Temporal variations in microbial biomass C and cellulolytic enzyme activity in arable soils: effects of organic matter input

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Abstract

Temporal variations in soil microbial biomass C concentration and in activity of extracellular enzymes of the cellulolytic complex were investigated in a field experiment after eight years of cultivation with either low organic matter input (low-OM) or high organic matter input (high-OM). The cultivation systems differed in whether their source of fertiliser was mainly mineral or organic, in whether a winter cover crop was grown, and whether straw was mulched or removed. Sampling occurred at approximately monthly intervals, over a period of two years. Distinct temporal variations in microbial biomass C concentration and activity of extracellular enzymes of the cellulolytic complex were observed. The temporal pattern was generally similar in the low-OM and high-OM cultivation systems. Temporal variations may have been driven by environmental factors (e.g., temperature and moisture) and crop growth, i.e. by factors common to both systems but not differences in organic matter input. Pronounced and constant increases in β-glucosidase activity (40%) and endocellulase activity (30%) in high-OM were detected across all sampling periods. The increases in microbial biomass C concentration and celllobiohydrolase activity varied over the sampling periods (0–60% and 24–92%, respectively). Over the experimental period a mean of 148 ± 6.0 μg biomass C g⁻¹, a β-glucosidase activity of 123 ± 3.3 nmol g⁻¹ h⁻¹, a celllobiohydrolase activity of 122 ± 2.4 nmol g⁻¹ h⁻¹ and an endocellulase activity of 33.8 ± 0.9 nmol g⁻¹ h⁻¹ were recorded in the low-OM soil (0–20 cm). The corresponding means in the high-OM treatment were 189 ± 6.6 μg biomass C g⁻¹, a β-glucosidase activity of 174 ± 4.1 nmol g⁻¹ h⁻¹, a celllobiohydrolase activity of 173 ± 3.4 nmol g⁻¹ h⁻¹ and an endocellulase activity of 44.2 ± 1.1 nmol g⁻¹ h⁻¹. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Integrated arable farming system; Soil quality indicators; Microbial biomass; Extracellular cellulase; Fluorogenic compound assay

1. Introduction

Soils managed with organic inputs generally have larger and more active microbial populations than those managed with mineral fertilisers (Dick, 1984; Doran et al., 1987; Powlson et al., 1987; Werner and Dindal, 1990; Fauci and Dick, 1994). Soil microbial biomass and activity are greatly stimulated by the addition of manure through modification of soil physical characteristics and addition of readily available C and N sources (Reganold, 1988; Fraser et al., 1988).
Since microorganisms are more responsive to changes in the soil environment than is the soil organic matter (Powlson et al., 1987), effects of experimental treatments are more readily detectable in the microbial fractions (Dick, 1994). Previous studies have compared temporal responses of microbial biomass and soil enzymes under different management or different temperature and moisture regimes (Martyniuk and Wagner, 1978; Lynch and Panting, 1982; Kaiser and Heinemeyer, 1993; Joergensen et al., 1994; Ritz et al., 1997; Jensen et al., 1997). McGill et al. (1986) proposed that seasonal temporal changes in soil microbial biomass are directly linked to the turnover of organic matter and the cycling of nutrients in soil. Temporal changes in the size of microbial pool may enhance the efficiency of N use in agricultural systems (Granatstein et al., 1987; Bonde et al., 1988; Bremer and van Kessel, 1992).

Doran and Parkin (1994) proposed a set of soil quality indicators that are sensitive to changes in soil management and integrate biological, physical, and chemical properties. Biological methods used to assess soil quality should reflect cropping and management histories in long-term studies. In discussing soil quality indicators, Karlen et al. (1997) included soil microbial biomass C and extracellular enzyme activity as biological indicators.

There is considerable interest in adopting alternative agricultural practices such as organic or biodynamic and low-input systems in the belief that the present conventional agricultural system using mineral fertilisers has detrimental effects on physical, chemical and biological properties of soils (Nguyen et al., 1995). Long-term benefits from the use of crop rotations and animal manure include better soil tilth, improved water infiltration, increased soil organic matter content, and increased biological activity (Wardle, 1992).

