Agronomy Research **8** (1) 827–836, 2010

Microbiological activity of soil against the background of differentiated irrigation and fertilization in apple (Malus domestica) orchard after replantation

K. Styła[1], A. Sawicka[2]

[1]Institute for Agricultural and Forest Environment Polish Academy of Sciences, Poznań, Poland; e-mail: styla.katarzyna@gmail.com
[2]Department of Agricultural Microbiology, University of Life Science, Poznań, Poland

Abstract. The effect of differentiated irrigation and fertilization on the number of microorganisms in the soil of an apple (*Malus domestica*) orchard after replantation was investigated. 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, and W_2 - maintenance of soil moisture at the level of -0.01MPA of water potential. Three fertilization combinations were used, i.e. 65 kg N/ha, 65 kg N/ha and 95 kg/ha K_2O as well as 130 kg N/ha and 190 kg/ha K_2O. The highest number of fungi was noted in the 130 kg N/ha and 190 kg/ha combination. The highest number of actinomycetes, *Azotobacter*, proteolytic bacteria, phosphate solubilizing bacteria, and the total number of bacteria were found of 65kg N/ha and 95kg/ha combination. A high number of fungi was observed in W_0 combination. The highest number of actinomycetes, *Azotobacter*, proteolytic bacteria, phosphate solubilizing bacteria, and the total number of bacteria were confirmed in the W_1 and W_2 combination. A high number of actinomycetes, *Azotobacter*, proteolytic bacteria, phosphate solubilizing bacteria, and the total number of bacteria and lower number of fungi in control object (Nowina and virgin soil) were recorded.

Keywords: soil fatigue, replantation, apple orchard, microbiological activity, irrigation, fertilization

INTRODUCTION

Apple (*Malus domestica*) replant disease (ARD) is distributed worldwide and is often encountered in establishing new orchards on old sites (Yao et al., 2006). Symptoms include death of fine feeder roots, stunted growth above- and below-ground, and reduced fruit yields (Rumberger et al., 2007). Replant disease, also called "soil sickness", causes a significant problem in fruit orchards in the temperate climate zone. It may increase because of intensification of fruit production, which is usually followed by replanting orchards in the same place (Szczygieł & Zepp, 1998). Replant disease is one of the components of replant problem, which is caused by biotic and abiotic factors. (When the problem is caused by biotic factors, it is referred to as replant disease.) Causes of replant disease are fungi, bacteria, actinomycetes, nematodes, and their interactions. (Utkhede & Smith, 1994). In 1968, a work was published calling attention to the connection between fluorescent bacteria from *Pseudomonas* genus

(Utkhede & Smith 1994; Rutkowski et al., 2000a) and *Bacillus subtilis* (Utkhede & Smith, 1994; Rutkowski et al. 2000a) with a limited growth of apple tree roots as a result of root top necrosis. Actinomycetes might be the reason for apple tree replantation disease (Otto & Winkler, 1993; Hoestra, 1994; Otto et al., 1994a, Otto et al., 1994b; Utkhede & Smith, 1994). Other causes cited by researchers include fungi from the following genera: *Pythium, Thielaviopsis, Rosellinia, Phytophthora, Cylindrocarpon, Fusarium, Rhizoctoni, Penicillium* and *Alternaria* (Rutkowski et al., 2000b; Benizri et al., 2005). 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).

According to Szczygieł (2003), the limitation of negative results of replantation disease can be exerted by rational fertilization and irrigation. Barabasz & Vořišek (2002); Zydlik & Pacholak (2004) believe that agrotechnical treatments such as fertilization (including organic and mineral fertilization with nitrogen) as well as irrigation have a significant effect on the activity of soil microorganisms.

Recently replant disease has become a major problem in Poland. Therefore, knowledge of the problem may be a great help in avoiding its negative effects on the productivity of newly established orchards (Szczygieł & Zepp, 1998).

The objective of the present work was the investigation of soil microbiological activity by the determination of the number of fungi, actinomycetes, bacteria from *Azotobacter* genus, proteolytic bacteria, phosphate solubilizing bacteria and the total number of bacteria in the soil of an apple (*Malus domestica*) orchard after replantation, depending on the applied irrigation and fertilization levels.

