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PLANT, SOIL AND ENVIRONMENT

Former Rostlinná výroba

The importance of long-term field experiments for soil science and environmental research – a review

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ABSTRACT

An insufficient use of the results of long-term field experiments is not responsible and it means harm to research capacities. Long-term experiments are very expensive, but under the condition of comprehensive and coordinated evaluation they still represent the most cost-effective research method. With the knowledge obtained on the basis of long-term field experiments the farmers could double the yields in the last decades, improve the quality of the products and the environmental protection and secure the sufficient human nutrition. Nevertheless, if there are more than 400 000 diet-related deaths annually in Germany, it is not because of the lack of food, but in the opposite, because the food is too plentiful, too good and too cheap. If a farmer fertilized his plants and fed his animals in such a way we humans nourish ourselves, he would bankrupt within a few months, because: 1. The yields in plant and livestock production would be dramatically reduced and 2. veterinary surgeon costs would become priceless. On the occasion of the 60th anniversary of the establishment of the long-term field experiment in Thyrow in Germany, an international conference took place in Berlin, in June 1997. The participants of this conference proclaimed the memorandum *For the maintenance and the comprehensive use of European long-term field experiments*, which was signed by many scientists responsible for the maintenance of the long-term field experiments in 14 countries. In the concluding part of this memorandum the following points were emphasized: Contribute to the maintenance of the European long-term field trials, as they are essential for agricultural and environmental research. Support the efforts aiming at more extensive and cooperative use of the long-term field trials, which are a basis of the research on sustainable land use. Help to use the scientific knowledge originating in the long-term field trials to increase food production by means of maintenance of the soil quality and protection of natural resources. Contribute to keep the long-term field trials available and functioning effectively as a scientific heritage for future generations.

Keywords: long-term experiments; optimal carbon content; carbon sequestration; environmental protection

Soil is one of the most important bases of human life. Therefore, soil research is one of our most important tasks at present, as well as in future. Liebig formulated it more than 160 years ago: "Always and at all times it was the soil and its fertility that decided about well-being and health of the people." We live on the soil of what the soil gives. But our soils are in danger; seven million hectares of agricultural land and 9 million hectares of forests are irreparably lost worldwide every year.

Soil science is very expensive because:

- Soils are extremely diverse and it is necessary to treat them in a specific manner. Any recommendation has to be fitted to the specific soil conditions.
- Soil characteristics have large time and spatial variability.
- Changes of soil characteristics can be often proved and quantified only after decades.
- There is a strong dependence of the soil processes on the weather conditions in a given year.
- It is necessary to verify all the results suitable for practical application in long-term field experiments.

New knowledge suitable for practical application that has been verified in long-term field experiments is urgently needed and thus the long-term field experiments, in particular long-term fertilization experiments, are indispensable. However, the problems specified above are a reason why the

research on the field of humus chemistry is often limited to the laboratory.

With view of the value of soil and long-term experiments I would like to refer to the proclamation of the *soil of the year* made for the first time in Germany on the occasion of the World Soil Day. A selected group of soil scientists' representatives from the German Soil Science Society and the National Association of Soil chose from numerous candidates Chernozem, black earth, as the *soil of the year 2005*.

A more pronounced awareness of soil among the population and more engagement of the political decision-makers regarding soil protection should be initiated within this context. Black earths belong to the most fertile and most productive soils; they fulfil not only the production function, but also all ecological functions in an outstanding way.

The Static Fertilization Experiment Bad Lauchstädt is one of the most important long-term experiments in the world and it is studies black earth. This experiment allows a comprehensive understanding of the characteristics and potential of Chernozem, and it thereby establishes the need for sustainable protection of these soils. The highest yields achieved so far at this location indicate the yield potential and allow the estimations of the possibilities for further yield increases at these soils (Table 1).

So, even in the Central German dry region, under consideration of by-products such as straw and sugar beet leaves, up to 20 t/ha of dry mass can be harvested annually from black earth locations. This represents a decrease for the atmosphere of around 8 tonnes of carbon or approx. 30 tonnes of CO_2 per ha, provided that this carbon is sensibly used.

The importance of long-term field experiments

A large part of the problems that have not been sufficiently clarified yet can be solved only by using long-term field experiments. They include among others:

- The supply of soils with soil organic matter and the elaboration of suitable methods to determine optimal humus contents and the factors of the humus balance. Since many decades, we have optimal values for all macro- and micronutrients in the soil, we have also limit values for pollutants, however, we have no optimal values for the most important elements in soil, i.e. carbon and nitrogen.
- The effect of crop rotations on the crop yields, soil health and chemical, physical and biological soil characteristics.
- The effect of various management systems, in particular fertilization, on the nutrient leaching and the ecological soil functions.
- The explanations of the interrelations between land use systems and environment with consideration of hydrosphere and atmosphere.
- Research of the effects of climatic changes on soil properties.
- Indirect quantification of the atmospheric N deposition.
- Evidence of the nutrient efficiency of different fertilization systems by means of nutrient balances.

We owe predominantly to the results of the long-term field experiments for the contemporary knowledge regarding the sustainable land use. Long-term field experiments will also be indispensable in future, as they cannot be replaced by new analytical techniques or models; on the contrary, they are an indispensable basis for the calibration and validation of these techniques.

Overview of the long-term experiments of the world

In the research and politics only current problems stand in the foreground. The needs of future generations are not, or not sufficiently, considered. It should be therefore highly acknowledged that the scientists recognised the need of long-term field experiments (LTE) more than 100 years ago. Thanks to them and to the following generations that maintained the experiments we have a number of long-term field experiments that are invaluable today for the contemporary research and sustainable land use.

In this century, we already had the 100th anniversary of the Static Fertilization Experiment Bad Lauchstädt (Germany) in 2002, the 45th anniversary of the long-term experiments in Groß Kreutz (Germany) in 2004, and the 50th anniversary of long-term experiments in the Czech Republic in 2005.

The value of long-term field experiments increased internationally in the last decades. Overviews of the existing long-term field experiments were accomplished and a number of international projects dealing with the data collection and evaluation were launched.

Table 1. Maximum yields in the last 10 years in long-term experiments on the site Bad Lauchstädt

Crop	Yield (t/ha)
Corn (86% dry matter)	
Winter wheat	12.2
Winter barley	11.3
Winter rye	11.2
Spring barley	10.3
Maize	15.1
Corn (91% dry matter)	
Winter rape	6.5
Sunflower	5.4
Dry matter	
Silage maize	26
Fodder beet	29
Potato	17
Sugar	
Sugar beet	16

At present there are around 600 long-term experiments (LTE) worldwide with duration of more than 20 years, including also grassland trials. This number appears to be very high, however, considering the great diversity of the soil and climate conditions and the fact that each experiment is specific only for local (or very similar) conditions. Generally accepted results may only be expected by the compilation of the most diverse soil and climate conditions: from the sandy soil to the black earth and from dry regions with only 500 mm annual precipitation to mountain regions with more than 1000 mm of rainfall. In addition, the great multiplicity of the treatment combinations has to be considered, of which crop rotation, fertilization and cultivation are only some of the most important.

Therefore, international cooperation in this area manifold practiced is still necessary. The oldest and most important long-term field experiments in the world with duration > 100 years are shown in (Table 2). Some disadvantages of these old experiments are partly balanced by the advantage of their long duration. This concerns mainly the experimental design and the choice of the treatment factors. Nearly all of the so-called classical LTE were designed without randomisation, lacking in precision. For example, the Broadbalk field, the oldest experiment in the world, has, beside the treatment without fertilization, only one level of the factor organic fertilization which is with 35 t FYM/ha annually, far outside the practical range of application.

The Morrow Plots have had so many changes of the treatments in the course of 130 years that today only few variants represent the entire duration of the experiment. These are disadvantages that can be frequently found. Changes of treatments as well as of constant factors in long-term experiments should be considered and discussed very carefully within the statistical analyses. Such modifications of the experimental design are justified only if in the preceding decades the accumulated standard of knowledge was not substantially impaired and an increase in knowledge transfer can be expected after such experimental rearrangement. But, it has

Table 2. The most important long-term field experiments in the world with duration > 100 years

Location	Country	Start
Rothamsted (Broadbalk, Park Gras a.o.)	UK	1843
Grignon	France	1875
Illinois (Morrow Plots)	USA	1876
Halle/Saale (Eternal Rye)	Germany	1878
Columbia (Sanborn fields)	USA	1888
Dakota	USA	1892
Askov (Sandmarken and Lermarken)	Denmark	1894
Auburn	USA	1896
Bad Lauchstädt (Static Fertilization Experiment)	Germany	1902
Dikopshof	Germany	1904

to be also considered that the steady state of soil organic carbon can be reached after decades, in some cases after more than 100 years.

In the second half of the past century long-term experiments were established in many countries. One important advantage of these experiments is that they are designed in a modern, randomized experimental design with quantitatively gradated treatment factors that include scientifically and practically relevant orders of magnitude. Among these experiments belong also experiments in the Czech Republic, in Prague, Lukavec, Viglas, Čáslav, Ivanovice and Pohořelice, as well as similar field experiments in Hungary, Russia, Sweden, Germany and other countries.

A further significant benefit is that not only single experiments but also the whole series of experiments were planned and carried out. The scientific aim is to record experimental results of different local conditions with the same experimental design to obtain generally acceptable statements. In addition, there are very intensive and successful efforts concerning experimental data collection and utilization for comprehensive evaluations in the international scientific community.

I would like to mention the Soil Organic Matter Network (SOMNET) in the context of the Global Change and Terrestrial Ecosystems (GCTE), the series of the international organic nitrogen fertilization long-term experiments (IOSDV), the work of the All-Russian Research Institute of Agronomy in Moscow, the All India Coordinated Research Project of the ICAR (India), and the coordination centre in Australia.

Examples for analysis

The long-term field experiments remarkably contributed to the present knowledge of soil fertility. Thanks to this knowledge, among others, crop yields and soil fertility significantly increased. At present, SOM once again moved into the focus of interest. Practically usable solutions are urgently needed. Reasons for this are: 1. the efforts of the European Union to establish, in context of cross compliance, measurable parameters for supplying the soil with organic matter and subsequent controlling for the estimation/evaluation of good agricultural practice, 2. incorrect orientations of the European Union and

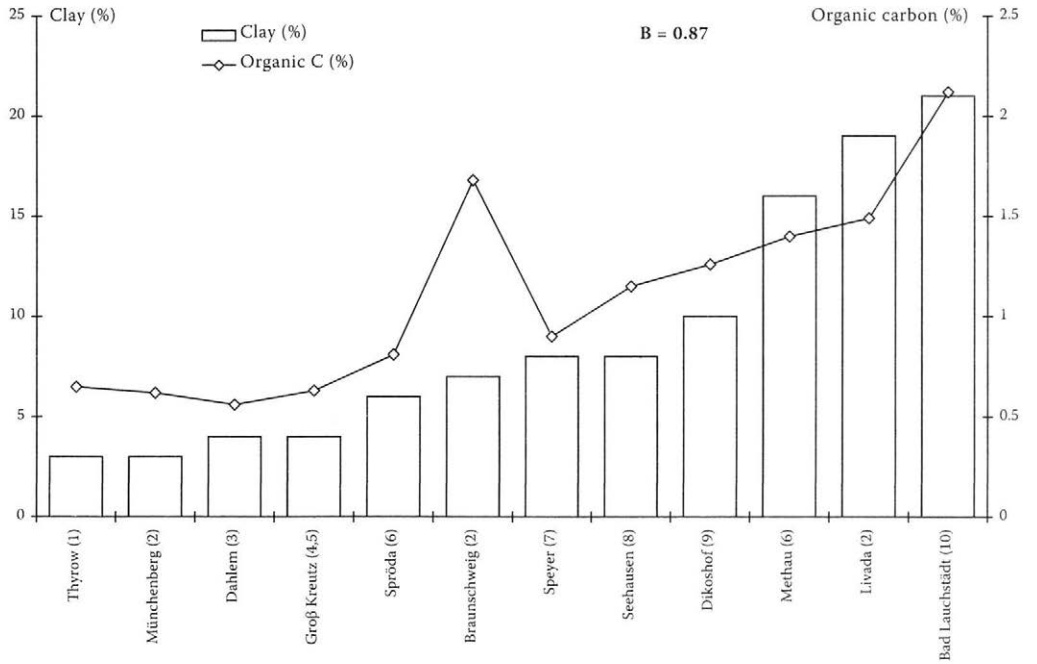

Figure 1. Relations between clay content and optimal organic carbon content (0–30 cm) in long-term experiments (1) Lettau and Ellmer (1997), (2) Rogasik et al. (2004), (3) Krzysch and Caesar (1992), (4) Asmus (1995) Zimmer and Roschke (2001), (5) Prystav and Zimmer (2002), (6) Albert (1999), (7) Bischoff and Emmerling (2003), (8) Leithold et al. (1997), (9) Schellberg et al. (1999), (10) Körschens et al. (1994), Rathke et al. (2002)

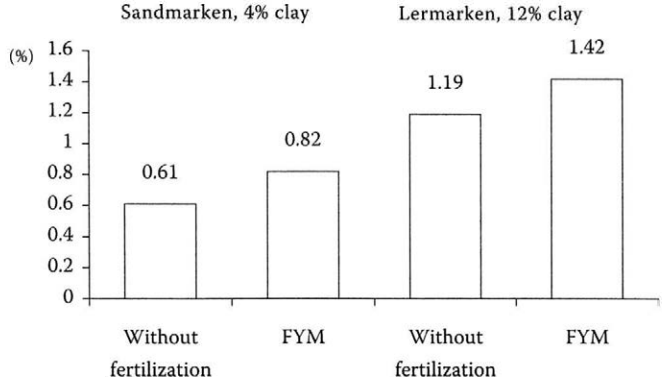

Figure 2. Organic carbon content (%) depending on clay content and manuring in Askov (Christensen 1989)

other countries regarding the supply of soils with organic matter, which leads to irritations.

There are some other examples to be mentioned:

1. In the report of the Commission to the Council, the European Parliament, the economic and social Committee as well as to the Committee of the Regions they say: "According to the agrarian scientists soils with a content of organic substance less than 3.6% are in a preliminary state of desert." This statement is scientifically not valid and disagrees with all the knowledge in soil science.
2. Montanarella and Rusco (2002) of the European Commission joint research Centre, Institutes for Environment and Sustainability, Soil and Waste unit, European Soil Office (which are likewise organizations of the European Union), divide the carbon content of the European soils into the following four classes: high (> 6), medium (2–6%), low (1–2%), very low (< 1%). This classification is subjective and without any practical importance. Not reliable and unproven is however the conclusion that in many soils of southern Europe an intensive cultivation led to a process of soil degradation.
3. The limit values for the content of SOM: 1. Clay content in the soil < 13%: humus content > 1%. 2. Clay content in the soil > 13%: humus content > 1.5% given in the Direktzahlungen – Verpflichtungenverordnung – DirektZahlVerpflV (Direct payment obligation) are equally misleading. The limit value of 1% humus is correct only for light sandy soils with a clay content of less than 5%. On the other hand, soils with a clay content of more than 13% and only 1.5% humus can become impoverished.

