 cfu kg^{-1} in the second year and from 350.25 to 4485.90 cfu kg^{-1} in the last year. Results of the presented work agree with the results obtained by Bis (2002) and Pacholak et al. (2004). Those authors observed an increase of microorganisms in spring and autumn. Malicki (1980) stressed a distinct drop of bacteria number during summer in comparison with spring and autumn. Smoliński et al. (1997) noticed a high number of microorganisms in autumn in comparison with the spring term.

The activities of dehydrogenase, protease and urease were high in autumn or in spring, but low in summer (Table 4). The highest dehydrogenase activity was found in the last year (from 0.43 to 2.42 cm^3 kg^{-1}) and the lowest in the second year (from 0.19 to 0.58 cm^3 kg^{-1}). The highest protease activity was recorded in the last year (from 1.25 to 12.08 mg kg^{-1}) and the lowest in the second year (from 1.65 to 3.47 mg kg^{-1}). The highest urease activity was found in the first year (from 1.17 to 6.42 μmol g^{-1}) and the lowest in the second year (from 0.74 to 2.82 μmol g^{-1}). Similar results in the activity of enzymes depending on the sampling time were obtained by Bielińska & Domżał (1998). Those authors found that enzymes are comparatively active at the end of spring, while in summer their activity decreases. In autumn, their activity increases again. Bielińska (2001) believes that the decrease of enzymatic activity in summer may be connected with an intensive uptake of nutritive components by the growing trees. On the other hand, the high enzymatic activity of soil in spring can be explained by optimal temperatures with a sufficient level of moisture (Bielińska & Domżał, 1998). These conditions may have a favourable effect on the development of microflora (Bielińska & Domżał, 1998). On the other hand, in autumn, plant residuals which remained after harvest can be the stimulating factor of the increase of enzyme activity. However, results obtained in our present work are different from those shown by Koper & Piotrowska (1999). According to those authors, enzymes were most active in summer (July, August). Bielińska (2001) associated the intensification of enzyme activity in summer with the increase of root mass in that period, which generated the maximal secretion of enzymes to the rhyzosphere directly from the roots, as well as with the bacteria and fungi developing in the root zone.

The highest CO_2 emission was observed in the summers of 2003 and 2005 (Table 4). A high CO_2 emission was found in the last year (29.32 to 46.86 mg kg^{-1}) and low in the second year (from 15.35 to 27.95 mg kg^{-1}). Wojnowska et al. (1993) and Dziadowiec & Kaczmarek (1998) also observed a high respiration rate of soil in summer. Mo et al. (2005) noted that the daily soil CO_2 emission was moderate in late spring, increased sharply in summer and decreased in autumn. According to those authors, the soil temperature exerted principle control on the seasonal and annual variation of soil respiration. According to Vanhala (2002) the soil respiration rates decreased during spring and summer, the minimum values occurring at the end of August.

Table 2. Effect of sampling time and irrigation on the number of fungi (F), actinomycetes (A) and *Azotobacter* (Azb) in the soil of apple tree orchard after replantation in the years 2003-2005 (cfu kg^{-1}d.m. soil).