MATERIALS AND METHODS

Studies were conducted from 2003–05 in an apple (*Malus domestica*) 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 (N), 65 kg N/ha and 95 kg/ha K_2O (NK) as well as 130 kg N/ha and 190 kg/ha K_2O (2N2K). 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 grown before, referred to as 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 mass soil. In the studied soil samples, the following microorganisms were estimated: the 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 with tricalcium phosphate during 10 days incubation at 28ºC (Rodina, 1968). The total number of bacteria was made on 2% agar medium on soil extract with K_2HPO_4 during 14 days incubation at 28ºC (Löchnis, 1920). Soil pH was measured in soil-water (1:2.5 v/v) suspensions by potentiometric method. The total organic carbon (TOC) was analyzed on the Total Organic Carbon Analyzer (TOC 5050A) with Solid Sample Module (SSM-5000A) produced by Shimadzu (Japan). Dissolved organic carbon (DOC) was analyzed on TOC 5050A equipment produced by Shimadzu (Japan). For the investigation of DOC, soil samples were heated in redistilled water at 100ºC for two hours under a reflux condenser. Extracts were separated by the mean filter paper and analyzed on TOC 5050A facilities Twice-distilled water from silica glass equipment was used (Smolander & Kitunen, 2002). The number of microorganisms was analyzed in 5 replications in soil with natural moisture. Obtained results were subject to analysis of variance and differences between mean values were estimated by *Tukey's* test. Dependences between the number of microorganisms, and the biochemical and chemical properties were estimated using multiple regression.

The mean temperatures and total rainfall data during the period of vegetation of the apple orchard are presented in Table 1.

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 2005, the respective values were 15.2°C and 301.4 mm.

Table 1. Weather characteristics.

Months	2003		2004		2005	
	Temp (ºC)	Precip (mm)	Temp (ºC)	Precip (mm)	Temp (ºC)	Precip (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

RESULTS AND DISCUSSION

The data in Table 2 clearly show that combinations intensively fertilized with nitrogen and potassium (2N2K) caused an increase of fungi number; on the other hand, lower doses of fertilization (NK) contributed to the increased number of actinomycetes, bacteria from *Azotobacter* genus, proteolytic bacteria, phosphate solubilizing bacteria, and the total number of bacteria. The number of fungi ranged from 45.00 to 59.29 cfu kg^{-1} in N combination and from 32.63 to 164.03 cfu kg^{-1} in NK combination and from 55.70 to 133.14 cfu kg^{-1} in 2N2K combination. The number of actinomycetes ranged from 889.76 to 1066.51 cfu kg^{-1} in 2N2K combination and from 390.12 to 675.21 cfu kg^{-1} in N combination. The number of *Azotobacter* ranged from 1.00 to 4.00 cfu kg^{-1} in N combination and from 5.00 to 16.00 cfu kg^{-1} in NK combination and from 2.00 to 16.00 in 2N2K combination. The number of proteolytic bacteria ranged from 43.64 to 170.84 cfu kg^{-1} in N combination, from 81.11 to 112.24 cfu kg^{-1} in NK combination and from 59.12 to 104.70 cfu kg^{-1} in 2N2K combination. The number of phosphate solubilizing bacteria ranged from 224.50 to 843.45 cfu kg^{-1} in N combination, from 686.21 to 1135.77 cfu kg^{-1} in NK combination and from 303.23 to 1179.30 cfu kg^{-1} in 2N2K combination. The total number of bacteria ranged from 1927.06 to 3298.36 cfu kg^{-1} in N combination, from 3068.88 to 4397.70 cfu kg^{-1} in NK combination and from 2214.23 to 4334.05 cfu kg^{-1} in 2N2K combination. At the same time, a higher number of actinomycetes, *Azotobacter,* proteolytic bacteria, phosphate solubilizing bacteria and the total number of bacteria, but a low number of fungi was recorded on control objects – on Nowina (soil earlier used for agriculture) of Virgin Soil – earlier used as fallow. The number of fungi amounted to 77.96 cfu kg^{-1} in Nowina and 63.42 cfu kg^{-1} in virgin soil. The number of actinomycetes was 1824.65 cfu kg^{-1} in Nowina and 942.86 cfu kg^{-1} in virgin soil. The number of *Azotobacter* was 4.00 cfu kg^{-1} in Nowina and 30.00 cfu kg^{-1} in virgin soil. The number of proteolytic bacteria was 119.92 cfu kg^{-1} in Nowina and 134.79 cfu kg^{-1} in virgin soil. The number of phosphate solubilizing bacteria was 1467.95 cfu kg^{-1} in Nowina and 1367.65 cfu kg^{-1} in virgin soil. The total number of bacteria was 5701.88 cfu kg^{-1} in Nowina and 5458.48 cfu kg^{-1} in virgin soil.