Here, we report the effects of up to eight years of high (high-OM) and low (low-OM) organic matter input in arable farming systems on microbial biomass carbon concentrations and selected cellulolytic enzyme activity in the soil. The systems being compared in a long-term field trial are cereal rotations that differ in their source of fertiliser (mineral or organic), in whether a catch crop is grown, and whether the straw is mulched or removed. In the high-OM system only a small part of the nutrient supply is provided by mineral fertilisation and the nutrient supply to crops and soil microorganisms will be mainly from organic inputs. The objective of this research was to determine the extent of temporal variations in microbial biomass C concentration, cellulobiodyrolase, β-glucosidase and endocellulase activity over two successive years. A second objective was to evaluate the effects of high and low organic matter input on soil quality indicators after eight years of cultivation.

2. Materials and methods

2.1. Site and soil

The study was part of a field experiment on the integrated arable farming initiated in 1987, located at Research Centre Foulum, Denmark. It is situated at (56°30′ N and 9°34′ E, 50 m above mean sea level). The soil is a loamy sand with 67% sand and 8% clay. Mean annual (1961–1990) temperature is 7.3°C and ranges from −0.5°C in January to 15.4°C in July. Average mean annual (1961–1990) rainfall is 626 mm, of which 50% falls in autumn and winter.

Previously these fields were uniformly cultivated, and had no organic manure additions for 14 years before the start of the experiment. In the spring of 1988, randomized blocks were established, with three replicate blocks per treatment. The treatments differed mainly in the form of nutrients applied (Rasmussen and Hansen, 1994). This study included a high-OM treatment with high inputs of organic matter in the form of pig slurry, a catch crop and incorporation of straw, and a low-OM treatment with mineral fertilisers, no catch crop and straw removed (short stubble remaining) at harvest (Table 1). Both high- and low-input treatments received a reduced input of pesticides (Secher et al., 1995). Each spring, 30 t pig slurry ha⁻¹ were applied to the high-OM treatment, supplemented by ammonium nitrate (AN) evenly spread over the surface (Table 1). The slurry was surface-applied, then ploughed in. Straw left after harvest was cut before mulching. The low-OM treatment received mineral fertilisers (NPK and AN) in the spring. In the high-OM treatment, a four-year crop rotation of winter wheat, spring barley with a catch crop sown...
after harvest, spring barley undersown with a catch crop in the spring, and pea in the first and spring rape in the second crop rotation was practised. Fodder rape (*Brassica napus* L. *ssp. oleifera* (Metzg.) Sinsk.), or tansy-leaf phacelia (*Phacelia tanacetifolia*) was sown after harvest of the first spring barley crop in the rotation was harvested. Perennial ryegrass (*Lolium perenne* L.) was undersown in the second spring barley crop in the crop rotation. In the low-OM treatment a four-year crop rotation of winter wheat, spring barley, spring barley and pea in the first and spring rape in the second crop rotation was practised. No catch crop was sown in the low-OM treatment. In 1995 and 1996 both treatments were sown to a spring barley, as the main crop. In the high-OM treatment fodder rape was sown in autumn 1995 as a catch crop and perennial ryegrass was the undersown catch crop in 1996.

Soil was sampled (0–20 cm) on 20 occasions between May 1995 and December 1996. On each sampling date, four soil cores (2.5-cm diameter) were collected at random from each block and pooled to give one sample per replicate. Freshly sampled soil was thoroughly mixed, and roots and visible plant residues removed. Soil was frozen (approximately −18°C) for later determination of microbial biomass C and enzyme activity. Gravimetric water content was determined by drying duplicate 10 g portions of soil at 105°C for 24 h.

### 2.2. Biomass C

Microbial biomass C was determined by the fumigation–extraction method Vance et al. (1987). Soluble C and chloroform labile C (C solubilized by CHCl₃ during an 18 h fumigation period) were extracted with 0.5 M K₂SO₄ in a soil : solution ratio of 1 : 3 (wt : v). The extract was centrifuged and the supernatant filtered through a combusted (250°C, 5 h) Whatman GF/C filter and a 0.22 μm Millipore Millex-GP filter unit. Organic C in all 0.5 M K₂SO₄ extracts was determined by an automated UV persulphate oxidation procedure using a Dohrmann DC-180 Carbon Analyzer (Wu et al., 1990). Biomass C was calculated as soluble C in fumigated minus soluble C in non-fumigated soil using 0.45 as the $k_c$-factor (Kaiser et al., 1992) and determined in duplicate.