Sampling time	Combination of irrigation	2003			2004			2005		
		F x10^6	**A x10^6**	**Azb x10^3**	**F x10^6**	**A x10^6**	**Azb x10^3**	**F x10^6**	**A x10^6**	**Azb x10^3**
Intensive growth of apple trees	W_0	105.48bc*	455.58a*	9ab*	182.22c*	856.69bc*	0a*	102.52bc*	510.16a*	12a*
	W_1	38.83a	771.07ab	1a	102.73b	1141.02c	0a	35.12a-c	386.41a	2a
	W_2	33.58a	517.44a	1a	23.74a	1696.59d	5ab	16.53a	445.30a	7a
Fruiting/ripering	W_0	130.63c	692.57a	1a	72.73.ab	132.16a	0a	55.61a-c	558.69a	2a
	W_1	57.35ab	378.49a	4a	57.57ab	365.92a	0a	31.83ab	436.71a	11a
	W_2	41.05a	1094.57ab	0a	130.66bc	533.97ab	0a	31.53ab	450.63a	11a
Harvesting of fruits	W_0	238.85d	1096.30ab	1a	78.96ab	471.66ab	1a	260.01d	592.42a	6a
	W_1	79.38abc	1490.37b	29b	58.11ab	161.34a	2ab	113.77c	1152.45ab	50b
	W_2	55.34ab	1484.88b	8ab	19.48a	380.33a	2ab	48.09a-c	1662.72b	4a
Means for level of irrigation	W_0	158.32b	748.15a	3a	111.30b	486.84a	0a	139.38b	553.76a	6a
	W_1	58.52a	879.97a	11a	72.81ab	556.09a	1ab	60.24a	658.69a	21b
	W_2	43.32a	1032.30a	3a	57.96a	870.30b	2b	92.05a	852.88a	7a
Means for sampling time	Intensive growth of apple trees	59.30a	581.36a	4ab	102.90a	1231.43b	2a	51.39a	447.46a	7a
	Fruiting/ripering	76.34a	721.88a	2a	86.99ab	344.01a	0a	39.66a	482.01a	8a
	Harvesting of fruits	124.52b	1357.19b	12b	52.18b	337.78a	2a	140.62b	1135.86b	20b

* Means marked with the same letters did not differ significantly at the probability of P=0.05
cfu - colony forming units

Table 3. Effect of sampling time and irrigation on the number of proteolytic bacteria (PB), phosphate solubilizing bacteria (PSB) and the total number of bacteria in the soil of apple tree orchard after replantation in the years 2003-2005 (cfu kg^{-1}d.m. soil).

Sampling time	Combination of irrigation	2003			2004			2005		
		PB x10^6	PSB x10^6	TNB x10^6	PB x10^6	PSB x10^6	TNB x10^6	PB x10^6	PSB x10^6	TNB x10^6
Intensive growth of apple trees	W_0	50.54a*	224.20b*	2755.84a*	49.98a*	533.85ab*	2927.42a-c*	96.41ab*	28.45b*	702.38ab*
	W_1	146.49b	985.56a	2700.82a	80.64a	1157.59bc	3541.15bc	129.92ab	147.66ab	350.25a
	W_2	266.12c	654.36a-c	2551.19a	77.45a	1853.67c	6783.39e	184.49a	186.57ab	401.31ab
Fruiting/ripering	W_0	15.17a	348.76bc	2810.03a	27.25a	297.18a	668.45d	70.33a	286.26ab	1233.33a-c
	W_1	39.61a	458.79a-c	3507.50a	63.34a	941.60ab	2582.76a-d	130.67ab	527.67ab	660.20ab
	W_2	62.51a	693.05a-c	3948.51a	211.72b	1857.55c	3992.95c	125.62ab	618.05a	1791.10bc
Harvesting of fruits	W_0	25.88a	730.41a-c	4539.35a	35.44a	325.61ab	1589.60abd	180.62a	611.07a	2372.71c
	W_1	40.04a	982.85a	4349.31a	40.58a	609.40ab	1107.41ad	160.66ab	1292.46c	4485.90d
	W_2	30.06a	892.66ac	3643.21a	61.21a	547.21ab	2884.50a-c	144.12ab	1406.40c	4256.83d
Means for combination of irrigation	W_0	30.53a	434.46b	3368.41a	37.56a	385.55a	1728.50a	115.79a	308.59b	1436.00a
	W_1	75.38b	809.07a	3519.21a	61.52a	902.86b	2410.44a	140.42a	655.93a	1832.11ab
	W_2	119.57c	746.69a	3380.97a	116.79b	1419.48c	4553.61b	151.41a	737.01a	2149.75b
Means for sampling time	Intensive growth of apple trees	154.39a	621.37ab	2669.28a	69.36ab	1181.70a	4417.32b	136.94ab	120.89a	484.65a
	Fruiting/ripering	39.10b	500.20a	3422.01ab	100.77b	1032.11a	2414.72a	108.87a	477.33b	1228.21b
	Harvesting of fruits	31.99b	868.64b	4177.29b	45.74a	494.07b	1860.51a	161.80b	1103.31c	3705.15c

* Means marked with the same letters did not differ significantly at the probability of P=0.05 cfu colony forming units

Table 4. Effect of sampling time and irrigation on the dehydrogenase (ADh), protease, (AP) urease activity (AU) and emission CO_2 (CO_2) in the soil of apple tree orchard after replantation in the years 2003-2005.