Thus, it was found that on objects originating from the replanted orchard, there occurs a decrease of microflora which has a favourable effect on the growth and development of plants, but the number of fungi increases; (one can suppose that the fungi are toxinogenic).

Barabasz & Vořišek (2002) believe that rational mineral fertilization has a favourable effect on plant yielding. It must be stressed, however, that incorrect agrotechnical treatments and irrational application of fertilization may cause disturbances in the functioning of the whole agrosystem and contribute to the development in soil environments of different noxious compounds (nitrozoamines, mycotoxins) acting unfavourably on soil microorganisms, on the cultivated plants as well as on the fertility of arable soils. Myśków & Kobus (1986), Wołoszyk & Nowak (1993); Myśków et al. (1996) believe that repeated mineral fertilization, particularly with high doses of nitrogen, can cause strong acidification of soils and increase the development of fungi. Myśków et al. (1996) have found that the development in soil of microflora communities depends also on the type of the applied nitrogen fertilizer. Those authors confirmed in their studies the presence of the greatest number of fungi in soil fertilized with ammonium sulphate.

Table 2. Effect of irrigation and fertilization on the numbers of microorganisms in the soil of the apple tree orchard after replantation in cfu kg^{-1} d.m. soil (means in years 2003–05).

Levels of irrigation	*Levels of fertilization*	*F x 10^6*	*A x 10^6*	*Azb. x 10^3*	*PB x 10^6*	*PSB x 10^6*	*TNB x 10^6*
W_0	N	59.29a*	578.59a*	4b*	43.64b*	224.50d*	1927.06a*
	NK	164.03d	889.76ac	8ab	81.11ab	686.21de	3068.88ab
	2N2K	133.14cd	574.65bc	2a	59.12ab	303.23bce	2214.23a
W_1	N	47.69ab	390.12b	1b	70.33ab	531.54bde	2325.43a
	NK	46.77ab	1025.71a,c	16b	109.39a	1135.77a	3639.35ab
	2N2K	97.09bc	678.93a-c	16b	97.57ab	786.37a-c	2847.75ab
W_2	N	45.00ab	675.21a-c	2a	170.84c	843.45a-c	3298.36ab
	NK	32.63a	1066.51a	5ab	112.24ac	968.81ac	4397.70b
	2N2K	55.70ab	1079.12a	6ab	104.70a	1179.30a	4334.05b
Control I	Nowina	77.96ab	1824.65c	4ab	119.92ab	1467.95a	5701.88c
Control II	Virgin soil	63.42ab	942.86ab	30b	134.79b	1367.56a	5458.47c
Means for fertilization	N	67.17a	442.08b	1a	94.94ab	533.16a	2516.95a
	NK	81.14ab	992.33a	10b	100.91ab	930.26ab	3701.98b
	2N2K	95.31b	777.57a	8ab	87.13a	756.30bc	3132.01ab
Means for irrigation	W_0	135.32a	573.43a	4a	61.29a	404.65c	2403.39a
	W_1	63.85b	698.25ab	11b	92.43ab	817.89a	2937.51a
	W_2	44.44b	940.28b	4a	129.26b	997.19ab	4010.04b