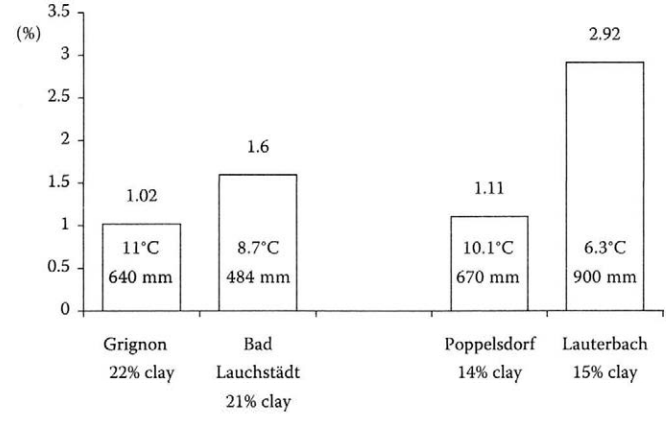

Figure 3. Organic carbon content (%) in selected long-term field experiments depending on clay content and climatic conditions, without fertilization

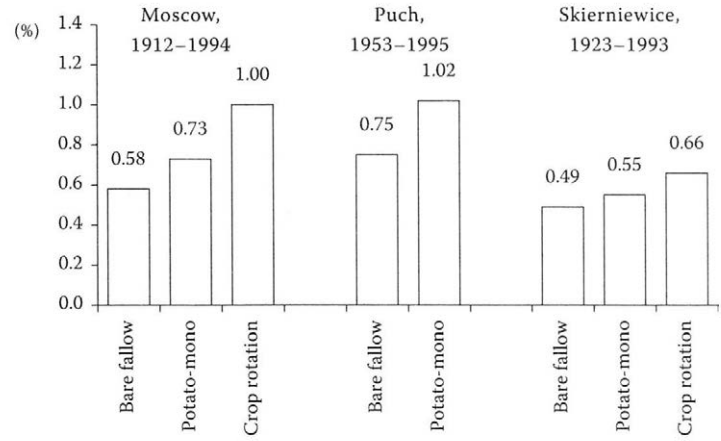

Figure 4. Organic carbon content (%) in selected long-term field experiments depending on crop rotation, without fertilization (Kirjushin 1997, Krauss et al. 1997, Mercik 1993)

The clearing-up of the questions of the supply of soils with organic substance is necessary because of sustainable land use and environmental protection. In the following paragraphs, there are some current results from long-term experiments that disprove the aforementioned statements.

First orientation values for optimal contents of the soil organic matter for sand and loamy soils without groundwater influence were already published 20 years ago. They prove a close correlation between the soil content of clay and organic carbon; results for that were already presented

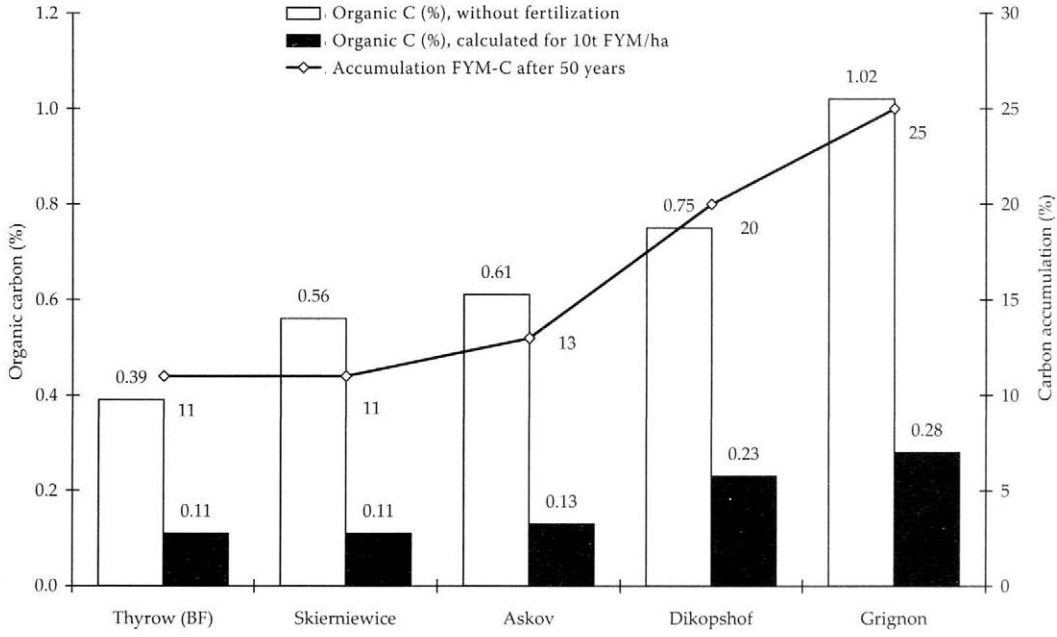

Figure 5. Influence of FYM on the carbon content and the percentage accumulation of the FYM-carbon given in soil in 5 long-term field experiments

several times (Körschens et al. 1986, Körschens and Schulz 1999, Körschens et al. 2005). A newer experimental data including further international long-term experiments confirm these statements. Figures 1–5 demonstrate these relations.

Figure 1 shows the optimal carbon content of 12 LTE and the correlation of the C-content and the clay content. In the LTE of Dahlem, Dikopfshof and Livada the content is estimated with a fertilization with 10 t FYM/ha and NPK, in all other cases the optimal value is derived from the results of LTE of the above-mentioned authors and their experiences after many decades on the site. One exception is Braunschweig, where the experimental field was woodland with a relatively high carbon content several decades ago. Figure 2 concerns the influence of different clay content on the carbon content under the same climatic conditions.

The distance between both sites amounts only few kilometres, i.e. the climatic conditions are alike. Also the next four sites (Figure 3) demonstrate the influence of clay and different climatic conditions. The differences are very high and so the difference between Poppelsdorf and Lauterbach amounts roughly 1.8% carbon. Normally, the differences in the middle of Germany are not so great, around 7 up to 10 degrees and 500 to 800 mm rainfall. Figure 4 shows the influence of different crops and bare fallow on three different sites.

The greatest differences between bare fallow and crop rotation were observed in Moscow with 0.42% carbon. Of course, we have many factors that more or less affect the C-content in soil and some exceptions can always be found. However, there is no doubt that clay content is one of the leading factors that determines the optimal C-content.

A further problem that is very important for the environment is so-called carbon sequestration. The possibility to use the soil as a carbon sink has been often discussed. It was shown that too high humus content in soil does not bring advantages either for the yield formation or for the environment. Figure 5 shows the accumulation of FYM-carbon in five different LTE after 50 years. In the case of light sandy soils only 11% of carbon was accumulated, in loamy soils it was 25%. If the flow equilibrium is reached, the C-accumulation is equal to zero. Because FYM is limited in Germany, a lot of straw has been used as organic manure. But this source of carbon can be used in a much better way for energy production, if the optimal content of carbon in soil is reached.

The reduction of the CO_2 concentration in atmosphere by means of carbon sequestration to mineral soils (arable land) is practically impossible. The necessary input of primary organic matter largely exceeds the C quantity that can be accumulated.

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Steady state of the soil organic matter in the long-term field experiments

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ABSTRACT

Organic C content in the topsoil of the selected plots in the long-term field experiments pursued in the Research Institute of Crop Production in Prague has been evaluated. Steady state of the soil organic matter was well documented in the bare fallow mini-plot field experiment founded in 1958. The organic C content slightly dicreased and remained constant in mineral fertilised and non-fertilised plots. The manured plots showed three phases in the organic C content: accumulation, equilibrium and decline, after the manuring was stopped in 1989. The cropped long-term field experiments lasting for 20 to 50 years showed that both organic and mineral fertilisation increased the organic C content in the topsoil. The effect of mineral fertilisation on the organic C content in the topsoil was positive in most experiments; however, the increment was smaller than that in the variants manured with farmyard manure. Combined organic and mineral fertilisation has the most favourable effect on the organic C content in the topsoil.

Keywords: long-term field experiments; soil organic matter; soil organic carbon; manuring; mineral fertilisation

Soil organic matter (SOM) has been recognised as the main factor of the soil fertility for a long time. It is a source of plant nutrients, it supports soil biota and soil biological activity and it is an important factor affecting the physical and chemical soil properties. From the agricultural point of view, the most important role of the soil organic matter is its contribution to the soil productivity (yields of the cultivated crops) and the long-term yield stability (sustainability of the crop production). Therefore, the goal of a proper agricultural land use was the maintenance or increase of the SOM content. A general consideration was that the higher the SOM content, the better soil quality.

In order to meet this goal (maintenance or increase in the SOM content) several methods were developed among which the balance methods were the most common. It became a basis for the fertilisation and mineral plant nutrition. It was applied in the estimation of the soil organic matter balance and widely used in general ecology, as well.

A number of more or less sophisticated methods based on the results of the pot and field experiments were developed (Neuberg 1990, Bielek and Jurčová 1997, 1999).

While the balance principle was suitable in mineral plant nutrition and it has been used in practical agriculture till the present time, its application in the SOM dynamics has been much more complicated and much less suitable. The estimation of the SOM mineralization (analogue to consumption or uptake) and the SOM input are much more difficult. The SOM and plant and animal residues and products, so called primary organic matter (POM), are of many different kinds, they have rather different tissue and chemical structure and they are more or less stabilised due to their interactions with mineral soil particles. For these reasons, the concepts of "humification coefficients" and "subsequent effect" of the organic fertilisers have to be implemented. However, the most important shortcoming of the balance methods is the constant values of the organic matter balance over longer time periods. Particularly this shortcoming of the balance methods and the results of the long-term field experiments induced a new principle of the

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SOM turnover, so called "steady state" of the SOM (Kubát and Lipavský 1996, Kubát et al. 1999a, b, 2001, 2002, 2003, Lipavský et al. 2002)

The aim of this contribution is to demonstrate the steady state of the SOM in the long-term field experiments pursued by the Research Institute of Crop Production in Prague (RICP).

MATERIAL AND METHODS

Mini-plot black fallow experiment in Prague-Ruzyne

The experiment was founded on Luvi-haplic Chernozem in Prague-Ruzyne in 1958. It consists of 7 plots 1.5 × 2 m that have been treated once a year as follows:

I. control – untreated soil (control)
II. manured with 80 tons of composted manure per ha per year, mixed within 10 cm layer (FYM)
III. manured with 160 tons of composted manure per ha per year, mixed within 20 cm layer (2FYM)
IV. fertilised with mineral fertilisers (ammonium sulphate, mono calcium phosphate and potassium chloride) in the equivalent doses of N, P, and K as in II, mixed within 10 cm layer (NPK)
V. the same fertilisers in doses equivalent to III, mixed within 20 cm layer (2NPK)
VI. no fertilisers tilled to 10 cm (tilled)
VII. no fertilisers tilled to 20 cm (tilled)

In 1971, the depth of tillage was changed to 200 mm in all plots (except plot I) and ammonium sulphate was substituted by urea. Second change occurred in 1989. No manure and fertilisers have been applied and the plots have not been tilled since then. The plots have been maintained bare and weeds have been removed mechanically over the whole time of the experiment.

Soil samples were taken twice a year till 1979 and once a year since then. Depth of sampling was 20 cm in all plots. The soil samples were sieved through 2 mm sieve and air-dried at laboratory temperature. Oxidisable carbon content (C_{ox}) was determined in air-dried soil samples by wet combustion according to Alten et al. (1935).

Further long-term field experiments

In the RICP, there is a number of long-term field experiments, in different sites (soil and climate conditions), and some of them are already 50 years old. Other field experiments were established in seventies, so they are more than 30 years old. Supposedly, all these experiments might have already reached the steady state of organic C content in the topsoil. The basic site characteristics are presented in Table 1.

We have selected four basic variants in each experiment to estimate the organic C steady state level: non fertilised control (control), mineral fertilised variants (NPK), manured variants (FYM) and organic and mineral fertilised variants (FYM + NPK), in close to optimum level. Similar doses (as far as possible) of organic and mineral fertilisation were selected in each experiment.

The soil samples were taken twice a year (or once a year) sieved through 2 mm sieve and air-dried at laboratory temperature. Oxidisable carbon content (C_{ox}) was determined in air-dried soil

Table 1. Soil and climate characteristics of the sites in which the long-term field experiments were pursued

Station	Altitude (m)	Taxonomical unit	Abb.	Texture class	Parent material	pH (KCl)	pH (H_2O)	Average (°C)	Average (mm)
Ivanovice	225	Luvi-haplic Chernozem	Chl	loam	loess	6.6	7.1	8.8	549
Čáslav	240	Orthic Greyzem	Mo	loam	loess	6.9	7.56	8.9	555
Hněvčeves	265	Orthic Luvisol	Lo	clay-loam	loess	6.9	7.4	8.2	573
Kostelec	290	Orthic Luvisol	Lo	sandy-loam	loess	5.6	6.8	7.6	681
Ruzyně	340	Orthic Luvisol	Lo	clay-loam	loess	7.0	7.8	7.9	472
Trutnov	427	Eutric Cambisol	Be	sandy-loam	perm.-carb. sediments	6.6	6.4	7.5	750
Humpolec	525	Stagno-gleyic Cambisol	Bgs	sandy-loam	gneiss	6.9	7.6	6.5	667
Pernolec	530	Stagno-gleyic Cambisol	Bgs	sandy-loam	gneiss	6.8	7.5	7.1	559
Lukavec	610	Stagno-gleyic Cambisol	Bgs	sandy-loam	gneiss	5.8	6.2	6.8	667
Vysoké nad Jizerou	670	Dystric Cambisol	Bd	loamy-sand	gneiss	6.8	7.5	6.5	995

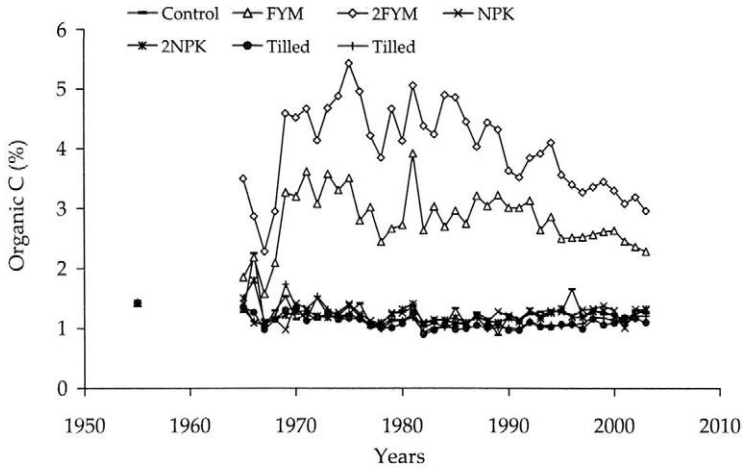

Figure 1. Total organic C content in the topsoil (% C)

samples by wet combustion according to Alten et al. (1935). The results obtained in the time period 1994 to 2004 have been evaluated in this paper. Microsoft Excel programme was used for the statistical evaluation.

RESULTS AND DISCUSSION

Mini-plot black fallow experiment in Prague-Ruzyne

The results of the black fallow mini-plot field experiment in the RICP, Prague-Ruzyne provide a very clear example of the SOM dynamics and balance. Figure 1 shows total organic C (C_{ox}) in the soil samples over the whole time period. Apparently, the organic C content slightly diminished in mineral fertilised and non-fertilised plots during the first years and it remained approximately constant over the rest of the experiment. The FYM plots clearly show three phases: accumulation, equilibrium and the decline.

The total organic C content increased about 2.5 times in plot II and about 3.5 times in plot III, as compared to the initial C content, during this time period (Figure 2). Accumulation phase lasted for about 16 years, till 1974. The SOM accumulation followed quite well the exponential function. Values of the correlation coefficients were 0.6336 and 0.5856 in variants 2FYM and FYM, respectively.