### 2.3. Enzyme assays

The activity of β-glucosidase (EC 3.2.1.21), cellobiohydrolase (EC 3.2.1.91) and endocellulase (EC 3.2.1.4) were measured in field moist samples by the use of different fluorogenic substrates (see below). During hydrolysis of the substrates, the aglycone 4-methylumbelliflorone (4-MU) is formed, which is highly fluorescent at an alkaline pH. The assays were performed according to the methods described by Tilbeurgh and Clayssens (1985), with the following

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**Table 1:** Soil properties, treatments, crop rotation and OM inputs

<table>
<thead>
<tr>
<th></th>
<th>High-OM</th>
<th>Low-OM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year of initiation</td>
<td>1988</td>
<td>1988</td>
</tr>
<tr>
<td>Soil properties in 1995</td>
<td>Total C 1.7 (%), Total N 0.13 (%), CEC 9 (meq 100 g⁻¹), pH-CaCl₂ 5.9</td>
<td>Total C 1.6 (%), Total N 0.13 (%), CEC 6 (meq 100 g⁻¹), pH-CaCl₂ 5.7</td>
</tr>
<tr>
<td>Fertilizer practice</td>
<td>Pig slurry (30 t) and AN a, (80 kg mineral N and 30 kg organic N ha⁻¹)</td>
<td>NPK and AN (90 kg N ha⁻¹)</td>
</tr>
<tr>
<td>Ploughing</td>
<td>Mouldboard ploughed, spring straw</td>
<td>Mouldboard ploughed, spring straw</td>
</tr>
<tr>
<td>Mulching</td>
<td>winter wheat, spring barley, fodder rape c, spring barley/ryegrass d, spring rape</td>
<td>Straw removed, winter wheat, spring barley, spring barley, spring rape</td>
</tr>
<tr>
<td>Crop rotationb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Annual input of OM e</td>
<td>6477 kg ha⁻¹</td>
<td>0</td>
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</table>

a Ammonium nitrate.
b Crops at sampling time underlined.
c Catch crop.
d Undersown catch crop.
e Average annual input of OM in the form of straw, ryegrass and pig slurry, stubble and roots not included.
modifications. The β-glucosidase activity was determined by the addition of 2.0 ml of 0.05 M acetate buffer, (pH 5.0) to 20–80 mg soil. The mixture was left for five minutes in polyethylene tubes at 30°C. The enzymatic reaction was initiated by the addition of 0.5 ml substrate solution, and incubated for 20 min in a water bath at 30°C. The substrate concentration of 4-methylumbelliferyl β-D-glycoside was 100 μM. The reaction was stopped by the addition of 2.5 ml 96% ethanol (v/v). The mixture was left for 20–30 min at 5°C, then transferred into centrifuge tubes and centrifuged at 5°C for 10 min at 1500 g. The supernatant (4.5 ml) was transferred to a second polyethylene tube, and 0.5 ml 2.5 M Tris buffer (pH 10) was added to convert 4-MU into its fluorescent anionic form. Fluorescence was determined with a Perkin-Elmer fluorometer (LS50) at 448 nm emission and 365 nm excitation.

The activity of cellobiohydrolase was determined using 4-methylumbelliferyl β-D-cellobioside as substrate. Soil samples of 50 mg were pre-incubated with 2.0 ml 0.05 M acetate buffer, (pH 5.0) for five minutes, at 30°C. The assay conditions and separation of method for 4-MU were the same as described above for β-glucosidase. The substrate concentration in the assay was 100 μM. Endocellulase activity was determined using 4-methylumbelliferyl β-D-cellotrioside as the substrate. The assay was performed in the same way as described above, but the incubation period was increased to 50 min. Soil samples of 50 mg were used and the substrate concentration in the assay was 25 μM. All enzyme activity measurements were determined in six replicates.