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

Explanations: F – fungi, A – actinomycetes, Azb. – *Azotobacter,* PB – proteolytic bacteria, PSB – phosphate solubilizing bacteria, TNB – the total number of bacteria

cfu – colony forming units

According to Kucharski & Niewolak (1997), the effect of fertilization on the microbiological status of soil is associated with the form of fertilizers (mineral or organic), their dose and type, as well as with the cultivation status. Kucharski et al. (1996) also believe that an imbalance of homeostasia may be connected not only with the dose of nitrogen, but also with the complex of soil agricultural suitability. Soils of the particular complexes differ by physical and chemical properties, and they in turn are responsible for biological properties (Kucharski et al., 1996). According to Kucharski et al. (1996), fertilization with mineral nitrogen increases the populations of bacteria, actinomycetes and fungi as a result of the improvement of nitrogen availability in the soil and causes changes in the physical and chemical properties. According to Kucharski et al. (1996), nitrogen fertilization exerts an effect on microorganisms, mainly by affecting soil pH.

According to Myśków et al. (1996) and Gostkowska et al. (1997) high mineral fertilization, particularly with nitrogen as well as the use of pesticides can constitute factors favouring the occurrence of toxinogenic fungi. Smyk et al. (1989) confirm also the observations of Gostkowska et al. (1997). Smyk et al. (1989) believe that under the influence of the use of great amounts of chemical agents and high doses of nitrogen fertilizers, the qualitative composition of biocenoses is subject to modification – there follows a recession of bacteria from the genera: *Arthrobacter, Azotobacter and Streptomycetes*, being the hitherto existing dominants from the group of autochthonous microflora, and the domination in microbiocenoses is taken over by other species – mainly by fungi from *Deuteromycetes* class.

Gawrońska et al. (1992) and Koper et al. (2003) showed that plant cultivation for many years on the same site has a negative effect on the development of bacteria including also „phosphoric" bacteria. Also Bis (2002) found, in soil environments subject to soil sickness (fatigue), a higher frequency of toxinogenic fungi in comparison with soils in optimal conditions. According to Bis (2002), plant cultivation in monoculture causes changes in the quantitative and qualitative composition of fungi, and mutated toxinogenic strains exert through their metabolites a destructive effect on the biological balance of soil biocenoses.

The amount and level of water in the soil exert the basic effect on the growth and development of microorganisms living in it. The data in Table 2 show that intensive irrigation (-0.01 MPa) exerted an effect on the decrease in number of fungi and on the increase in number of the remaining microorganisms in the soil. The number of fungi ranged from 59.29 to 133.14 cfu kg^{-1} in W_0 combination and from 32.63 to 55.70 cfu kg^{-1} in W_2 combination. The number of actinomycetes ranged from 675.21 to 1079.12 cfu kg^{-1} in W_2 combination and from 574.65 to 889.76 cfu kg^{-1} in W_0 combination. The number of *Azotobacter* ranged from 1.00 to 16.00 in W_1 combination, from 2.00 to 8.00 cfu kg^{-1} in W_0 combination and from 2.00 to 6.00 cfu kg^{-1} in W_2 combination. The number of proteolytic bacteria ranged from 104.70 to 170.84 cfu kg^{-1} in W_2 combination and from 43.64 to 81.11 cfu kg^{-1} in W_0 combination. The number of phosphate solubilizing bacteria ranged from 843.45 to 1179.30 cfu kg^{-1} in W_2 combination and from 224.50 to 686.21 cfu kg^{-1} in W_0 combination. The total number of bacteria ranged from 3298.36 to 4397.70 cfu kg^{-1} in W_2 combination, from 1927.06 to 3068.88 cfu kg^{-1} in W_0 combination and from 1927.06 to 3298.36 cfu kg^{-1} in N combination.

Kobus (1995) found that fungi can develop in very low moisture; this has been confirmed by the results of the present work. Similar relations between soil moisture and the number of microorganisms, particularly in reference to the number of fungi were obtained by Rutkowski et al. (2000b). Also Paul & Clark (2000) believe that bacteria and actinomycetes take up more water than fungi.