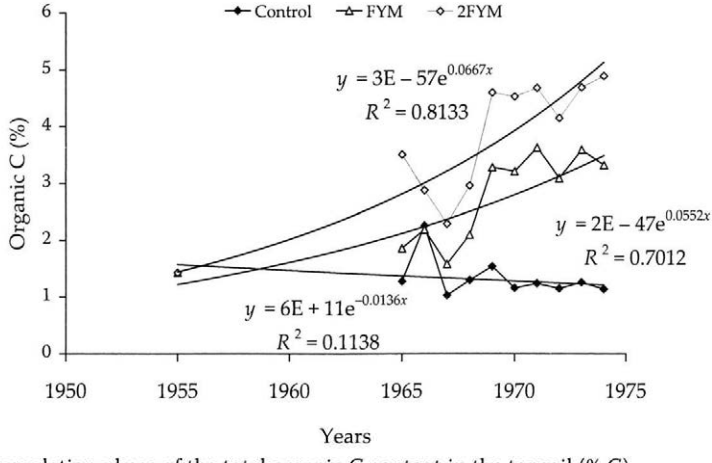

Figure 2. Accumulation phase of the total organic C content in the topsoil (% C)

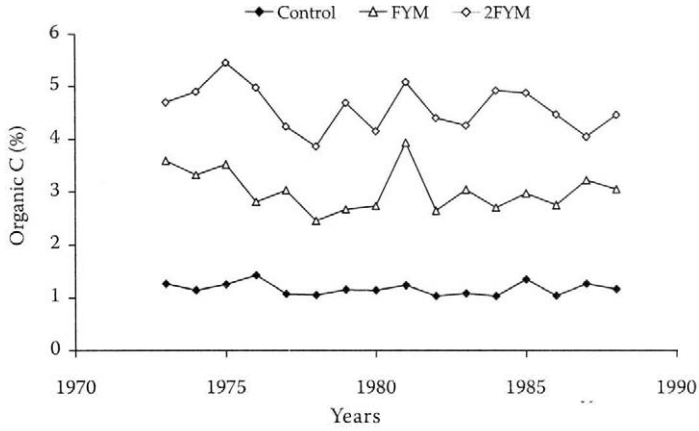

Figure 3. Equilibrium phase of the total organic C content in the topsoil (% C)

The accumulation phase was followed by the equilibrium phase (steady state) from about 1975 till 1989, when the annual fertilisation and tillage was stopped. The results are presented in Figure 3. Annual variability in the organic fertilised variants is much higher than in the control variants, mainly due to the difficulty of perfect spreading of the FYM in such high doses.

After the manuring, mineral fertilisation and tillage were stopped in 1990, and the decline phase of the organic C content started. As Figure 4 shows, the decline of the organic C content can be well expressed by linear regression, with correlation coefficients 0.7388 and 0.790 in plot III and II, respectively. The organic C content in the control variant remains approximately constant during this time period, as well. From the regression lines, we can calculate the approximate time of the end of the SOM decline, in which the C content should reach the equilibrium value in the control plot. The calculated dates are 2022 and 2019 for variant III and II, respectively. The long lasting changes in the SOM content will thus take about 32 and 29 years in variant III and II, respectively. These results are in agreement with the results published by Körschens (1997) who estimated the transition phase in the Static long-term experiment in Bad Lauchstädt to 28 years.

The difference between the results of the SOM balance method and the SOM steady state can be well demonstrated in Figure 5. The normative of the need of the organic matter according to the methodology of plant nutrition (Neuberg 1990) in most unfavourable conditions (80% root crops, 20% grain crops and no fodder crops) would be

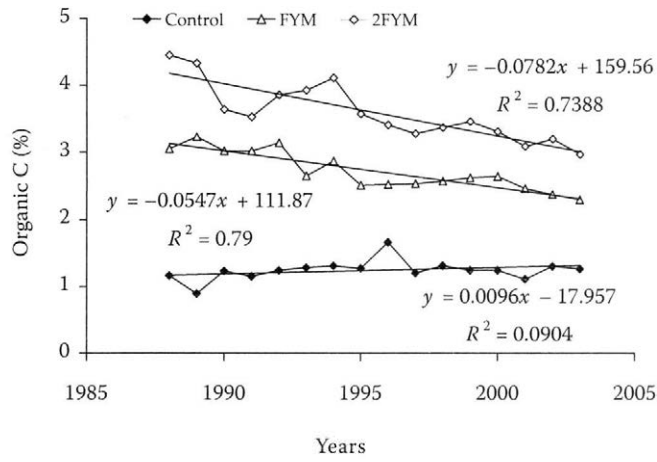

Figure 4. Decline phase of the total organic C content in the topsoil (% C)

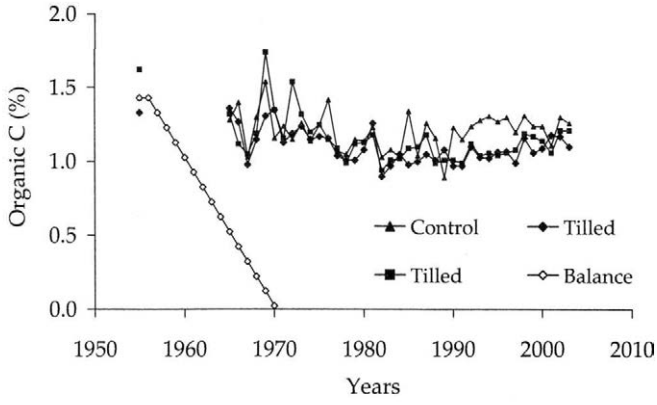

Figure 5. Organic C content in the non-fertilised plots and the balance values (% C)

2.50 t OM/ha/a. Certainly, bare fallow without any input of organic matter is still less favourable for the SOM balance. Using the normative deficit of the organic matter estimated for the 80% root crops and 20% grain crops, we can calculate that the SOM would be completely mineralised within about 12 years. However, this was not the case, as shows Figure 5. Steady state in the minimum level is about 1.2% C in the given soil.

Further long-term field experiments pursued in the RICP

The differentiation of the organic C level in different variants is presented in Figure 6. Minimum organic C level (control variants) differed in individual sites. The lowest values were found in Luvisols (Kostelec and Hněvčeves) and the highest in Chernozems (Ivanovice).

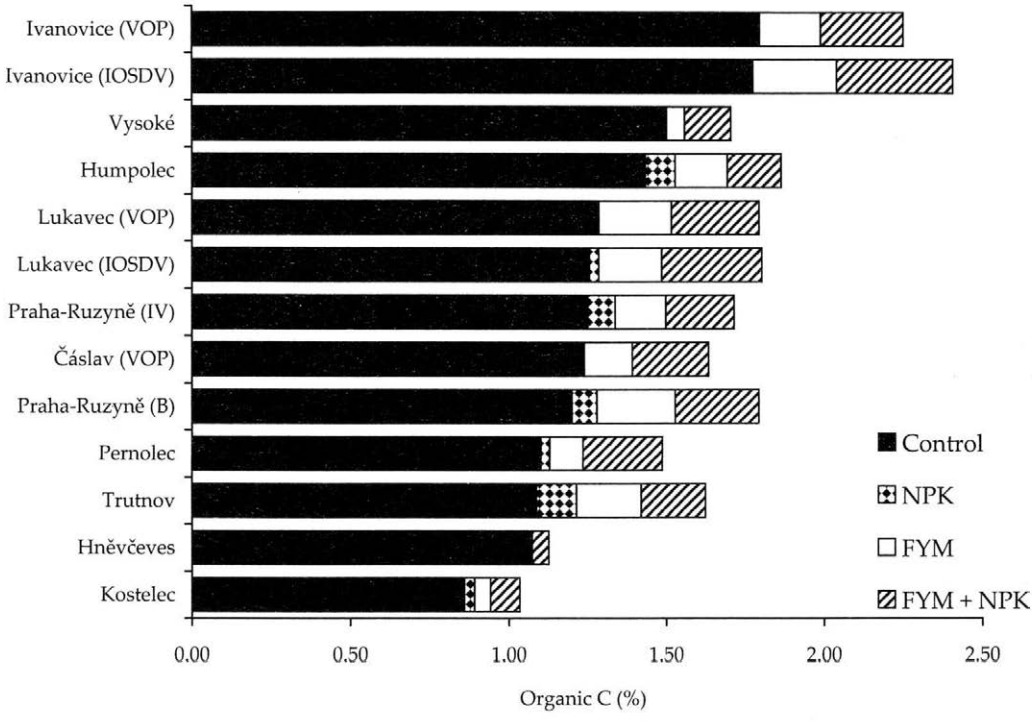

Figure 6. Differentiation of the organic C content in the topsoil in the long-term field experiments (% C)

The long-term organic and mineral fertilisation increased the organic C content in the topsoil. The effect of mineral NPK fertilisation (NPK variants) on the organic C content in the topsoil was positive in most experiments, however, the increment was smaller than that in the plots fertilised by farmyard manure (FYM variants), although the average input of plant nutrients was about a half of that in the NPK variants. The combined organic and mineral (FYM + NPK) fertilisation have caused the highest organic C levels in the topsoil. As the selected FYM + NPK variants represent close to optimum plant nutrition from the point of view of productivity (crop yields and yield stability), the organic C level in the topsoil can also be considered as optimum.

The changes of the organic matter content in soils proved to be long-lasting. Annual differences in the soil organic matter level usually do not exceed the variability of the soil sampling and analyses. They can only be detected in the long-term field experiments. For the same reason, steady state of the soil organic matter could only be documented in the long-term field experiments.

The long-term field experiments are essential for the understanding of soil functions, soil processes and also sustainability of agricultural land use. Results of the long-term field experiments provided basic knowledge for the development of the farming practices in different soil and climate conditions, suitability of the cultivated crops, crop rotation, and organic and mineral fertilisation. Databases of the long-term field experiments became an inevitable source of data needed for the calibration and validation of the mathematical models simulating soil processes and plant growth. Long-term field experiments are also valuable experimental objects for agronomical and environmental research.

Most of the scientists who established long-term field experiments could not have conceived that the experiments will continue for decades and will become a real and extensive basis of our present knowledge of soil properties and processes. The maintenance of the long-term field experiments and the evaluation of their results have brought new unexpected knowledge and, for sure, the long-term field experiments will generate new ideas and will be inevitable for the testing of these new ideas in agronomy and ecology in future, as well.

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Dynamics of the soil organic matter in crop rotation and long-term monoculture

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ABSTRACT

The field experiment, focused on the response of the most important plants cultivated in Poland to monocultural cultivation, has been carried out at the Experimental Station at Bałcyny since 1967. The experiment is located on Luvisols formed from silty light loam. Twelve plant species are cultivated in crop rotations: A) potato – oats – flax – winter rye – faba bean – winter triticale; B) sugar beet – maize – spring barley – pea – winter rape – winter wheat, and each of the species in monocultures. The content of organic matter in the soil plough layer (0–250 mm) in 1992, 1998 and 2004 is presented. Fractionation of humus was carried out with the Duchaufour and Jacquin method. Under the conditions of the crop rotation a slight increase occurred in the content of organic matter; similar tendencies appeared in most monoculture fields. The lowest content of organic C was recorded under the conditions of continuous cultivation of pea, maize and faba bean. Humins and humus compounds (extraction II and III) had a dominant share among the determined fractions. Humic acids prevailed over fulvic acids in the crop rotation cultivations and in the most monocultural fields. However, prevalence of fulvic acids was found under the conditions of continuous cultivation of sugar beet, potato and winter rye.

Keywords: organic matter; humus; organic carbon; humic acids; fulvic acids; crop rotation; monoculture

The content of organic matter in soil is one of the significant factors limiting the level of plant production. Frequent decrement of humus and loss in soil nutrients occur under the conditions of production specialization and universal non-manure economy. The most serious decrements are caused by root crops, while the effect of corns is slightly weaker, and that of papilionaceous plants is regenerative (Fotyma 1988, Zawiślak et al. 1988, Puła and Łabza 2004). One of the methods of maintaining soil fertility is applying crop rotation with the share of humus-producing plants, which activate soil organisms thanks to various crop residues they provide. Rational crop rotation in the field economy is one of the main factors enabling an increase in the resources of organic matter and nutrients or maintaining them at a proper level (Římovský 1987, Siuta 1988, Rychcik et al. 2004).

With regard to the threats brought about by monocultural cultivation of plants, the Chair of Agricultural Systems of the University of Warmia and Mazury in Olsztyn carried out field investigations in the years 1992–2004 concerning the influence of the succession of species on the organic matter resources in soil and its fractional composition.

MATERIAL AND METHODS

The field experiment, focused on the response of economically most important plants cultivated in Poland to monocultural cultivation, has been carried out at the Experimental Station at Bałcyny, which belongs to the University of Warmia and Mazury in Olsztyn, since 1967. The experiment is located in a slightly undulating area, on Luvisols, formed from silty light loam. The content of clay particles (< 0.002 mm) in the arable layer ranges within 2–4%, that of floatable particles (0.02–0.002 mm) ranges within 17–22%, and that of silt (0.1–0.02 mm) – 26–39%. Annual rainfall in the region (north-east Poland) amounts to 596 mm, average air temperature is 7.3°C, and the growing season lasts around 210 days.

Twelve plant species were cultivated in two 6-plot crop rotations:

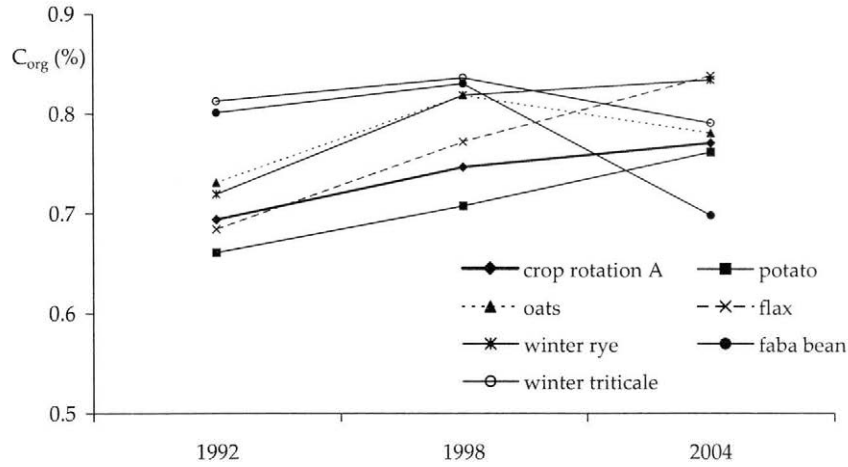

Figure 1. Changes of organic carbon content in crop rotation A and monocultures

A) potato – oats – flax – winter rye – faba bean – winter triticale;
B) sugar beet – maize – spring barley – pea – winter rape – winter wheat, and each of the above-mentioned species in an increasing monoculture.

Manure in the crop rotation was used once in the rotation under potato or sugar beet in a dose of 30 t/ha, and in the monoculture – under every species, 15 t/ha every three years. In both systems, mineral fertilization and pesticide protection was used according to the plants' requirements, and cultivars were replaced after every rotation.

The paper presents the dynamics of the organic matter content in the soil arable layer (0–250 mm) after completing the succeeding 6-plot crop rotations in 1992, 1998 and 2004. The content of organic carbon was estimated with the Tiurin method and sorptive properties (exchangeable cations) – according to the Kappen method. Fractionation of humus compounds was carried out with the Duchaufour and Jacquin method (1966) in the

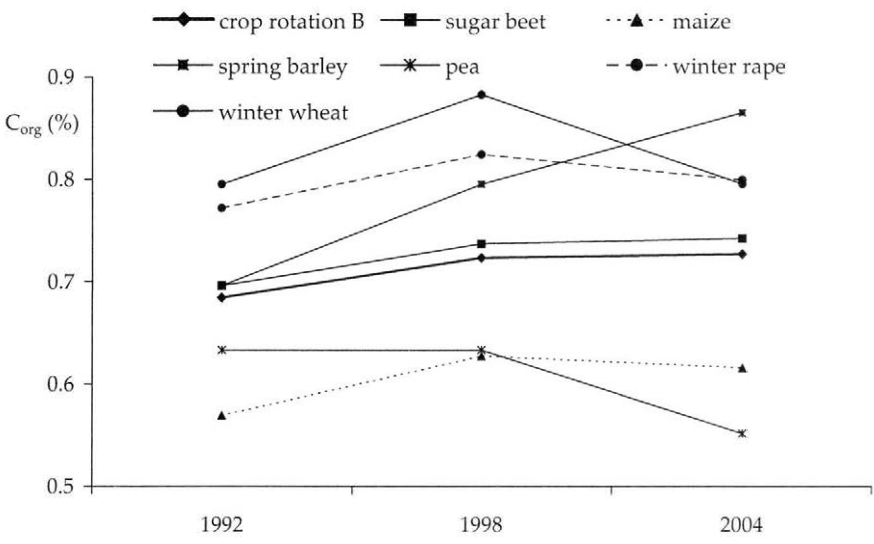

Figure 2. Changes of organic carbon content in crop rotation B and monocultures

Kuźnicki and Skłodowski modification (1968) educing the following fractions:

- extraction I – active humus compounds extracted with 0.1 mol $Na_4P_2O_7/dm^3$ + 7.5% Na_2SO_4 (pH = 7.0);
- extraction II – humus compounds bounded with cations educed during the extraction of 0.1 mol $Na_4P_2O_7/dm^3$;
- extraction III – strongly bounded humus compounds educed during the extraction of 0.1 mol $NaOH/dm^3$.