Sampling time	Combination of irrigation	2003				2004				2005			
		ADh ($cm^3 kg$)	AP ($mg\ kg^{-1}$)	AU ($\mu mol\ g^{-1}$)	CO_2 ($mg\ kg^{-1}$)	ADh ($cm^3\ kg^{-1}$)	AP ($mg\ kg^{1}$)	AU ($\mu molg^{1}$)	CO_2 ($mg\ kg^{-1}$)	ADh ($cm^3\ kg^{1}$)	AP ($mg\ kg^{1}$)	AU ($\mu mo\ g^{-1}$)	CO_2 ($mg\ kg^{-1}$)
Intensive growth of apple trees	W_0	0.63ab*	3.11a-c*	2.48a-c*	23.31a*	0.34ab*	2.36a*	2.82b*	20.96a*	0.48a*	1.51a*	2.38a*	41.36ab*
	W_1	0.97a-c	6.36b-d	2.73a-c	26.35a	0.58b	3.47a	1.53a,c	23.94a	0.43a	2.62ab	1.73a	36.09ab
	W_2	0.84a-c	6.72c,d	3.81b-d	24.63a	0.38ab	3.27a	1.76a-c	24.86a	0.67a	1.25a	1.86a	37.81ab
Fruiting/ripering	W_0	0.49a	2.61a	1.81a,b	22.22a	0.58b	2.03a	1.65a,b	15.35a	0.63a	4.21ab	1.98a	35.97ab
	W_1	0.92a-c	2.12a	1.17a	51.21c	0.19a	1.65a	0.74d	27.95a	1.22ab	8.59bc	2,42a	34.60a
	W_2	0.60a,b	3.40a-c	1.61a	45.71bc	0.38ab	2.29a	0.76d	19.82a	2.02b	3.86ab	2.40a	46.86b
Harvesting of fruits	W_0	1.53b-d	2.24a	5.06d,e	25.89a	0.38ab	1.73a	1.93a,b	23.60a	0.55a	3.95ab	4.19b	34.14a
	W_1	1.70c,d	7.49d	4,21cd	22.91a	0.27a	2.04a	2.08a,b	22.22a	2.42b	10.70c	3.68b	29.90a
	W_2	2.19d	5.26a-d	6.42e	27.27ab	0.37ab	2.03a	1.17c,d	27.50a	1.62a,b	12.08c	3.46b	32.88a
Means for level irrigation	W_0	0.88a	2.65b	3.12ab	23.81b	0.44a	2.04a	1.95b	19.97a	0.55b	3.22a	2.85a	37.16ab
	W_1	1.20a	5.32a	2.70a	33.49a	0.35a	2.39a	1.45a	24.71a	1.35a	7.30b	2.61a	33.53a
	W_2	1.21a	5.13a	3.95b	32.54a	0.38a	2.53a	1.23a	24.06a	1.44a	5.73ab	2.57a	39.18b
Means for sampling time	Intensive growth of apple trees	0.81a	5.40a	3.00b	24.76a	0.34a	3.03b	1.86a	23.25a	0.52b	1.79a	1.99a	38.42a
	Fruiting/ripering	0.67a	2.71b	1.53a	39.72b	0.38a	1.99a	1.05b	21.04a	1.29a	5.55b	2.26a	39.14a
	Harvesting of fruits	1.81b	4.99a	5.23c	25.36a	0.44a	1.93a	1.73a	24.44a	1.53a	8.91c	3.77b	32.31b

* Means marked with the same letters did not differ significantly at the probability of P=0.05