Soil organic matter affects biochemical, chemical, biological and physical soil properties that control soil microbial activity (Dou et al., 2007). Table 3 shows the highest pH value, the contents of TOC and DOC in NK and 2N2K combination, while in N combination pH value, TOC and DOC were the lowest. The pH value ranged from 3.63 to 4.91 in N combination, from 5.28 to 6.71 in NK combination and from 5.19 to 6.49 in 2N2K combination. The content of TOC ranged from 8.55 to 11.42 g kg^{-1} in 2N2K combination and from 8.09 to 9.71 g kg^{-1} in N combination. The concentrations of DOC ranged from 0.39 to 0.61 g kg^{-1} in 2N2K combination and from 0.37 to 0.46 g kg^{-1} in N combination. A high pH value was observed in W_2 combination (from 4.91 to 6.71) and low in W_0 combination (from 3.63 to 5.28). The highest content of TOC was found in W_1 (from 8.09 to 11.42 g kg^{-1}) and W_0 combination (from 8.60 to 9.93 g kg^{-1}) and the lowest in W_2 combination (from 8.37 to 8.96 g kg^{-1}). A high concentration of DOC was found in W_0 combination (from 0.46 to 0.51 g kg^{-1}) and low in W_2 combination (from 0.37 to 0.39 g kg^{-1}).

Table 3. Effect of irrigation and fertilization on the pH, the contents of the total organic carbon (TOC) and dissolved organic carbon (DOC) in the soil of the apple tree orchard after replantation (means with years 2003–2005).

Levels of irrigation	*Levels of fertilization*	*pH*	*TOC (g kg^{-1} d.m. soil)*	*DOC (g kg^{-1} d.m. soil)*
W_0	N	3.63f*	9.71ab*	0.46ab*
	NK	5.28ab	8.60a	0.51bc
	2N2K	5.19ab	9.93ab	0.48a-c
W_1	N	4.48c	8.09a	0.38ab
	NK	5.64bd	9.48ab	0.43ab
	2N2K	5.87d	11.42b	0.61c
W_2	N	4.91ac	8.37a	0.37a
	NK	6.71e	8.96a	0.38ab
	2N2K	6.49e	8.55a	0.39ab
Control I	Nowina	5.81b	11.01b	0.47ab
Control II	Virgin soil	6.14b	10.46b	0.51bc
Means for fertilization	N	4.34a	8.72a	0.40a
	NK	5.88b	9.02ab	0.44ab
	2N2K	5.85b	9.97b	0.49b
Means for irrigation	W_0	4.70a	9.41a	0.48a
	W_1	5.33b	9.66a	0.47a
	W_2	6.03c	8.63a	0.38b

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

High microbiological activity in combinations with mean (NK) and intensive (2N2K) fertilization levels and irrigation combination could consist in the higher concentration of chemical compounds (TOC, DOC and pH value) in that object. In NK and 2N2K and irrigation combination were noted high content of TOC, DOC and pH, similar to the number of microorganisms in soil. This work confirms, therefore, the increase of microbiological activity of soil in those combinations which at the same time have demonstrated favourable chemical properties of soil. It is well known that active microbial biomass is strongly associated with the pH values of the soil (Meysner et al., 2006). Also Kobus (1995) and Bis (2002) believe that bacteria generally develop better in the environment with a neutral reaction or slightly alkaline reaction, while fungi develop well with lower pH values. The obtained significant correlation coefficients confirm also significant dependences between the amount of microorganism activity and pH and the content of TOC and DOC (Table 4).

Table 4. Correlation coefficients between chemical properties and the number of microorganisms.

Groups microorganisms	*Chemical properties soil*		
	pH	TOC	DOC
Fungi	-0.18*	-0.04	0.11***
Actinomycetes	0.41*	-0.00	-0.08
Azotobacter	0.35*	0.16**	0.29*
Proteolytic bacteria	0.21*	-0.02	-0.21*
Phosphate solubilizing bacteria	0.35*	-0.0	-0.10
The total number of bacteria	0.31*	-0.1	-0.19*

** significant levels p <0.001, ** p< 0.01, *** p<0.05*

CONCLUSIONS

1. The higher number of microorganisms in control combinations in comparison with their smaller number in fertilized objects originating from the apple orchard after replantation may indicate lower biological activity and soil fatigue in the apple orchard after replantation.