Carbon of humic acids (CH) was determined with the Tiurin method after precipitation (at pH 1.0). Carbon of fulvic acids (CF) was calculated based on the difference between the carbon content in particular fractions and carbon of humic acids (CH).

The results obtained were subject to the analysis of variance, and the significance of the differences was determined using the *t*-Duncan test at the significance level of $P = 0.05$.

RESULTS AND DISCUSSION

After twelve years of investigation, a slight increase in the content of organic carbon was recorded in both 6-field crop rotations (Figures 1 and 2). The increase in crop rotation A was greater and amounted to 0.08%, while in crop rotation B it was only 0.04%. Under the conditions of monoculture, the lowest amount of organic carbon in 1992 was found in the monocultures of maize (0.57%), pea (0.63%), and potato (0.66%), while the highest in the field of winter triticale (0.81%), winter wheat and faba bean (0.80% each). In 1998 after six years of investigation, an increase in the organic carbon content was recorded in all experimental fields. Similarly, after the next investigation cycle com-

Table 1. Content of various fractions of organic carbon (% of dry soil matter)

	C_{org}	Extraction							Humins
		I	II			III			
		CI	CII	C_{H2}	C_{F2}	CIII	C_{H3}	C_{F3}	
Crop rotation – A (average)	0.770	0.099	0.103	0.061	0.042	0.140	0.089	0.051	0.428
Potato	0.761	0.077	0.102	0.046	0.056	0.128	0.088	0.040	0.454
Oats	0.780	0.107	0.086	0.054	0.032	0.185	0.103	0.082	0.402
Flax	0.837	0.113	0.083	0.057	0.026	0.108	0.094	0.014	0.533
Winter rye	0.833	0.094	0.127	0.055	0.072	0.160	0.080	0.080	0.452
Faba bean	0.698	0.128	0.110	0.065	0.045	0.117	0.064	0.053	0.343
Winter triticale	0.790	0.088	0.124	0.087	0.037	0.143	0.094	0.049	0.435
Crop rotation – B (average)	0.727	0.098	0.107	0.059	0.048	0.129	0.079	0.050	0.394
Sugar beet	0.742	0.108	0.099	0.043	0.056	0.108	0.062	0.046	0.427
Maize	0.615	0.096	0.069	0.049	0.020	0.097	0.059	0.038	0.353
Spring barley	0.864	0.145	0.090	0.055	0.035	0.143	0.105	0.038	0.486
Pea	0.551	0.068	0.068	0.052	0.016	0.091	0.061	0.030	0.324
Winter rape	0.799	0.077	0.110	0.058	0.052	0.128	0.082	0.046	0.484
Winter wheat	0.795	0.105	0.122	0.065	0.057	0.164	0.094	0.070	0.404
$LSD_{0.05}$	0.054	0.012	0.011	0.008	0.015	0.013	0.005	0.010	0.052

Extraction I – active humus compounds extracted with 0.1 mol $Na_4P_2O_7/dm^3$ + 7.5% Na_2SO_4 (of pH = 7.0); extraction II – humus compounds bounded with cations educed during the extraction of 0.1 mol $Na_4P_2O_7/dm^3$; extraction III – strongly bounded humus compounds educed during the extraction of 0.1 mol $NaOH/dm^3$; C_F – carbon of fulvic acids; C_H – carbon of humic acids

Table 2. Humus composition in % of organic carbon

	$C_H:C_F$ ($C_H = 1$)	Extraction							Humins
		I	II			III			
		CI	CII	C_{H2}	C_{F2}	CIII	C_{H3}	C_{F3}	
Crop rotation – A (average)	0.61	12.93	13.28	7.92	5.36	18.13	11.56	6.56	55.67
Potato	0.72	10.12	13.40	6.04	7.35	16.82	11.56	5.26	59.66
Oats	0.73	13.72	11.02	6.92	4.10	23.72	13.20	10.51	51.54
Flax	0.26	13.50	9.92	6.81	3.11	12.90	11.23	1.67	63.68
Winter rye	1.13	11.28	15.25	6.60	8.64	19.21	9.60	9.61	54.26
Faba bean	0.76	18.33	15.75	9.31	6.45	16.76	9.17	7.59	49.16
Winter triticale	0.48	11.14	15.69	11.01	4.68	18.10	11.89	6.20	55.07
Crop rotation – B (average)	0.74	13.39	14.79	8.24	6.58	17.61	10.95	7.68	54.22
Sugar beet	0.97	14.92	13.39	5.79	7.54	14.55	8.35	6.20	57.14
Maize	0.54	15.60	11.22	7.96	3.25	15.77	9.56	6.18	57.41
Spring barley	0.46	16.75	10.42	6.36	4.06	16.55	12.15	4.40	56.28
Pea	0.41	12.34	12.34	9.43	2.90	16.51	11.07	5.44	58.81
Winter rape	0.70	9.64	13.76	7.26	6.51	16.02	10.26	5.76	60.58
Winter wheat	0.80	13.21	15.35	8.18	7.16	20.63	11.82	8.80	50.81
$LSD_{0.05}$	–	2.03	1.21	1.18	1.74	1.68	0.81	1.27	3.22

Extraction I – active humus compounds extracted with 0.1 mol $Na_4P_2O_7/dm^3$ + 7.5% Na_2SO_4 (of pH = 7.0); extraction II – humus compounds bounded with cations educed during the extraction of 0.1 mol $Na_4P_2O_7/dm^3$; extraction III – strongly bounded humus compounds educed during the extraction of 0.1 mol $NaOH/dm^3$; C_F – carbon of fulvic acids; C_H – carbon of humic acids

pleted in 2004, a further increase in organic carbon content was recorded, although to a lower extent. On the other hand, its decrease was observed in the fields of the monoculture of winter wheat, winter triticale, oats, pea and faba bean, and in the case of papilionaceous plants lower values than the initial ones of 1992 were proved. A connection between the choice of cultivated plant species and the organic carbon balance was indicated by Římovský (1987), Fotyma (1988), Zawiślak et al. (1988) and Rychcik et al. (2004).

From the research of numerous authors (Gonet 1989, Strączyńska 1993, Horáček et al. 2001, Puła and Łabza 2004, Růžek et al. 2005) it results that manuring influences the content of organic carbon and its fractional composition. It is assumed that systematic manuring increases the content of humic acids and humins. Humic acid particles have a less condensed nucleus while the number of lateral chains of aliphatic character increases (Pisarek 1993, Strączyńska 1993). Apart from fertilization and the kind of crop, the system of plant sequence is of a significant importance for the amount of organic matter in the soil. A characterization of soil humus compounds at the plough layer (0–250 mm) in the discussed field experiment was conducted based on the data contained in Tables 1 and 2. The content of soil organic carbon in the crop rotation showed inconsiderable variation and, on average, amounted to 0.770% in A, and 0.727% in B, which in terms of humus (multiply by 1.724) constitutes 1.327 and 1.253%, respectively. However, significant differences in the amount of organic carbon were recorded in the case of monocultures (Table 1); its highest content was recorded in the soil of spring barley, flax and winter rye, and the lowest in the case of pea, maize and faba bean.

When analyzing the content of the determined fractions, it was found that the fraction of humins had the largest share (from 49.16%) in the faba bean monoculture up to 63.68% in the flax continuous cultivation field (Table 2). In the crop rotation fields, similar values were achieved (55.67% in A and 54.22% in B, on average). On the other hand,

Table 3. Exchangeable cations in cmol(+)/kg

	Ca^{2+}	Mg^{2+}	K^{+}	Na^{+}	Sum
Crop rotation – A (average)	3.46	0.68	0.33	0.04	4.51
Potato	2.16	0.49	0.36	0.03	3.04
Oats	4.12	0.78	0.41	0.06	5.37
Flax	3.87	0.79	0.46	0.05	5.17
Winter rye	2.16	0.49	0.23	0.02	2.90
Faba bean	2.83	0.69	0.41	0.03	3.96
Winter triticale	3.12	0.61	0.28	0.03	4.04
Crop rotation – B (average)	3.22	0.63	0.32	0.04	4.21
Sugar beet	2.99	0.61	0.41	0.03	4.04
Maize	2.83	0.70	0.26	0.03	3.82
Spring barley	3.99	0.79	0.61	0.04	5.43
Pea	3.18	0.74	0.47	0.03	4.42
Winter rape	3.87	0.85	0.62	0.05	5.39
Winter wheat	3.37	0.72	0.44	0.04	4.57

significant differences in the CI fraction content were recorded in monocultures – from 18.33 in the case of faba bean, 16.75 in spring barley, 15.60 in maize to only 10.12 in potato and 9.64 in winter rape. Humus compounds, forming bonds with cations and humus-mineral bonds (extraction II and III) occurred in a very wide range of values from 22.82% in the flax monoculture field to 35.98% in the winter wheat continuous cultivation. A distinct domination of humic acids over fulvic acids became pronounced in the crop rotations, and the ratio CH:CF was 1:0.61 in A and 1:0.74 in B. A similar correlation appeared in the soil of most monoculture fields except for winter rye, where the CH:CF relation was 1:1.13. The predominance of fulvic acids over humic acids (extraction II) in the continuous cultivation of sugar beet, potato and winter rye is noteworthy, too. On the other hand, a low proportion of CF was found in the flax monoculture soil. The formation of high-molecular humus combinations with a prevalence of humic acids over fulvic acids is probably favoured by a high content of exchangeable calcium as shown in Table 3 (Pisarek 1993, Wójciak et al. 2000).

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Respiration activity of the soil samples from the long-term field experiments in Prague

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ABSTRACT

Respiration activity of the soil samples from the topsoil of several variants of the long-term field experiments in Prague was evaluated over a nine-year period (1995–2003). Selected fields differing in crop rotation and selected variants, in which the doses of organic and mineral fertilisers were similar as far as possible, allow the evaluation of the effect of cultivated crops (crop rotation) and of the organic and mineral fertilisation on the respiration activity. Both reactive and basal respiration activity were higher in the field alternatively cropped with spring wheat and sugar beet, in comparison to the nine-year crop rotation. The effect of organic and/or mineral fertilisation showed minor differences among the selected variants in the reactive and basal respiration activity. Statistically significant differences among the variants with different fertilisation were found mainly in the potential respiration activity. The ratio between the values of the potential and basal respiration activity indicates the stability of the soil organic matter. According to this criterion, stability of the soil organic matter was higher in the fields cropped in a nine-year crop rotation than in the field B alternatively cropped with spring wheat and sugar beet. Organic and mainly mineral fertilisation increased the stability of the soil organic matter.

Keywords: long-term field experiments; soil organic matter; soil respiration activity; organic and mineral fertilisation

Respiration activity of soil belongs among the most important characteristics of the soil biological activity. Usually measured as CO_2 emissions (in laboratory or *in situ*), it is a strong indicator of the soil metabolism and ecological soil functions (Tesařová and Gloser 1976, Šantrůčková 1993). It reflects the intensity of the soil organic matter decomposition and mineralization and the incidence of the microorganisms in soil, and it is often used for the biomass determination (Anderson and Domsch 1978, 1990). Růžek et al. (2004, 2005) showed close relationships between the soil respiration activity, microbial biomass C and total organic C content in most of the investigated soils. Respiration activity of the soil samples amended with easily available substrate (potential respiration activity) may be used as a criterion of the soil organic matter stability, for the estimation of the plant nutrient availability or for the indication of the overall quality of the physical soil properties (Novák 1963, Novák et al. 1963, Novák and Apfelthaler 1964). Respiration activity was frequently used to evaluate soil quality, soil fertility or soil contamination with organic pollutants or heavy metals (Brookes 1995, Mikanová et al. 1996, Kubát et al. 1996, 1999, 2001, 2002) and for the evaluation of the effect of the change in land use (Vořišek et al. 2002). *In situ* measurements of soil respiration activity (CO_2 production) belongs among the basic characteristics of the carbon cycle and its sequestration to soil.

For all these reasons, soil respiration activity is a valuable tool in determination of the effect of agricultural land use on the soil quality and sustainability of farming systems.

Soil respiration activity is, however, rather variable depending on a number of biotic and abiotic factors, mainly on the physical and chemical soil properties, abundance and diversity of the soil microorganisms, substrate availability, aeration,

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soil temperature and moisture. Due to the high variability and seasonal dynamics of the respiration activity, long-term investigations are needed to evaluate the effect of different farming systems (e.g. organic and mineral fertilisation, crop rotation) on the respiration activity.

In this contribution, the effect of crop rotation and of organic and mineral fertilisation on the soil respiration activity was evaluated in the long-term field experiments in Prague, over nine-year crop rotation (1995–2003).

MATERIAL AND METHODS

The long-term field experiments in Prague were established in 1955 with the aim to investigate the effect of various fertilisation systems on the yields, nutrient uptake and the soil quality. The experiments were started on six independent fields, five of which exist until present. The site characteristics and experimental design were described in more details in our earlier paper (Kubát et al. 2003).

Four variants (Nil, NPK, FYM and FYM + NPK) from three fields (III, IV and B) were selected for more detailed investigations, as shown in Table 1.

We evaluated the results of nine-year investigations, over the period from 1995 to 2003. Soil samples were taken from the upper layer 0–200 mm twice a year, in the spring and in the autumn, they were mixed, sieved through 2 mm sieve and stored in refrigerator at 4°C. The analyses were performed as soon as possible, usually the next day. No preincubation of the soil samples was applied.

The respiration activity was measured as CO_2 production after incubation of the non-amended soil samples in glass vessels at 28°C for 3 days (reactive respiration) and the CO_2 production after the next 4 days (basal respiration). Simultaneously, potential NG respiration of the soil samples amended with glucose and ammonium sulphate was determined after 20 hours of incubation at 28°C. The results were calculated to the dry matter of the soil samples and evaluated as differences between mean values obtained over the nine-year period.

The data were evaluated by means of *t*-tests for paired samples that is available in the MS Excel.

RESULTS AND DISCUSSION

Selection of the fields differing in crop rotation and the selection of variants, in which the doses of organic and mineral fertilisers were similar (as far as possible), allows the evaluation of the effect of cultivated crops (crop rotation) and of the organic and mineral fertilisation on the respiration activity. The difference between field III and field IV is just in the shift of the crops in rotation, which may represent different results of the interaction between the cultivated crop and the climate conditions in the given year and also the variability in field experiments.

Table 1. Selected fields and variants

	Crop rotation	Variants	Average N doses (kg N/ha)
Field III	9 years: lucerne, lucerne, winter wheat, sugar beet, spring barley, potatoes, winter wheat, sugar beet, spring barley	Nil	0
		NPK	64.6
		FYM	38.6
		FYM + NPK	103.2
Field IV	9 years: lucerne, lucerne, winter wheat, sugar beet, spring barley, potatoes, winter wheat, sugar beet, spring barley	Nil	0
		NPK	64.6
		FYM	38.6
		FYM + NPK	103.2
Field B	alternatively sugar beet and spring wheat since 1965	Nil	0
		NPK	100
		FYM	57
		FYM + NPK	157

Table 2. Effect of crop rotation on the reactive respiration activity in the soil samples from the selected fields and variants; values of *t*-test for dependent samples (*t*-crit 0.05 = 2.131, *t*-crit 0.01 = 2.947)

Fields (Variants)	B (Nil)	B (NPK)	B (FYM)	B (FYM + NPK)	III (Nil)	III (NPK)	III (FYM)	III (FYM + NPK)
IV (Nil)	15.18				−2.96			
IV (NPK)		8.2				−0.04		
IV (FYM)			10.85				0.47	
IV (FYM + NPK)				9.43				−3.13
III (Nil)	17.08							
III (NPK)		5.56						
III (FYM)			8.31					
III (FYM + NPK)				9.07				

Reactive respiration activity

The average values and standard deviations of the reactive respiration activities in the selected fields and variants over the period 1995–2003 are presented in Figure 1. Results of the statistical evaluation by means of the *t*-test for dependent samples are shown in Tables 2 and 3.