The amount and level of water in the soil exert the basic effect on the growth and development of microorganisms living in it. The highest number of fungi was found in the soil of the combination without irrigation - W_0 (55.61 to 260.01 cfu kg^{-1}) and the lowest number of fungi was observed in W_1 and W_2 combination (from 16.53 to 130.33 cfu kg^{-1}). A high number of *Azotobacter,* actinomycetes, proteolytic bacteria, phosphate solubilizing bacteria almost always were observed in the soil in the irrigation combination - W_1 or W_2 (Table 2, 3). The number of actinomycetes ranged from 132.16 to 1096.30 cfu kg^{-1} in W_0 combination, from 161.34 to 1490.37 cfu kg^{-1} in W_1 combination and from 380.33 to 1696.59 cfu kg^{-1} in W_2 combination. The number of *Azotobacter* ranged from 0 to 12 cfu kg^{-1} in W_0 combination, from 0 to 50 cfu kg^{-1} in W_1 combination and from 0 to 11 cfu kg^{-1} in W_2 combination. The number of proteolytic bacteria ranged from 15.17 to 180.62 cfu kg^{-1} in W_0 combination, from 39.69 to 160.66 cfu kg^{-1} in W_1 combination and from 30.06 to 266.12 cfu kg^{-1} in W_2 combination. The number of phosphate solubilizing bacteria ranged from 28.45 to 730.41 cfu kg^{-1} in W_0 combination, from 147.66 to 1292.46 cfu kg^{-1} in W_1 combination and from 186.57 to 1857.55 cfu kg^{-1} in W_2 combination. The total number of bacteria ranged from 668.45 to 4539.35 cfu kg^{-1} in W_0 combination, from 350.25 to 4485.90 cfu kg^{-1} in W_1 combination and from 401.31 to 6783.39 cfu kg^{-1} in W_2 combination.

According to Bielińska (2001), adequate soil moisture is the basic condition for the action of soil enzymes. Water determines the physiological status of microorganisms and plants, and it is also necessary to maintain soil enzymes in catalytically active status. However, the highest activity of the studied enzymes and CO_2 emission was recorded rather in the irrigation combinations, i.e. in W_1 or W_2 (Table 4). Dehydrogenase activity ranged from 0.19 to 2.42 cm^3 kg^{-1} in irrigation combination (W_1, W_2) and from 0.34 to 1.53 cm^3 kg^{-1} in W_0 combination. Protease activity ranged from 1.25 to 12.08 mg kg^{-1} in irrigation combination (W_1, W_2) and from 1.51 to 4.21 mg kg^{-1} in W_0 combination. Urease activity ranged from 1.65 to 5.06 μmol g^{-1} in W_0 combination, from 0.74 to 4.21 μmol g^{-1} in W_1 combination and from 0.76 to 6.42 μmol g^{-1} in W_2 combination. CO_2 emission ranged from 15.35 to 41.36 mg kg^{-1} in W_0 combination, from 22.22 to 51.21 mg kg^{-1} in W_1 combination and from 19.82 to 46.86 mg kg^{-1} in W_2 combination. Bielińska (2001) obtained only a significant dependence between the moisture and dehydrogenase activity and further pointed to the fact that dehydrogenases showed a higher sensitivity to environmental stress than phosphotases, ureases and proteases being extracellular enzymes creating complexes with soil colloids.

Soil organic matter affects biochemical, chemical, biological and physical soil properties that control soil microbial activity (Dou et al., 2007). A high pH was observed in autumn and in spring, while in summer, pH was low. The highest pH in summer was recorded in the last year. In the following years, pH ranged from 4.79 to 5.90 in 2003, from 3.42 to 6.38 in the second year and from 4.35 to 6.51 in the last year. The highest pH was found in W_2 combination (from 5.83 to 6.35) and the lowest without any irrigation - W_0 (from 4.37 to 4.93). The highest TOC content has been confirmed in summer in the second and last year. A high content of TOC was found in the last year (from 7.03 to 17.32 g kg^{-1}) and low in the second year (from 5.45 to 8.92 g kg^{-1}). However, the highest TOC content was almost always recorded in irrigation combination - W_1 (from 7.03 to 17.32 g kg^{-1}) (Table 5).