2. The high number of microorganisms in the combination with a mean fertilization level (NK) and an intensive fertilization level (2N2K) is the effect of more favourable chemical properties of the soil (i.e. higher values of: pH, content of TOC and DOC) existing in those combinations.

3. The smaller number of fungi in control objects and their increased number in fertilized combinations may indicate that there occur unfavourable changes in the soil caused by replantation.

4. Irrigation had a favourable effect on the increased number of typical bacteria and actinomycetes, while exerting an unfavourable effect on the number of fungi in the soil.

REFERENCES

Barabasz, W. & Vořišek K. 2002. Biodiversity of microorganisms in soil environments. In *Activity of microorganisms in different environments*. Kraków, pp. 23–34 (in Polish).

Benizri, E., Piutti, S., Verger, S., Pagès, L., Vercambre, G., Poessel, J.L. & Michelot, P. 2005. Replant disease: Bacterial community structure and diversity in peach rhizosphere as determined by metabolic and genetic finger printing. *Soil Biol. Biochem.,* **37**, 1738–1746.

Bis, H. 2002. Occurrence of toxinogenic fungi in soil environment. In *Activity of microorganisms in different environments*. Kraków, pp. 35–42 (in Polish).

Dou, F., Wright, A.L. & Hons F.M. 2007. Depth distribution of soil organic C and N after long-term soybean cropping in Texas. *Soil & Tillage Research,* **94**, 530–536.

Fenglerowa, W. 1965. Simple method for counting *Azotobacter* in soil samples. *Acta Microbiol. Polon*. **14**, 203.

Gawrońska, A., Kulińska, D., Lenart, S. & Jaśkowska, H. 1992. The effect of the maize monoculture on the biological properties of soil and on the yields of plants. *Pol. J. Soil Sci*. **25**, 89–94.

Gostkowska, K., Szwed, A., Furczak, J. & Iglik, H. 1997. Quantitative and qualitative changes of organisms in soil used for pomiculture enriched with organic waste material. In *Microorganisms in environment. Occurrence, activity and importance*. Kraków, pp. 181–193 (in Polish).

Grabińska-Łoniewska, A. 1999. *Laboratory Exercises in General Microbiology* Oficyna Wydawnicza Politechniki Warszawskiej, Warszawa, 223 (in Polish).

Hoestra, H. 1994. Ecology and pathology of replant problems. *Acta Hort*. **365,** 1–10**.**

Kobus, J. 1995. Biological processes and soil fertility formation. *Zesz. Probl. Post. Nauk Roln.* **421a**, 209–219 (in Polish).

Koper, J., Piotrowska, A. & Siwik-Ziomek, A. 2003. Influence of long-term mineral-organic fertilization on the value of biological indicator of soil fecundity. *Zesz. Probl. Post. Nauk Roln*. **494**, 207–213 (in Polish)

Kucharski, J., Ciećko, Z., Niewolak, T. & Niklewska-Larska, T. 1996. Activity of microorganisms in soils counted to different complexes of agricultural soil suitability fertilized with mineral nitrogen. *Acta Acad. Agricult. Tech. Olst. Agricult*. **62,** 25–35 (in Polish).

Kucharski, J. & Niewolak, T. 1997. The effect of cultivation system of cereals on the activity of soil enzymes. In *Microorganisms in environment. Occurrence, activity and importance* Kraków, 349–356 (in Polish)

Löchnis, F. 1920. *Landwirtschaftlich bakteriologisches Praktikum*. Berlin.

Martin, J.P. 1950. Use of acid, rose bengal and streptomycin in the plate method for estimating soil fungi. *Soil Science*. **69,** 215–232.

Meysner, T., Szajdak, L. & Kuś J. 2006. Impact of the farming systems on the content of biologically active substances and the forms of nitrogen in the soils. *Agronomy Research,* **4** (2), 531–542.