Average values of the reactive respiration activity show a very distinctive effect of the crop rotation on both the unfertilised variants and the variants fertilised with organic and mineral fertilisers. The values of the reactive respiration activity are approximately 2.5 times higher in field B (alternative cropping with spring wheat and sugar beet) than in fields III and IV (nine-year crop rotation). The differences between selected variants in field B and appropriate variants in both fields III and IV were statistically highly significant (Table 2). Statistically highly significant differences in the reactive respiration activity were also found between the Nil variants and FYM + NPK variants in the fields III and IV.

Organic fertilisation increased the reactive respiration activities in relation to the non-fertilised controls in all fields. However, the differences were statistically significant in the fields B and III. Mineral fertilisation increased the reactive respiration activity in the crop rotation (fields III and IV)

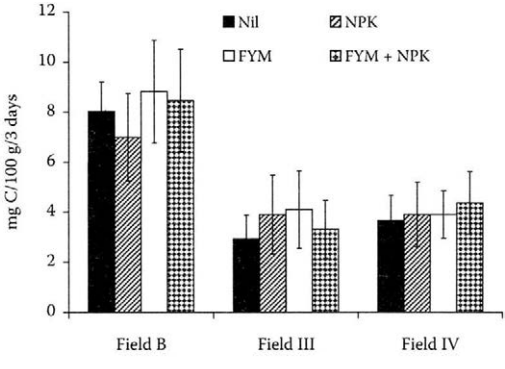

Figure 1. Reactive respiration activity of the soil samples from the selected fields and variants; average values 1995–2003 (mg C/100 g dry soil/3 days)

Table 3. Effect of organic and mineral fertilisation on the reactive respiration activity in the soil samples from the selected fields and variants; values of *t*-test for dependent samples

Variants	Nil	NPK	FYM
Field B: *t*-crit 0.05 = 2.131; *t*-crit 0.01 = 2.947			
FYM + NPK	−1.52	−5.74	1.31
FYM	−2.67	−6.01	
NPK	4.18		
Field III: *t*-crit 0.05 = 2.120; *t*-crit 0.01 = 2.921			
FYM + NPK	−2.19	2.13	3.07
FYM	−5.03	−0.83	
NPK	−4.11		
Field IV: *t*-crit 0.05 = 2.131; *t*-crit 0.01 = 2.947			
FYM + NPK	−2.98	−2.32	−1.63
FYM	−1.79	0.01	
NPK	−1.3		

Table 4. Effect of crop rotation on the reactive respiration activity in the soil samples from the selected fields and variants; values of *t*-test for dependent samples (*t*-crit 0.05 = 2.131, *t*-crit 0.01 = 2.947)

Fields (Variants)	B (Nil)	B (NPK)	B (FYM)	B (FYM + NPK)	III (Nil)	III (NPK)	III (FYM)	III (FYM + NPK)
IV (Nil)	5.69				−1.08			
IV (NPK)		3.22				−1.18		
IV (FYM)			8.23				0.12	
IV (FYM + NPK)				6.35				−2.07
III (Nil)	6.26							
III (NPK)		3.47						
III (FYM)			6.23					
III (FYM + NPK)				7.59				

and decreased it in the field B. The differences were statistically significant in the fields B and III and not significant in the field IV (Table 3).

A combined organic and mineral fertilisation (variants FYM + NPK) increased the reactive respiration activity as compared to the non-fertilised controls in all fields. The differences are statistically highly significant in the field IV, significant in the field III and not significant in the field B (Table 3). In relation to the organic fertilisation (FYM variants), the effect of combined organic and mineral fertilisation was positive in the field IV (not significantly) and negative in fields B and III (highly significantly in field III and not significant in field B). In relation to the mineral fertilisation (NPK variants), the effect of combined organic and mineral fertilisation on the reactive respiration activity was positive in the field B (highly significantly) and in the field III (significantly) and negative in the field IV (significantly).

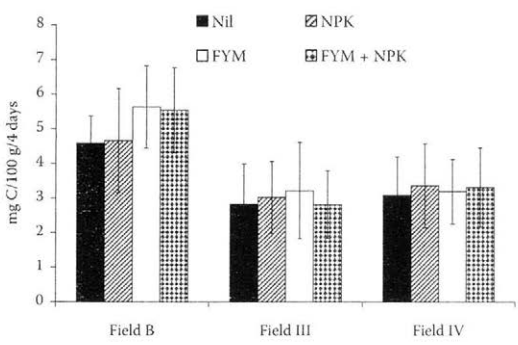

Figure 2. Basal respiration activity of the soil samples from the selected fields and variants; average values 1995–2003 (mg C/100 g dry soil/4 days)

Basal respiration activity

Average values of the basal respiration activity of the soil samples (Figure 2) were similar to those of the reactive respiration activity in the fields III and IV (under nine-year crop rotation). The basal respiration activity was much lower than

Table 5. Effect of organic and mineral fertilisation on the basal respiration activity in the soil samples from the selected fields and variants; values of *t*-test for dependent samples

Variants	Nil	NPK	FYM
Field B: *t*-crit 0.05 = 2.131; *t*-crit 0.01 = 2.947			
FYM + NPK	−4.89	−4.46	0.45
FYM	−5.8	−3.04	
NPK	−0.28		
Field III: *t*-crit 0.05 = 2.120; *t*-crit 0.01 = 2.921			
FYM + NPK	0.1	0.87	2.26
FYM	−1.94	−0.81	
NPK	−0.79		
Field IV: *t*-crit 0.05 = 2.131; *t*-crit 0.01 = 2.947			
FYM + NPK	−1.03	0.27	−0.45
FYM	−0.47	0.58	
NPK	−1.00		

Table 6. Effect of crop rotation on the potential respiration activity in the soil samples from the selected fields and variants; values of *t*-test for dependent samples (*t*-crit 0.05 = 2.145, *t*-crit 0.01 = 2.977)

Fields (Variants)	B (Nil)	B (NPK)	B (FYM)	B (FYM + NPK)	III (Nil)	III (NPK)	III (FYM)	III (FYM + NPK)
IV (Nil)	3.35				−0.57			
IV (NPK)		5.24				−0.31		
IV (FYM)			4.61				−0.73	
IV (FYM + NPK)				4.28				−4.06
III (Nil)	4.43							
III (NPK)		6.06						
III (FYM)			5.61					
III (FYM + NPK)				6.93				

the reactive in the field B. Nevertheless, the average values of the basal respiration activity were about 1.5 times higher in the field B (alternative growing of spring wheat and sugar beet) than in the fields III and IV, in all variants of organic and mineral fertilisation. The differences are statistically highly significant in all variants, while there is no significant difference between respective variants in fields III and IV (Table 4).

Organic fertilisation increased the basal respiration activity as compared to the non-fertilised control. The difference was statistically highly significant in the field B (alternative growing of spring wheat and sugar beet) and not significant in the fields III and IV. Mineral fertilisation slightly increased the basal respiration activity, the differences, however, were not statistically significant (Table 5).

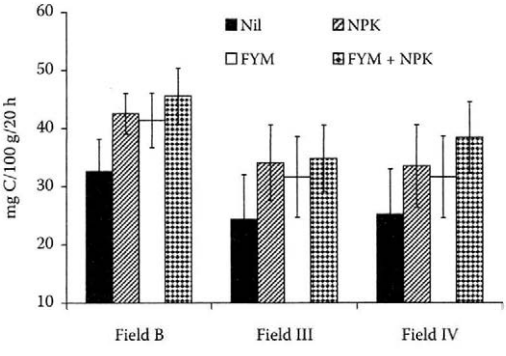

Figure 3. Potential respiration activity of the soil samples from the selected fields and variants; average values 1995–2003 (mg C/100 g dry soil/20 hours)

Combined organic and mineral fertilisation increased the basal respiration activity as compared to the non-fertilised controls in the fields B and IV. The difference was highly significant in field B.

Potential respiration activity

Potential respiration activity represents the ability of the soil microorganisms to mineralise easily available substrate. It was determined after the incubation of soil samples amended with glucose and ammonium sulphate.

The average values over the period 1995 to 2003 are presented in Figure 3. The highest values were found in the field B (alternative growing of spring wheat and sugar beet), similarly to the reactive and basal respiration activity. Differences were much smaller (about 30%), although statistically highly significant (Table 6).

As compared to the Nil variants, both organic and mineral fertilisation increased the potential respiration activity in all the fields. The differences were statistically highly significant. The effect of mineral fertilisation was stronger than that of the FYM fertilisation. However, the difference between the NPK and FYM variants was significant in the field III, not in the fields B and IV (Table 7).

Combined organic and mineral fertilisation increased the potential respiration activity in relation to the non-fertilised controls and to single organic or mineral fertilisation, in all the fields. The differences were highly significant, except for the NPK plots in the field III (Table 7).

Ratio of potential and basal respiration activity (index of the soil organic matter stability)

Ratio between the potential and basal respiration activity reflects the ability of the soil micro-organisms to mineralise easily decomposable substrate (glucose). If this ability is high and simultaneously the basal respiration activity is low, it is due to the lack of available substrate in soil samples and thus indicates an increased stability of the soil organic matter (SOM). As Figure 4 shows, the stability of soil organic matter was higher in the fields III and IV (nine-year crop rotation) than in the field B (alternative growing of spring wheat and sugar beet). The results also showed a positive effect of organic and mainly mineral fertilisation on the SOM stabilisation. The combined organic and mineral fertilisation seems to reflect additive effects of organic and mineral fertilisation in the fields III and IV (crop rotation).

Cultivated crops and crop rotation substantially affect the organic matter transformations in soil and its biological activity (Weiskopf et al. 1988, Heisler 1998). Classical crop rotations usually increase soil organic matter content and stimulate soil biological activity in comparison with reduced rotations or even with monoculture. Ailincai et al. (1997) reported a worsening of the chemical soil properties as a result of reduced crop rotation. On the other hand, Graenitz and Bauer (2000) found a higher mineralization activity in soil in the plots with monoculture than in the plots with crop rotation. Similar conclusion was reported by Pavolic (1992). Our study showed that both reactive and basal respiration activity was higher in the field alternatively cropped by spring wheat and sugar beet, in comparison to the nine-year crop rotation.

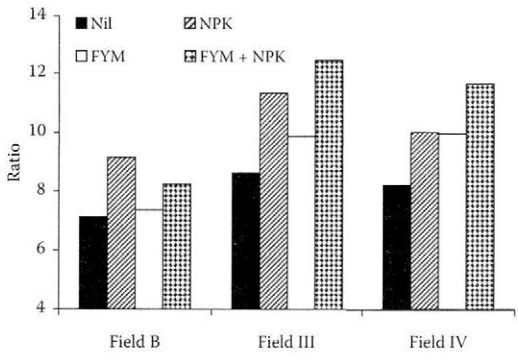

Figure 4. Average values of the ratio between potential and basal respiration activities over the time period 1995–2003

The differences were significant for all variants of organic and mineral fertilisation as well as for the Nil variants. The same results were found for the potential respiration activity.

Organic fertilisation contributes to the soil organic matter accumulation and turnover (Mercik et al. 1995, Körschens 1997, Kubát et al. 1999). Obviously, an increased soil organic matter turnover and accumulation enhance respiration activity in soils as it was shown by Kandeler and Eder (1990), Mercik et al. (1995) or Raupp and Lockretz (1997).

Mineral fertilisation, was however reported to contribute to losses of the soil organic matter (Haider et al. 1990) and to inhibit biological activity in soil (Kandeler and Eder 1990).

The evaluation of the effect of organic and/or mineral fertilisation in our experiments showed minor differences among the selected variants in the reactive and basal respiration activity. Statistically significant differences among the variants with different fertilisation were found mainly in the potential respiration activity. Mineral fertilisation alone and combined organic and mineral fertilisation showed significantly higher values of the potential respiration activity as compared to the non-fertilised control.

Ratio between the values of the potential and basal respiration activity indicates the stability of the soil organic matter. According to this criterion, stability of the soil organic matter was higher in the fields cropped in nine-year crop rotation than in the

Table 7. Effect of organic and mineral fertilisation on the potential respiration activity in the soil samples from the selected fields and variants; values of *t*-test for dependent samples

Variants	Nil	NPK	FYM
Field B: *t*-crit 0.05 = 2.131; *t*-crit 0.01 = 2.947			
FYM + NPK	−12.46	−5.36	−4.6
FYM	−12.71	1.75	
NPK	−11.63		
Field III: *t*-crit 0.05 = 2.120; *t*-crit 0.01 = 2.921			
FYM + NPK	−7.84	−0.95	−3.88
FYM	−6.15	2.73	
NPK	−9.32		
Field IV: *t*-crit 0.05 = 2.131; *t*-crit 0.01 = 2.947			
FYM + NPK	−8.42	−6.74	−7.6
FYM	−4.62	2.04	
NPK	−5.56		

field B alternatively cropped with spring wheat and sugar beet. Organic and mainly mineral fertilisation increased the stability of soil organic matter.

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Microbial, chemical and textural parameters of main soil taxonomical units of Czech Republic

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ABSTRACT

In the period 1991–2005 twelve soil taxonomical units covering 85 per cent of the total area of Czech Republic were tested at 163 localities of arable and grassed soils. The total number of analyzed soil samples reached 3 865; the lowest number was for Phaeozems and the highest for Cambisols and Haplic Luvisols. Ten soil microbial parameters were determined: microbial biomass carbon (MBC, 314.36–559.12 mg/kg), 0.5 mol/l K_2SO_4 extractable carbon (CE, 30.96–48.36 mg/kg), basal respiration (BR, 0.25–0.74 mg CO_2/h/100 g), potential respiration with glucose (2.53–4.18 mg CO_2/h/100 g), actual content of N-NH_4^+ (15.19–20.28 mg N-NH_4^+/100 g), potential ammonification with peptone (134.23–193.39 mg N-NH_4^+/24 h/100 g), actual content of N-NO_3^- (1.81–2.78 mg N-NO_3^-/100 g), potential nitrification (3.47–19.01 mg N-NO_3^-/8 days/100 g), and three ratios: CE/MBC, MBC/C_{org} and BR/MBC (qCO_2 = metabolic quotient). Following parameters were also tested: soil moisture, soil organic carbon (C_{org}, 0.95–1.81%), total nitrogen (N_t, 0.09–0.20%), ratio C_{org}/N_t, pH(H_2O), pH(KCl), sand, silt, clay and the quality of humus substances. The above-mentioned data do not include urban soils that are usually characterized with extremely low values.

Keywords: soil taxonomical units; soil organic carbon; microbial biomass; K_2SO_4 extractable C; respiration; ammonification; nitrification

The quality of our environment (Pierzynski et al. 1990) is a function of both natural phenomena and human activities. Soils are often the interface between human activities and those parts of environment that should be preserved and protected. Soils play an important role in environmental quality, as they can be a source, sink, or interacting medium for many contaminants. Proper soil management is an important step in maintaining and even improving environmental quality.

Soil is a heterogeneous, discontinuous and structured environment (Nannipieri et al. 1990) dominated by a solid phase, wherein microbial life exists in discrete microhabitats, and whose chemical, physical, and biological characteristics differ both in time and space. These characteristics are very often amalgamated into the term "soil quality". According to Pierzynski et al. (1990) soil quality includes physical properties (bulk density, texture, water-holding capacity, etc.), chemical properties (concentrations of organic and inorganic constituents) that can heavily influence soil fertility and biological activity as well as some other important soil properties. Similar chemical and physical characteristics were recommended by Pappendick (1991), who suggested biological tests such as microbial biomass, respiration, metabolic quotient (respiration/biomass), N mineralization, vegetation cover, earthworm's abundance.