2.4. Statistical models and analysis

A split-plot design with soil treatment as the main plot treatment and sampling date as the sub-plot treatment was used. Within each block the 20 measurements of each of the four variables, microbial biomass C concentration, cellobiohydrolase, β-glucosidase and endocellulase activity, were analysed for serial correlation by means of standard semi-variogram techniques (Cressie, 1991). Based on these analyses it was concluded that the serial correlation was negligible for all four variables, and so it was ignored in subsequent analyses. A linear statistical model with systematic interaction between treatment and sampling date and with random block effect was accepted. The correlation analysis did, however, indicate some variance heterogeneity between treatment groups, and even between blocks. However, only variance heterogeneity between treatment for the variables β-glucosidase and endocellulase activity was significant (p < 0.05). The analyses were performed by means of the SAS-procedure PROC MIXED (SAS Institute Inc., 1996a) using the REML-methodology (Searle et al., 1992; Diggle et al., 1994).

In the subsequent statistical analyses the systematic part of the models, i.e. treatment and sampling date effect were analysed. For microbial biomass C concentration and cellobiohydrolase activity for which variance homogeneity was applicable, the classical analysis of variance was used (PROC GLM, SAS Institute Inc., 1989). For the effects of β-glucosidase and endocellulase, iterative REML-methods (PROC MIXED, SAS Institute Inc., 1996b) were applied to allow for treatment specific variances. In both models, the sampling date specific treatment effect and the difference between treatments of the average level per year of the four variables were formulated as contrasts, and hence analysed by standard techniques. The average level per year, for the four variables, was approximated by a trapeze-sum, in order to account for the non-equidistant measurement of time points.

In order to test for a temporal trend over the two-year observation period, a linear time trend was added to the systematic part of the model. This linear time trend had a treatment specific intercept for all variables, whereas the slope was treatment specific for microbial biomass concentration and cellobiohydrolase activity, but the same across treatments for endocellulase and β-glucosidase activity in accordance with the statistical model derived above. Furthermore, either the sampling date factor or the sampling date × treatment interaction factor was treated as a random effect in order to test the significance of the added linear term.

3. Results

Mean monthly rainfall and air temperature over the two-year observation period are shown in Fig. 1. Total precipitation for the two-year sampling period was 1010 mm, 242 mm below the long-term average.
Autumn 1995 and 1996 were both relatively wet, while the spring of 1996 as well as the summers of 1995 and 1996 were extremely dry (Fig. 1). The mean temperature for the experimental period was 7.2°C, which is comparable with the long-term average. The soil was frozen from December 1995 to April 1996, with a mean temperature of \(-3.8°C\), and a mean temperature of \(-2.8°C\) in November and December 1996 (Fig. 1). The prolonged periods of frost in 1995 caused slow and weak development of the catch crop (fodder rape) in the high-OM treatment.

With the exception of a higher CEC for high-OM treatment, there was little or no difference in soil properties between treatments (Table 1). Percentage total carbon levels were similar for the soils of both treatments.

Significant effects of both treatment and sampling date were found for all four variables studied \((p < 0.05)\). For microbial biomass C concentration and cellobiohydrolase activity the treatment and the sampling date effects interacted significantly, which resulted in the treatment effect varying over the sampling period. The interaction between sampling date and treatment implied that the model-predicted level for each sampling date and treatment was equal to the average of the true measurements for sampling date and treatment (Fig. 2(a) and (b)). For \(\beta\)-glucosidase and endocellulase activity the treatment effect was constant over the sampling period and as a result the predicted levels differed from the measured averages for \(\beta\)-glucosidase and endocellulase activity (Fig. 2(c) and (d)). In the treatment with high-OM input the soil had consistently higher biomass C levels than low-OM soil when all sampling dates were considered together \((p < 0.01)\). We found no significant differences between the summer and autumn sampling dates of the respective years (Fig. 2(a)). There was no significant linear temporal trend in microbial biomass C concentration over the two-year period. Mean annual biomass C concentration, calculated by the trapezium on the data in Fig. 2(a), differed significantly with organic matter input (Table 2).

The high-OM treatment had consistently higher cellobiohydrolase activity (24–92%) than the low-OM treatment at all sampling dates \((p < 0.001)\). The difference between treatments was significant when
Fig. 2. Measured and model-predicted average levels of microbial biomass C concentration and extracellular enzyme activity in soils with cereal rotation, with low-OM and high-OM organic matter input, April 1995–December 1996: (a) Microbial biomass C concentration; (b) Cellulohydrolase activity; (c) β-glucosidase activity; (d) Endocellulase activity. (●) measured and (——) predicted values in low-OM treatment, (○) measured and (- - -) predicted values in high-OM treatment.