Myśków, W. & Kobus, J. 1986. International Symposium on Soil Biology and Conservation of the Biosphere (Sopron. 1985). *Post. Mikrobiol*. **25,** 243–255 (in Polish).

Myśków, W., Stachyra, A., Zięba, S. & Masiak, D. 1996. Biological activity of soil as indicator of its fertility and fruitfulness. *Rocz. Glebozn*. **47**, 89–99 (in Polish).

Otto, G. & Winkler, H. 1993. Colonization of rootlets of apple seedlings from replant soils by actinomycetes and endotrophic mycorrhiza. *Acta Hort*. **324**, 53–59.

Otto, G., Winkler, H. & Szabó, K. 1994a. Proof of actinomycetes in rootlets of Rosaceae from SARD soil – a contribution to the specificity of replant disease. *Acta Hort*. **363,** 43–48.

Otto, G., Winkler, H. & Szabó, K. 1994b. Influence of growth regulators on the infection of rootlets of apple seedlings in SARD soils by *Actinomycetes*. *Acta Hort*. **363**, 101–107.

Paul, E.A. & Clark, F.E. 2000. *Microbiology and biochemistry of soils*. Wyd. Uniwersytetu Marii Curie-Skłodowskiej, Lublin. (in Polish).

Rodina, A. 1986. *Microbiological methods of water investigations*. Państwowe Wydawnictwo Rolnicze i Leśne. Warszawa, pp. 63–350 (in Polish).

Rumberger, A., Merwin, I.A. & Thies, J.E. 2007. Microbial community development in the rhizosphere of apple trees at a replant disease site. *Soil Biol. Biochem.*, **39**, 1645–1654.

Rutkowski, K., Pacholak, E. & Sawicka, A. 2000a. Evaluation of the microbiological state of the soil under varied conditions of fertilization and irrigation in a replanted orchard. Part I. Number of bacteria.. *Prace Kom. Nauk Rol. i Kom. Nauk Leś. PTPN***. 89**, 175–183 (in Polish).

Rutkowski, K., Pacholak, E. & Sawicka, A. 2000b. Evaluation of the microbiological state of the soil under varied conditions of fertilization and irrigation in a replanted orchard. Part II. Number of fungi and actinomycetes. *Prace Kom. Nauk Rol. i Kom. Nauk Leś. PTPN*. **89**, 185–192 (in Polish).

Smolander, A. & Kitunen, V. 2002. Soil microbial activities and characteristics of dissolved organic C and N in relation to tree species. *Soil Biol. Biochem.* **34,** 651–660.

Smyk, B., Czachor, M. & Aziz, N.H. 1989. Occurrence of toxinogenic fungi in soil and their influence on the biological productivity of agroecosystems. *Zesz. Probl. Post. Nauk Roln.* **380,** 143–150 (in Polish).

Szczygieł, A. & Zepp, A.L. 1998. An occurrence and importance of apple replant disease in Polish orchard. *Acta Hort.* **477**, 99–101.

Szczygieł, A. 2003. Soil fatigue in orchard. Part I. *Owoce Warzywa Kwiaty*. **l3**, 16–17 (in Polish).

Utkhede, R.S. & Smith E.M. 1994. Biotic and abiotic causes of replant problems of fruit trees. *Acta Hort*. **363**, 25–31.

Wołoszyk, C. & Nowak, W. 1993. Changes in organic carbon and total nitrogen content in light soil under the influence of mineral fertilization of grasses in field cultivation. *Zesz. Probl. Post. Nauk Roln*. **411**, 85–90 (in Polish).

Yao, S., Merwin, I.A., Abawi, G.S. & Thies, J.E. 2006. Soil fumigation and compost amendment alter soil microbial community composition but do not improve tree growth or yield in an apple replant site. *Soil Biol. Biochem.,* **38**, 587–599.

Zydlik, Z. & Pacholak, E. 2004. Effect of fertilization and irrigation on soil microorganisms in a replanted apple orchard. Part III. Numbers of fungi and actinomycetes. *Prace Kom. Nauk Rol. Leś. PTPN*. **97**, 317–324 (in Polish).