Elliot et al. (1996) defined "soil quality" as "the capacity of soil to produce healthy and nutritious crops, resist erosion and reduce the impact of environmental stresses on plants". Similarly this term was described by Filip (2001) as "an integral value of the compositional structures and functions of terrestrial soils in relation to their different uses and to long-term environmental conditions on site".

Nielsen and Winding (2002) stressed that microorganisms are an essential part of living soil and have an utmost importance for soil health; as such they can be used as indicators of soil health. In their

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review there is a catalogue of microbial indicators of soil health. Some examples of recommended indicators are: functional and structural diversity of microorganisms, respiration, metabolic quotient, SOM decomposition, soil enzymes, N mineralization, nitrification, microbial biomass, bacterial DNA synthesis, presence of pathogens, etc.

The soil quality description and understanding have to be connected with quite complex studies of chemical, physical and biological properties of soils. The choice of specific tests (Nielsen and Winding 2002) will depend on the purpose of the study. Generally, as mentioned by Huber et al. (2001), the measurements related to early changes in organic matter and biological and microbial attributes are among the least monitored parameters in Europe.

Paul and Clark (1996) pointed out that the study of soil characteristics expanded from primary concern with N and soil organic matter (SOM) to several other areas as soil enzymes, rhizosphere microflora, degradation of xenobiotics, microbial ecology, transformations (cycling) of biogenic elements, genetic studies, etc. Soil microbiological studies thus contribute to sustainable resource management in agriculture and forestry.

There are several publications using microbial activities to describe how the soil characteristics can be influenced with contaminants or with different soil management. Wyszkowska et al. (2005) described a negative influence of nickel on dehydrogenase, urease and phosphatase activities. Earlier Kucharski and Wyszkowska (2004) mentioned a negative influence of copper on the number of soil microorganisms. Barajas-Aceves (2005) also used several methods (microbial biomass, soil ATP content, respiration and ammonification) for the study of the influence of Ni, Cu and Zn on soil biota.

There are limited data on the microbial characteristics of undisturbed soils. Šimon (2005) has determined in a long-term field experiment the influence of different fertilization on microbial biomass, respiration, content of C_{org} and N_t. This is in accordance with our former findings (Růžek et al. 2003, 2004) with the same characteristics on Cambisols and Luvisols.

The aim of this study was to collect a set of microbial, chemical and textural characteristics of the main soil taxonomical units in the Czech Republic. The aggregated data can be used for the evaluation of future results in this field.

MATERIAL AND METHODS

Soil samples from arable and grassed sites were collected in the years 1991–2005 covering the area of the Czech Republic (CR). Soil samples from the profile (0–200 mm) were collected using sampler *Eijkelkamp agrisearch equipment.* They were transported in the cooling box (temperature 6–12°C), adjusted, sieved (mesh 2 mm) and stored in refrigerator (4–6°C). The samples were pre-incubated

Table 1. Main soil taxonomical units in the database file and their frequency in the Czech Republic

Main soil taxonomical units	Number of localities	Number of soil samples[1] (arable and grassed soils)	Frequency in the Czech Republic[2] (in per cent of the total area)
Rendzic Leptosols (LP)	6	102	0.23
Regosols, Arenosols (RG, AR)	6	176	1.91
Fluvisols (FL)	5	53	2.90
Chernozems (CH)	29	602	5.75
Phaeozems, Vertisols (PH, VR)	6	23	2.78
Haplic Luvisols (ha LV)	31	957	7.87
Albeluvisols (AB)	13	288	2.35
Cambisols (CM)	46	692	53.03
Luvic Stagnosols	3	96	2.61
Cambic Stagnosols	2	171	4.99
Anthroposols (AT)	16	632	0.33
Urban soils, urban areas		73	

[1]0–200 mm, [2]Kozák et al. (1996)

in the room temperature (22 ± 2°C) 24 hours prior to analyses. Soil samples with original moisture were used for microbiological tests.

The following methods were used for soil samples characterisation:

- texture: sand, silt, clay content (ISO 112 77) by pipette method
- soil moisture gravimetrically (105°C)
- pH (H_2O), pH (0.2 mol/l KCl) according to Růžek et al. (2005)
- total nitrogen (N_t) – Kjeldahl method
- organic carbon (C_{org}, Sims and Haby 1971), modified by Růžek et al. (2005)
- humus quality (A 400/A 600) using the ratio of absorbances of sodium pyrophosphate soil extract at the wavelengths of 400 and 600 nm according to Pospíšil (1981)
- 0.5 mol/l K_2SO_4 extractable organic carbon (CE, Vance et al. 1987, Badalucco et al. 1992, Růžek et al. 2005) with colorimetric determination
- microbial biomass carbon (MBC) – re-hydration (RHD) technique on the base of K_2SO_4 extracts (65°C, 24 hours, k_C = 0.25, Blagodatskiy et al. 1987)
- respiration: basal (BR), potential with glucose (PRG), potential with glucose and $(NH_4)_2SO_4$ (PRNG) – CO_2 detection by Interpherometer Carl Zeiss, Jena (Novák and Apfelthaler 1964)
- metabolic quotient (qCO_2) – production of C-CO_2 calculated on MBC unit:

$$qCO_2 = (BR \times k_{C\text{-}CO_2}/MBC) \times 100$$

where: BR = basal respiration in mg/kg/h, $k_{C\text{-}CO_2}$ = 0.2727 (ratio of C in CO_2), MBC = microbial biomass carbon in mg/kg

- content of N-NH_4^+ (AA) released by 40% NaOH (modified Conway method)
- potential ammonification with peptone (PA), (Pokorná-Kozová et al. 1964)
- content of N-NO_3^- (AN), (Löbl and Novák 1964)
- potential nitrification (PN) with $(NH_4)_2SO_4$, 8 days incubation (Löbl and Novák 1964)

The following three ratios were calculated: C_{org}/N_t, $(MBC/C_{org}) \times 100$, $(CE/MBC) \times 100$.

Results were statistically evaluated using analyses of variance (multiple range tests) including Fisher's LSD method.

RESULTS AND DISCUSSION

Table 1 provides the information about soil taxonomical units, the number of localities and soil

Table 2. Chemical and textural soil characteristics (mean ± standard deviation)

Soil taxonomical unit	1)	2)	Soil moisture (%)	A 400/A 600	C_{org} (%)	N_t (%)	C_{org}:N_t	pH H_2O	pH KCl	Sand (%)	Silt (%)	Clay (%)
Rendzic Leptosols	6	102	15.93 ± 2.65	7.22 ± 1.03	1.38 ± 0.21	0.19 ± 0.03	7.58 ± 1.43	7.18 ± 0.23	6.45 ± 0.23	13.47 ± 10.30	57.73 ± 11.79	28.80 ± 6.87
Regosols	6	176	10.50 ± 2.92	6.61 ± 0.49	0.95 ± 0.14	0.09 ± 0.2	10.38 ± 2.14	6.92 ± 0.85	6.37 ± 0.98	49.20 ± 7.05	36.86 ± 9.49	13.94 ± 3.45
Fluvisols	5	53	15.63 ± 2.16	6.12 ± 0.67	1.28 ± 0.17	0.16 ± 0.03	7.20 ± 1.75	7.23 ± 0.40	6.40 ± 0.50	24.03 ± 10.60	58.14 ± 11.96	17.83 ± 2.07
Chernozems	29	602	15.39 ± 3.01	4.58 ± 0.43	1.52 ± 0.28	0.18 ± 0.03	8.71 ± 1.66	7.35 ± 0.44	6.68 ± 0.50	17.30 ± 10.41	56.26 ± 11.46	26.45 ± 7.00
Phaeozems	6	23	23.96 ± 2.16	5.02 ± 0.63	1.81 ± 0.46	0.20 ± 0.02	8.22 ± 1.90	7.13 ± 0.84	6.39 ± 0.98	30.47 ± 10.52	48.14 ± 11.69	21.39 ± 5.18
Haplic Luvisols	31	957	14.81 ± 2.68	5.75 ± 0.74	1.16 ± 0.22	0.15 ± 0.02	7.83 ± 1.77	6.88 ± 0.48	6.21 ± 0.58	14.93 ± 11.02	61.26 ± 12.04	23.34 ± 5.34
Albeluvisols	13	288	16.51 ± 2.58	7.52 ± 0.66	1.03 ± 0.15	0.14 ± 0.03	7.56 ± 1.70	6.36 ± 0.51	5.52 ± 0.58	15.26 ± 6.16	68.15 ± 8.83	16.58 ± 3.78
Cambisols	46	692	17.08 ± 4.46	6.94 ± 0.92	1.50 ± 0.43	0.18 ± 0.05	8.75 ± 1.77	6.65 ± 0.51	5.88 ± 0.61	30.22 ± 11.07	53.88 ± 14.51	15.90 ± 9.85
Luvic Stagnosols	3	96	16.99 ± 1.84	8.30 ± 1.27	1.10 ± 0.19	0.13 ± 0.02	8.67 ± 1.10	6.72 ± 0.21	6.06 ± 0.19	20.15 ± 11.35	63.49 ± 13.20	16.37 ± 3.43
Cambic Stagnosols	2	171	22.46 ± 2.22	7.03 ± 0.86	1.54 ± 0.37	0.17 ± 0.04	9.15 ± 1.57	6.90 ± 0.49	6.20 ± 0.56	13.41 ± 5.15	68.06 ± 7.07	18.53 ± 5.90
Anthroposols	16	632	16.52 ± 5.73	5.95 ± 0.80	1.72 ± 0.15	0.14 ± 0.07	10.26 ± 4.20	7.31 ± 0.55	6.69 ± 0.55	22.71 ± 13.34	45.78 ± 15.65	31.52 ± 12.76
Urban soils	–	73	16.06 ± 1.85	4.69 ± 0.65	0.62 ± 0.25	≥0.05	ND	7.96 ± 0.35	7.83 ± 0.29	69.13 ± 4.72	18.60 ± 5.44	12.27 ± 1.81

[1]number of localities, [2]number of soil samples; A 400/A 600 – humus quality, C_{org} – soil organic carbon, N_t – total nitrogen, ND – not determined

Table 3. Microbial soil characteristics (mean ± standard deviation)

Soil taxonomical unit	1	2	MBC[a]	CE/MBC[b]	MBC/C_{ox}[b]	CE[a]	qCO_2[c]
Rendzic Leptosols	6	102	434.88 ± 66.65	8.62 ± 3.35	3.22 ± 0.71	36.16 ± 10.95	0.31 ± 0.18
Regosols	6	176	314.36 ± 75.38	15.19 ± 6.35	3.35 ± 0.92	44.68 ± 12.43	0.23 ± 0.18
Fluvisols	5	53	421.42 ± 60.55	8.91 ± 3.19	3.37 ± 0.80	36.93 ± 11.78	0.30 ± 0.10
Chernozems	29	602	456.62 ± 135.52	8.84 ± 3.91	2.98 ± 0.66	38.56 ± 15.82	0.23 ± 0.09
Phaeozems	6	23	547.27 ± 112.49	6.75 ± 1.82	3.70 ± 0.74	36.26 ± 9.43	ND
Haplic Luvisols	31	957	388.03 ± 91.88	8.36 ± 3.44	3.46 ± 0.77	30.96 ± 11.74	0.27 ± 0.16
Albeluvisols	13	288	450.40 ± 90.87	8.17 ± 3.15	4.41 ± 0.81	36.19 ± 14.41	0.28 ± 0.14
Cambisols	46	692	489.35 ± 139.76	10.17 ± 3.93	3.38 ± 0.94	47.89 ± 19.12	0.27 ± 0.13
Luvic Stagnosols	3	96	421.47 ± 105.28	7.69 ± 3.74	3.84 ± 0.70	31.63 ± 14.73	0.30 ± 0.09
Cambic Stagnosols	2	171	559.12 ± 159.56	6.55 ± 2.62	3.67 ± 0.72	36.92 ± 20.56	0.33 ± 0.11
Anthroposols	16	632	396.55 ± 248.94	13.68 ± 12.32	3.11 ± 1.97	48.36 ± 41.34	0.31 ± 0.23
Urban soils		73	182.01 ± 82.31	54.09 ± 156.16	3.28 ± 1.76	48.55 ± 29.19	0.22 ± 0.21

1 – number of localities, 2 – number of soil samples; ND – not determined, MBC – microbial biomass carbon, CE – 0.5 mol/l K_2SO_4 extractable carbon, qCO_2 – metabolic quotient, BR – basal respiration, PRG – potential respiration with glucose, PRNG – potential respiration with NH_4^+ and glucose, AA – actual N-NH_4^+ content,

samples and also about the incidence of these units in the Czech Republic. Chemical and textural characteristics of tested soils are presented in Table 2.

Microbial biomass carbon-MBC (Tables 3, 4): The extremely low values (182 mg C/kg dry soil) were determined at urban soils, followed by Regosols (314 mg C/kg dry soil). Remaining ten soil units were at usual range reaching 388–559 mg C/kg dry soil, the highest levels were at Cambic Stagnosols and Phaeozems (> 500 mg C/kg dry soil). From the whole set only 2% of soil samples (Table 4) had less then 200 mg C/kg dry soil of MBC while 93% had more then 300 mg C/kg dry soil.

Ratio of microbial/soil organic carbon-MBC/C_{org} (Table 3): For this frequently used characteristic of arable and grassed soil the level of 2% is given as minimum (Dilly et al. 2005). Our soils started at a higher level (2.98%, Chernozems) reaching 4.41% (Albeluvisols). Remaining soil units were at quite a narrow range 3.11–3.84%.

K_2SO_4 extractable carbon-CE (Table 3): CE is easily available organic carbon and its low content in soil indicates an active microbial metabolism (Škoda et al. 1997). Haplic Luvisols and Luvic Stagnosols were characterized with the lowest level of CE (31 and 32 mg C/kg dry soil, respectively). Second group consisted of five soil taxonomical units with quite a narrow range 36–39 mg C/kg dry soil. The levels 45–49 mg C/kg dry soil of Regosols, Cambisols, Anthroposols and urban soils signaled worse immobilization of CE or/and less effective organic matter mineralization. The use of K_2SO_4 extractable carbon-CE for the study of annual and perennial pastures was reported by Milne and Haynes (2004) who found its significant correlation with other soil microbial characteristics (microbial biomass, basal respiration, arginine ammonification etc.).

Ratio K_2SO_4 extractable/microbial carbon-CE/MBC (Table 3): This parameter is often considered to be more valuable than CE alone because of its relation to the microbial activity. Cambisols are a good example of this statement. Their CE value (47.89 mg/kg dry soil) is very close to Anthroposols and Regosols but the ratio CE/MBC of Cambisols (10%) is characteristic for medium quality soils, while CE/MBC in Anthroposols (14%) and Regosols (15%) is characteristic for worse quality soils.

Basal respiration-BR (Tables 3, 5): The variation of BR of several soils (eight taxonomical units) is quite narrow (0.37–0.52 mg CO_2/h/100 g dry soil). Similar situation is shown in Table 5. 70% of localities were in the range 0.25–0.55 mg CO_2/h/100 g dry soil with prevailing frequency (46%) in the range 0.41–0.55 mg CO_2/h/100 g dry soil. Very low basal respiration activity (0.13 mg CO_2/h/100 g dry soil) of urban soils is connected with the lowest content of C_{org} (0.62%, Table 2). Similar low activity was determined for Regosols.