Table 5. Effect of sampling time and irrigation on the pH and the content of the total organic carbon (TOC) in the soil of the apple trees orchard after replantation in years 2003-2005.

Sampling time	Combination of irrigation	2003		2004		2005	
		pH	TOC (g kg^{-1})	pH	TOC (g kg^{-1})	pH	TOC (g kg^{-1})
Intensive growth of apple trees	W_0	4.76a*	8.31a*	5.14ab*	8.71ab*	4.35c*	9.12a*
	W_1	5.42a-c	8.96a	5.36a-c	8.92ab	4.94bc	9.73a
	W_2	5.80bc	8.18a	6.38c	8.47ab	5.85ab	7.69a
Fruiting/ripering	W_0	4.85ab	8.32a	3.42a	8.91ab	5.61ab	14.93b
	W_1	4.82ab	7.89a	4.76a	9.43b	6.31a	17.32c
	W_2	5.90c	7.87a	5.37a-c	8.20ab	6.71a	14.06b
Harvesting of fruits	W_0	4.79a	10.15a	4.55a	6.93ac	4.85bc	9.31a
	W_1	5.66a-c	10.20a	4.50a	7.50a-c	6.22a	7.03a
	W_2	5.79bc	9.82a	6.02bc	5.45c	6.51a	7.91a
Means for level irrigation	W_0	4.80a	8.92a	4.37a	8.18ab	4.93b	11.12ab
	W_1	5.30b	9.02a	4.87b	8.62b	5.82a	11.36b
	W_2	5.83c	8.62a	5.92c	7.37a	6.35a	9.89a
Means for sampling time	Intensive growth of apple trees	5.33a	8.48a	5.63c	8.70b	5.05b	8.85a
	Fruiting/ ripering	5.19a	8.03a	4.51a	8.85b	6.21a	15.44b
	Harvesting of fruits	5.41a	10.06b	5.02b	6.62a	5.86	8.08

* Means marked with the same letters did not differ significantly at the probability of P=0.05

High microbiological and biochemical activity in the irrigation combination could consist in the higher concentration of TOC in that object. In the irrigation combination were noted high content of TOC and pH or rather low in the combination without irrigation (W_0), similar to the number of microorganisms and enzyme activity in soil. This work confirms, therefore, the increase of microbiological and biochemical activity of soil in those combinations which at the same time have demonstrated favourable chemical properties of the soil. It is well known that active microbial biomass is strongly associated with the pH values of the soil (Meysner et al., 2006). The obtained significant correlation coefficients confirm also the significant dependences between the number of microorganisms, biochemical activity and pH and the content of TOC (Table 6). Chemical properties in the soil may have modified in a significant way the microbiological and biochemical activity in soil in the apple tree orchard after replantation.

Table 6. Correlation coefficients between pH, the content of total organic carbon (TOC), soil temperature and the biological and biochemical activity.

Parameters	Chemical properties soil		
	pH	TOC	Soil temperature
F	-0.18*	-0.04	-0.21*
A	0.41*	-0.00	-0.21*
Azb	0.35*	0.16**	-0.24*
PB	0.21*	-0.02	0.09
PSB	0.35*	-0.00	-0.08
TNB	0.31*	-0.10	-0.04
ADh	0.42*	0.15**	-0.32*
AP	0.47*	0.13***	-0.15**
AU	0.20*	0.06	-0.49*
CO_2	0.11***	0.06	0.13***

- significant at P <0.001, ** P< 0.01, *** P<0.05.

CONCLUSIONS

Microbiological and enzymatic activity and CO_2 emission of soil were differentiated in the vegetative season and in the particular years of studies. Periods of high activity of microorganisms, higher enzymatic activity of soil in the successive years of studies and in the particular terms may be connected with an increase of pH values and the content of total organic carbon in the soil. Therefore, microbiological and biochemical activities of soil environment are strongly connected with its reaction to and the content of organic matter in soil. Irrigation had a favourable effect on the increased number of typical bacteria, actinomycetes, and biochemical activity while an unfavourable effect of irrigation was exerted on the number of fungi in the soil.

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