BR[d]	PRG[d]	PRNG[d]	AA[e]	PA[f]	AN[g]	PN[h]
0.49 ± 0.28	4.18 ± 1.80	7.04 ± 3.71	20.28 ± 4.73	193.39 ± 38.71	2.78 ± 2.20	12.40 ± 4.64
0.25 ± 0.16	2.53 ± 1.49	8.04 ± 4.93	10.13 ± 3.83	134.23 ± 57.04	2.37 ± 2.62	3.47 ± 3.40
0.46 ± 0.15	3.40 ± 0.85	ND	19.14 ± 3.54	187.57 ± 36.59	2.66 ± 1.83	12.85 ± 5.36
0.37 ± 0.15	3.38 ± 1.44	12.41 ± 3.56	17.88 ± 5.14	184.01 ± 47.20	2.29 ± 1.74	19.01 ± 12.64
ND	ND	ND	ND	ND	ND	ND
0.39 ± 0.23	3.66 ± 1.69	10.37 ± 4.69	15.19 ± 5.01	171.69 ± 44.97	2.20 ± 2.00	8.22 ± 3.84
0.46 ± 0.24	3.43 ± 1.35	8.35 ± 5.17	17.10 ± 4.16	163.67 ± 46.63	2.17 ± 2.01	5.37 ± 4.75
0.47 ± 0.20	3.94 ± 1.47	11.48 ± 4.22	18.94 ± 7.21	182.16 ± 46.53	2.14 ± 1.73	8.40 ± 5.99
0.52 ± 0.25	3.79 ± 1.43	8.54 ± 2.44	15.55 ± 3.01	166.32 ± 40.62	2.17 ± 1.54	9.51 ± 8.20
0.74 ± 0.33	4.02 ± 1.16	13.06 ± 2.12	19.43 ± 4.64	186.07 ± 49.01	2.17 ± 2.08	14.95 ± 15.20
0.49 ± 0.46	3.53 ± 2.68	14.04 ± 10.56	16.42 ± 12.83	186.65 ± 77.73	1.81 ± 1.89	15.31 ± 23.27
0.13 ± 0.11	4.78 ± 2.42	ND	1.37 ± 1.29	111.88 ± 4.65	2.11 ± 1.90	1.03 ± 0.95

PA – potential ammonification with peptone, AN – actual $N\text{-}NO_3^-$ content, PN – potential nitrification with $(NH_4)_2SO_4$; [a]mg/kg dry soil, [b]%, [c]mg $C\text{-}CO_2$/h/100 mg MBC, [d]mg CO_2/h/100 g dry soil, [e]mg $N\text{-}NH_4^+$/100 g dry soil, [f]mg $N\text{-}NH_4^+$/24 h/100 g dry soil, [g]mg $N\text{-}NO_3^-$/100 g dry soil, [h]mg $N\text{-}NO_3^-$/8 days/100 g dry soil

Metabolic quotient-qCO_2 (Table 3): qCO_2 is a measure of soil microbial mineralization activities because the general production of CO_2 is accomplished by soil microorganisms. According to this parameter the tested soils can be divided into two groups. The first one with the low qCO_2 (0.22–0.23 mg $C\text{-}CO_2$/h/100 mg MBC) includes Regosols, urban soils and Chernozems. The presence of Chernozems in this group is caused by the high stability of their soil organic matter. The second group includes remaining taxonomical units in which the metabolic quotient ranges between 0.27–0.33 mg $C\text{-}CO_2$/h/100 mg MBC.

Potential respiration with glucose-PRG (Table 3): PRG can be valuable information about the possibility of soil microflora to increase the metabolic activity after amendment of easily available organic substances, especially if BR is low. In our tests the potential respiration was seven to ten times higher than the basal respiration activity with exception of urban soils where it was 37 times higher. Apparently, low BR was a result of available organic carbon shortage rather than a result of bad status of the microbial communities in urban soils.

Potential ammonification-PA (Table 3): The tested soils can be divided into two groups. The first one including urban soils and Regosols is characterized with low PA (112 and 134 mg $N\text{-}NH_4^+$/24 h/100 g dry soil, respectively). The rest of soil taxonomical units were quite homogenous (164–193 mg $N\text{-}NH_4^+$/24 h/100 g dry soil).

Potential nitrification-PN (Table 3): PN showed the highest variation in measured values. Similarly Smolders et al. (2001) stressed a wide variability of potential nitrification (0–21 mg N/kg/day) in uncontaminated soils. Urban soils were characterized with the lowest PN (1.03 mg $N\text{-}NO_3^-$/8 days/100 g dry soil) while Chernozems showed the highest values of the potential nitrification activity (19.01 mg $N\text{-}NO_3^-$/8 days/100 g dry soil). Remaining soil taxonomical units can be divided into two groups. Five units (Regosols, Albeluvisols, Haplic Luvisols, Cambisols and Luvic Stagnosols) were characterized with lower PN (3.47–9.51 mg $N\text{-}NO_3^-$/8 days/100 g dry soil), four units (Rendzic Leptosols, Fluvisols, Cambic Stagnosols, Anthroposols) showed a higher activity (12.40–15.31 mg $N\text{-}NO_3^-$/8 days/100 g dry soil).

Soil organic carbon-C_{org} (Tables 2, 6): The tested soils were quite variable in C_{org} content. Urban soils and Regosols had the C_{org} content lower than one per cent (0.62% and 0.95%, respectively); three soil taxonomical units (Cambic Stagnosols, Anthroposols, Phaeozems) higher than 1.50%. Remaining soil taxonomical units were in the range 1.00–1.50%, which is also prevailing level (53%) in the classification of localities. The frequency of localities with the C_{org} lower than 1% and in the

Table 4. Frequency ranges of microbial biomass carbon (MBC)[1] on 172 arable and grassed localities in the Czech Republic (1991–2005)

MBC (mg/kg)	Number of localities	Frequency (in per cent)
< 200.00	3	2
200.00–300.00	9	5
300.01–400.00	43	25
400.01–500.00	67	39
> 500.00	50	29

[1]re-hydration (RHD) method (0.5 mol/l K_2SO_4 extraction)

Table 5. Frequency ranges of basal CO_2 respiration (BR) on 33 arable and grassed localities in the Czech Republic (1991–2005)

BR[1]	Number of localities	Frequency (in per cent)
< 0.25	3	9
0.25–0.40	8	24
0.41–0.55	15	46
> 0.560	7	21

[1]mg CO_2/h/100 g oven-dried soil

Table 6. Ranges of frequency of C_{org} on 163 arable and grassed localities (3 865 soil samples) in the Czech Republic (1991–2005)

C_{org} (in per cent)	Number of localities	Frequency (in per cent)
< 1.00	32	19
1.00–1.50	86	53
1.51–2.00	30	18
2.01–2.50	9	6
> 2.50	6	4

Table 7. Frequency ranges of ratio C_{org}/N_t on 138 arable and grassed localities in the Czech Republic (1991–2005)

Ratio C_{org}/N_t	Number of localities	Frequency (in per cent)
< 6.00	26	19
6.01–8.00	64	47
8.01–10.00	35	25
10.01–12.00	10	7
> 12.00	3	2

range 1.51–2.00% was nearly the same (19% and 18%, respectively).

Ratio C_{org}/N_t (Tables 2, 7): Prevailing range (6.01–8.00) was determined at 47% of localities and it was typical as an average value for Fluvisols (7.20), Albeluvisols (7.56), Rendzic Leptosols (7.58) and Haplic Luvisols (7.83). It is interesting that the ratio lower than six had the frequency 19% (26 localities) and was spread among seven soil taxonomical units (especially Haplic Luvisols, Albeluvisols, Rendzic Leptosols – altogether 19 localities). A higher range of the C_{org}/N_t (> 10.00) ratio was typical for Anthroposols and Regosols.

Humus quality-A 400/A 600 (Table 2): Ratio of the absorbance of sodium pyrophosphate soil extract at the wavelengths of 400 and 600 nm was used as a measure of humus quality. According to Pospíšil (1981) values lower than 3.50 signalise prevailing content of humic acids. In our study the lowest value (4.58) was determined at Chernozems following by urban soils (4.69). The values higher than 4.50 indicate the dominance of fulvic acids. Majority of soil taxonomical units were in the range 5.00–7.50; strong fulvic acid dominance (8.30) at Luvic Stagnosols is characteristic for these soils.

Content of N-NH_4^+-AA (Table 3): This parameter was quite stabile (15.19–20.28 mg N-NH_4^+/100 g dry soil) with the exception of Regosols (10.13 mg N-NH_4^+/100 g dry soil) and urban soils (1.37 mg N-NH_4^+/100 g dry soil).

Content of N-NO_3^--AN (Table 3): Similarly like N-NH_4^+ the content of N-NO_3^- was quite stabile (1.81–2.78 mg N-NO_3^-/100 g dry soil).

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Nitrogen balance in the system plant – soil after urea fertilization combined with urease inhibitors

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ABSTRACT

The distribution of ^{15}N labelled urea in the system plant – soil under the influence of two urease inhibitors (samples of the group of phosphoric acid triamides) was investigated in a pot trial with oat. The yield and N-utilization of oat were increased by both inhibitors and the losses of fertilizer-N out of the system where reduced.

Keywords: ^{15}N urea; urease inhibitors; N-balance

Urea is used as a common, well-known fertilizer. After its application to the soil a fast conversion to ammonia and carbon dioxide with the help of the enzyme urease can be observed. Reaction extends according to following equation:

$$CO(NH_2)_2 + H_2O \xrightarrow{\text{urease}} 2NH_3 + CO_2$$

This fact limits the utilization of fertilizer-N and can produce gaseous N (ammonia) losses (Amberger 1996). It should be possible to inhibit this transformation process of urea to NH_3 and reduce ammonia losses by the use of urease inhibitors (Bremner and Douglas 1973, Schlegel et al. 1987, Zhao and Zhou 1991, Bremner 1995). These inhibitors can reduce the velocity of the hydrolysis of urea in the soil and increase therefore the fertilizing effect as well as the environmental protection (Michel and Wozniak 1998, Gioacchini et al. 2002). One point of the applied investigation was to examine the influence of urease inhibitors on the utilization and the whereabouts of fertilizer-N and calculate the N-balance.

MATERIAL AND METHODS

A base of the investigations was a pot trial with oats in which urea was labelled with the stable isotope ^{15}N, to find the origin of N in plants (fertilizer-N or soil-N) and to calculate a balance of the whereabouts of the applied urea-N with respect to the system plant – soil. Oat plants of the variety Revisor were set in Mitscherlich-pots. 6 kg of soil of the experimental station in Halle/S. (Haplic Phaeozem) were used for every pot. There were 3 variants with 4 replications established:

1. control with N, without urease-inhibitor
2. with N, with urease-inhibitor 1 (UI_1)
3. with N, with urease-inhibitor 2 (UI_2)

UI_1 and UI_2 are sample products of the group of phosphoric acid triamides.

The plants received a usual basic fertilization with macro and micro nutrients: 1.0 g P as $CaHPO_4.2\,H_2O$, 3.0 g K (1.1 g as KCl, 1.9 g as K_2SO_4), 0.4 g Mg as $MgSO_4.7\,H_2O$, 2.0 mg B as $Na_2B_4O_7.10\,H_2O$, 0.6 mg Mo as $(NH_4)_6\,Mo_2O_{24}.4\,H_2O$, 20 mg Cu as $CuSO_4.5\,H_2O$, 40 mg Mn as $MnSO_4$, 15 mg Zn as $ZnSO_4.7\,H_2O$.

0.7 g N/pot was given as nitrogen fertilizer; by mixing of ^{15}N-urea (95 at-% $^{15}N_{exc}$) an enrichment of 9.5 at-% $^{15}N_{exc}$ was reached. The application was carried out in liquid form on the soil surface. The humidity was 70% of max. water capacity before fertilizer application, after it was 60%.

The harvest time was at full ripeness stage of the oat plants. All plant und soil samples were dried at 60°C until constant mass was reached,

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followed by fine milling. Total nitrogen (N_t) was analysed in every sample by elementar analysis (Elementar Vario el) and the ^{15}N-abundance by emission spectrometer NOI 7 (Fischer Analytic).

Calculation basis for whereabouts of fertilizer-N

Fertilizer-N-utilization and N-balance are based upon following calculations:

fertilizer-N-utilization (%) = fertilizer-N-uptake of plant (mg)/fertilizer-N-amount (mg) × 100

fertilizer-N-share (%) = fertilizer-N-uptake of plant (mg)/total-N-uptake of plant (mg) × 100

fertilizer-N-balance-deficit (%) = fertilizer-N-amount (mg) – fertilizer-N-uptake of plant (mg) – fertilizer-N in soil (mg)

Calculation of fertilizer-born N:

fertilizer-N (mg N/pot) = ^{15}N-amount in plant (mg $^{15}N_{exc}$/pot) × 100/at-% $^{15}N_{exc}$-fertilizer

RESULTS AND DISCUSSION

First of all we can see that the use of urease-inhibitors increase the total dry matter of the plant (Figure 1). If we look at the composition of total dry matter some differences can be observed (Table 1). A main increment of dry matter can be found in the panicle fraction, but only in variant UI_1. The differences in culm fraction are smaller, but not significant.

Similar results were found in the case of N-uptake. Most of nitrogen increment exists in panicle

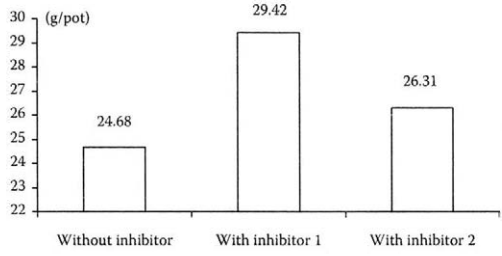

Figure 1. Dry matter at ripeness in a pot trial with oat in dependence on urease-inhibitor application

Table 1. Dry matter and N-uptake of separated plant organs

Variant	Dry matter yield (g/pot)			N-uptake (mg/pot)		
	panicle	leaf	culm	panicle	leaf	culm
Without UI	8.95 a	7.70 a	8.02 a	222 a	222 b	150 a
With UI_1	12.30 b	7.65 a	9.48 a	339 b	228 b	221 b
With UI_2	8.30 a	8.65 a	9.37 a	226 a	252 bc	241 b
LSD ($\alpha < 0.05$)	2.22	1.96	2.15	92	50	58

Numbers with the same letters mean no significant difference

Table 2. Fertilizer-N-utilization of oat plants after fertilizing with 0.7 g urea-N (9.5 at-% $^{15}N_{exc}$) to sowing (UI = urease-inhibitor)

Variant	Total-N in the plant (mg/pot)	Fertilizer-N in the plant (mg/pot)	Part of fertilizer-N on total-N in the plant (%)	Part of fertilizer-N in the plant on applied fertilizer-N (fertilizer-N-utilization) (%)
Without UI	594	297	50	42.4
UI_1	788	418	53	59.7
UI_2	719	403	56	57.6

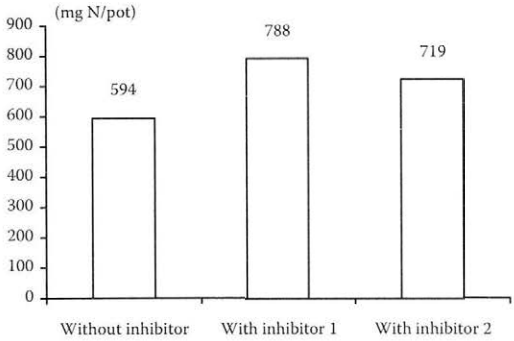

Figure 2. N-uptake of oat plants after urea fertilization with and without use of inhibitor (harvest at ripeness)

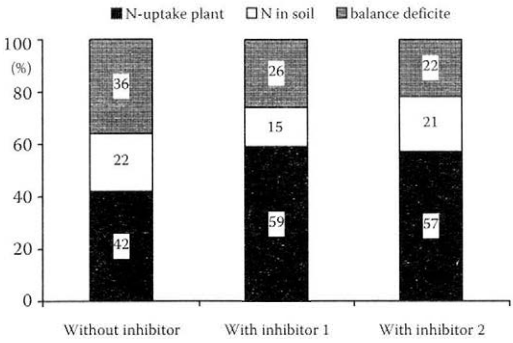

Figure 3. Balance of whereabouts of applied urea-N; pot trial with oat, application of 0.7 g urea-N (9.5 at-% $^{15}N_{exc}$)

fraction with a significant difference to control. But also the N-amount in culm of U1 is higher.

The total N-uptake of oat plants is shown in Figure 2. One reason was obviously an increased fertilizer-N-utilization by the oat plants, whereby the urea-N portion of total plant N was increased (Table 2). Former results with urease inhibitor NPPT (= N-(-*n*-butyl)-thio-phosphorsäure-triamid) (Murphy and Ferguson 1997) could thus be confirmed.

The balance of whereabouts of applied urea-N showed, that by an application of the two investigated urease inhibitors the part of urea-N in plant and soil increased, and the balance deficit (not detectable fertilizer-N) was reduced (Figure 3).

It is still a question which form the disappeared fertilizer-N exists in, and so further investigations are necessary.

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Population structure of microorganisms colonizing the soil environment of winter wheat

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ABSTRACT

A study of the population structure of the Hyphomycetes and prokaryotic organisms was conducted in the years 2001–2003. An exact experiment was performed in a randomized split-plot design, in four replications. Fungi of the genus *Penicillium* were the dominant species, which accounted for 44% and 22.3% of all isolates in cv. Sakwa and cv. Roma, respectively. Much higher counts of bacteria of the genus *Pseudomonas* were isolated from the rhizosphere of winter wheat cv. Roma than from the rhizosphere of cv. Sakwa. The population size of bacteria of the genus *Pseudomonas* and of the order *Actinomycetales* depended on the development phase of wheat plants.

Keywords: *Actinomycetales*; *Pseudomonas*; fungi; rhizosphere; wheat

Rhizosphere organisms play a key role in plant nutrition (Grayston et al. 1998). Results of numerous studies prove that secondary metabolites produced by antagonistic bacteria inhibit the development of soil pathogens (Bergsma-Vlami et al. 2005). As a result of root colonization by non-pathogenic rhizosphere microorganisms, such as plant growth promoting rhizobacteria (PGPR) or plant growth promoting fungi (PGPF), usually parasitic, plants acquire induced systemic resistance (ISR) (Kozłowska and Konieczny 2003). The occurrence and activity of soil microorganisms are affected by a variety of environmental factors (e.g. soil type, nutrient abundance, pH, moisture content) as well as plant-related factors (species, age). The objective of the present study was to determine the dynamics and population structure of fungi and bacteria of the genus *Pseudomonas* and of the order *Actinomycetales*, colonizing the rhizosphere of two winter wheat cultivars.

MATERIAL AND METHODS

Field trial

A study of the population structure of the Hyphomycetes and prokaryotic organisms was conducted in the years 2001–2003. An exact experiment was performed in a randomized split-plot design, in four replications, on a winter wheat plantation in Tomaszkowo near Olsztyn. Two winter wheat cultivars were used in the experiment: Roma and Sakwa. Winter wheat was grown on Eutric Cambisol (World Reference Base for Soil Resources 1998), formed from loamy silty sand underlain by light loam. The chemical properties of the soil are given in Table 1.

In 2001 and 2002 wheat was sown after winter triticale, and in 2003 after spring barley. The materials for microbiological analyses (root system + soil) were collected every 10 or 14 days. The development phases (phenophases) of wheat plants are shown in Table 2.

Microbiological analyses

At the laboratory soil was shaken off the roots. Roots with the associated rhizosphere soil (soil stuck to the roots) were cut into fragments. 10-gram root samples were transferred to 250 ml flasks filled with 90 ml sterilized water. The flasks were then shaken for 30 minutes in a shaker, type 358 S, at 180 rpm, and at an amplitude of 8. The suspension obtained was diluted and flooded with: Martin medium to isolate the Hyphomycetes, King B medium to isolate bacteria of the genus *Pseudomonas*, and William-Davies medium to isolate bacteria of the order *Actinomycetales* (Księżniak and Kobus

Table 1. The chemical properties of the soil

Years	pH	P	K	Mg	Ca	Carbon (%)	Nitrogen (%)	Salinity
		mg/100 g soil						
2000/2001	6.8	10.4	14.9	6.5	46	0.83	0.09	0.2
2001/2002	6.5	8.4	18.7	4.0	42	0.84	0.10	0.1
2002/2003	6.5	13.5	9.5	7.6	nt	1.05	0.08	nt

nt = not tested

Table 2. Phenophases of winter wheat plants

Years	Stem elongation	Ear formation	Maturity	
			milky	full
Zadoks' stages	GS 31	GS 55	GS 75-87	GS 92
2000/2001	07.05.	31.05.	12.07.	30.07.
2001/2002	06.05.	22.05.	01.07.	26.07.
2002/2003	16.05.	04.06.	04.07.	31.07.

1993). The colonies were counted after their counts stabilized on Petri dishes. The colonies of the Hyphomycetes were set in a slant cultures. Their taxonomic category was determined based on the appearance of conidiospores. Results were analyzed statistically using the Duncan test (Statistica ver. 6.0).

RESULTS AND DISCUSSION

A total of 429 fungal colonies were isolated from the rhizosphere of winter wheat in the years 2001–2003 (Tables 3 and 4). The fungal population that colonized the rhizosphere of winter wheat cv. Roma was much larger and diversified than that isolated from cv. Sakwa. Fungi of the genus *Penicillium* were the dominant species, which accounted for 44% and 22.3% of all isolates in cv. Sakwa and cv. Roma, respectively. It is possible that these species protected the roots of winter wheat cv. Sakwa against infestation (Shivanna et al. 1995). The roots of this cultivar were attacked to a much lower degree (data not published). Grayston et al. (1998) suggest that plant species may have a selective effect on the population structure of rhizosphere microorganisms. Results of our study show that plant varieties may also have a selective influence on rhizosphere fungi.

Potential plant pathogens of the genus *Fusarium* were sporadically found in the rhizosphere of the plants tested. It seems that these species occur primarily on root surface (Kurowski and Majchrzak 2000).

In 2001 and 2003 fungi of the genus *Cladosporium* were quite common in the rhizosphere of winter wheat, especially at late development stages. These species may contribute to an increase in the grain yield of cereals (Singh and Kapoor 1999). The population of rhizosphere fungi of wheat was larger than fungal communities observed in other crops, which indicates that this plant produces higher amounts of some compounds (Grayston et al. 1998).

The population size of bacteria of the order *Actinomycetales* varied widely (Figure 1) – 3×10^7 to 9×10^9 colony forming units (CFU) were isolated per gram of soil (fresh weight). Changes in bacterial counts were significant and related to the development phases of wheat plants and soil conditions. It was found that the growth of this bacterial group was affected by the soil pH to a relatively low degree. Bacteria of the order *Actinomycetales* developed mostly during flowering and at the milk stage of winter wheat.

Bacteria of the genus *Pseudomonas* occurred in great numbers in the rhizosphere of winter wheat, i.e. 2×10^7 to 13.2×10^7 colony forming units (CFU) per gram of soil fresh weight (Figure 2). Their count in the rhizosphere of winter wheat was much higher than in the soil (Grayston et al. 1998, Bergsma-Vlami et al. 2005). Much higher counts of bacteria

Table 3. Hyphomycetes fungi colonizing rhizosphere of winter wheat cv. Roma

Species of fungi	2001						2002						2003						Sum
	9.5.	18.5.	30.5.	20.6.	4.7.	11.7.	10.5.	23.5.	29.5.	11.6.	20.6.	27.6.	14.5.	1.6.	4.6.	17.5.	24.6.	8.7.	
Acremonium strictum W. Gams			13																13
Alternaria alternata (Fr.) Keissler																	1		1
Botrytis sp.					1														1
Chaetomium murorum Corda								1			1								2
Chaetomium sp.			2																2
Cladosporium cladosporioides (Fr.) de Vries				1		2													3
Cladosporium herbarum (Pers.) Link ex. S.F. Gray				1	2	2							1				12		18
Cladosporium macrocarpum Preuss				1													2		3
Coemansia pectinata (Coemans) Bainier				1															1
Doratomyces microsporus (Sacc.) Morton & Smith					2														2
Doratomyces spp.						3													3
Fusarium culmorum (W.G. Smith) Sacc.														1					1
Fusarium oxysporum Schlechtendahl														1					1
Fusarium poae (Peck) Wollenw.																	1		1
Gliocladium fimbriatum (Gilman and Abbott)														2	1				3
Gliomastix murorum (Corda) Hughes					4														4
Humicola fuscoatra Traaen			1					1			1								3
Leptothyrium sp.															1				1
Mucor hiemalis Wehmer								2			3								5
Mucor sp.								1			4					1		1	7
Myrothecium sp.																	1		1
Paecilomyces farinosus (Dickson ex Fr.) Brown et Smith							1												1
Paecilomyces variotii Bainier										1									1
Penicillium nigricans (Bain) Thom								1			1								2
Penicillium spp.	13		3	1	2	2	7	7	2	5	7	2	1		1	6			59
Periconiella sp.	2																		2
Phoma sp.						1													1
Pseudeutorotium sp.											1								1
Pyrenochaeta spp.	2				2			1											5
Scopulariopsis brevicaulis (Sacc.) Bain							1		34	1		35							71
Scopulariopsis brumptii Salvanet-Duval													1						1
Scopulariopsis chartarum (G. Smith) Morton et G. Smith																1			1
Scopulariopsis sp.		1																	1
Torula herbarum (Pers.) Link ex Fr.	11																		11
Trichoderma aureoviride Rifai					1														1
Trichoderma hamatum (Bon.) Bain.					2														2
Trichoderma harzianum Rifai				3	4														7
Trichoderma polysporum (Link ex Pers.) Rifai					1														1
Non-sporulating fungi			9		3	3						2					3		20
Total	28	1	28	8	24	13	9	14	36	7	18	39	3	4	3	8	20	1	264

Table 4. Hyphomycetes fungi colonizing rhizosphere of winter wheat cv. Sakwa

Species of fungi	2001						2002						2003						Sum
	9.5.	18.5.	30.5.	20.6.	4.7.	11.7.	10.5.	23.5.	29.5.	11.6.	20.6.	27.6.	14.5.	1.6.	4.6.	17.5.	24.6.	8.7.	
Acremonium strictum W. Gams			7																7
Alternaria alternata (Fr.) Keissler					3												2		5
Aureobasidium pullulans (De Bary) Arnaud						2		2		1									5
Chaetomium sp.				1									1						2
Cladosporium cladosporioides (Fr.) de Vries																	1		1
Cladosporium herbarum (Pers.) Link ex. S.F. Gray			2	7		1											3		13
Cladosporium macrocarpum Preuss																	1		1
Fusarium avenaceum (Fr.) Sacc.		1																	1
Fusarium culmorum (W.G. Smith) Sacc.														1	1				2
Gliocladium catenulatum Gilman and Abbott																	9		9
Mucor globosus Fischer							1			1									2
Mucor hiemalis Wehmer				1															1
Mucor sp.														1					1
Paecilomyces lilacinum (Thom) Samson														1					1
Papulaspora irregularis Hotson			5																5
Penicillium spp.	2		4	1	2	1	12		8	14		8		1	6	11	2	2	74
Phoma eupyrena Sacc.			8																8
Phoma sp.			4																4
Rhizopus nigricans Ehrenberg		4															1	1	6
Trichoderma harzianum Rifai				1															1
Trichoderma polysporum (Link ex Pers.) Rifai				1															1
Trichoderma viride Pers. ex. S.F. Gray		1																	1
Verticilium cephalosporum W. Gams																1			1
Wardomyces humicola Hennebert & Barron														1					1
Zygorhynchus heterogamus Vuillemin				3															3
Non-sporulating fungi			7													1	1		9
Total	2	6	37	15	5	4	13	2	8	16	0	8	1	5	7	13	20	3	165

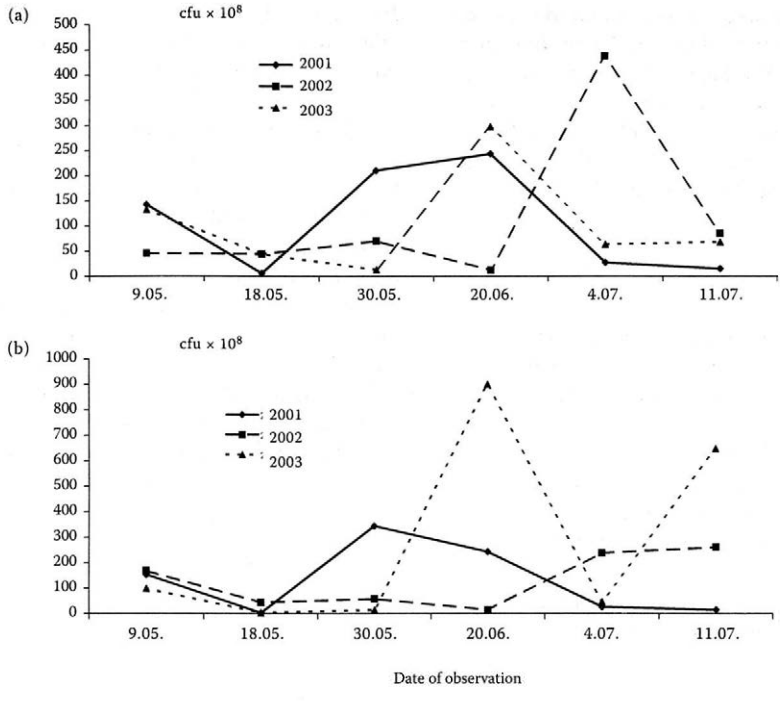

Figure 1. Bacteria of the order *Actinomycetales* colonizing rhizosphere of winter wheat cv. Roma (a) and cv. Sakwa (b)

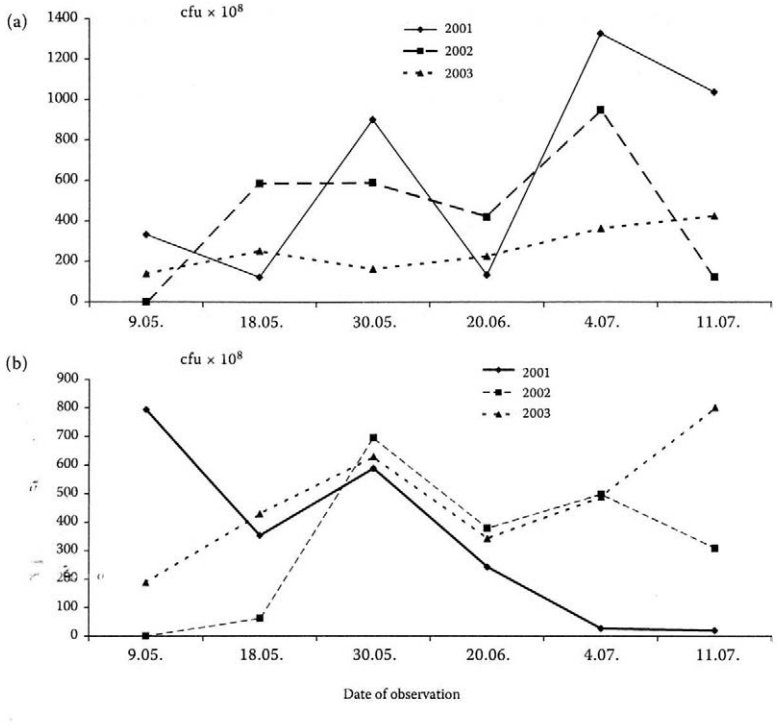

Figure 2. Bacteria of the genus *Pseudomonas* colonizing rhizosphere of winter wheat cv. Roma (a) and cv. Sakwa (b)

of the genus *Pseudomonas* were isolated from the rhizosphere of winter wheat cv. Roma than from the rhizosphere of cv. Sakwa. The population size of bacteria of the genus *Pseudomonas* depended on the development phase of wheat plants. Members of this genus dominated in cv. Roma at the milk stage, and in cv. Sakwa at the ear formation stage.

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