Table 2
Mean annual microbial biomass C concentration (MB-C), and extracellular enzymes activity in 1995 and 1996 in soils with cereal rotation, as related to low (low-OM) and high (high-OM) organic matter input

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<tbody>
<tr>
<td></td>
<td>Low-OM</td>
<td>High-OM</td>
<td>Low-OM</td>
<td>High-OM</td>
</tr>
<tr>
<td>Microbial biomass C (µg C g⁻¹ soil)</td>
<td>160.9</td>
<td>184.6xx a</td>
<td>140.4</td>
<td>182.8xxx b</td>
</tr>
<tr>
<td>Cellulohydrolase activity (nmol 4-MU g⁻¹ h⁻¹)</td>
<td>115.1</td>
<td>162.1xxx b</td>
<td>130.5</td>
<td>185.3xxx b</td>
</tr>
<tr>
<td>β-glucosidase activity (nmol 4-MU g⁻¹ h⁻¹)</td>
<td>114.5</td>
<td>165.7xxx b</td>
<td>133.5</td>
<td>184.5xxx b</td>
</tr>
<tr>
<td>Endocellulase activity (nmol 4-MU g⁻¹ h⁻¹)</td>
<td>30.6</td>
<td>41.1xxx b</td>
<td>38.5</td>
<td>49.0xxx b</td>
</tr>
</tbody>
</table>

Note: Means per treatment and experimental year were estimated by trapeze-sum method. The p-values were derived from statistical tests comparing the predicted yearly average level in low and high-OM level.

a p < 0.01.

b p < 0.001.
considering the whole entire time-course \( (p < 0.001) \), but not on all individual sampling dates (Fig. 2(b)). Soil with high-OM input had the greatest mean cellobiohydrolase activity in both years (Table 2). The overall temporal trend showed fairly uniform cellobiohydrolase activity for most of the sampling dates for both treatments, with a slight increase throughout the experimental period \( (p < 0.05) \), indicating increasing cellobiohydrolase activity between 1995 and 1996 in both treatments.

Temporal fluctuations in β-glucosidase activity were distinct in both treatments (Fig. 2(c)). There was a significant effect \( (p < 0.0001) \) on sampling date on β-glucosidase activity, demonstrated by low activity from August 1995 to April 1996 and relatively high activities on the other sampling dates (Fig. 2(c)). The effect of treatment was constant and significant over all sampling dates \( (p < 0.001) \). β-glucosidase activity followed a similar temporal pattern in the two treatments and was ca. 40% higher in the high-OM treatment than in the low-OM treatment on all sampling dates. There was no significant linear temporal trend in β-glucosidase activity. The average difference in β-glucosidase activity between high-OM and low-OM was highly significant (Table 2).

Temporal variations in endocellulase activity showed a different pattern from those for β-glucosidase activity, with the highest activity in the autumn/ winter and early summer sampling samplings (Fig. 2(d)). Despite temporal fluctuations of endocellulase activity the effect of treatment was constant and significant over all sampling dates \( (p < 0.001) \). On all sampling dates, the endocellulase activity in the high-OM treatment was ca. 30% higher in the low-OM treatment (Fig. 2(d)) and the average level over the two years differed significantly with organic matter input (Table 2). There was a consistent and significant temporal trend in endocellulase activity in both treatments \( (p < 0.05) \), indicating that endocellulase activity increased during the two years.

### 4. Discussion

#### 4.1. Temporal trends

Microbial biomass C concentration and extracellular soil enzymes of the cellulolytic complex displayed marked temporal variability, similar in both low- and high-OM treatments (Fig. 2). Different amounts of annual OM input (Table 1) did not have any large influence on temporal variations of microbial biomass C concentration and enzyme activity. This suggests that temporal variations may have been driven by climatic (e.g. temperature and moisture) factors and by crop growth, i.e. by factors common to both systems. Temporal changes in soil moisture, soil temperature, and C input from crop roots, rhizosphere products (i.e. root exudates, mucilage, sloughed cells, etc.), and crop residues can have a large effect on soil microbial biomass and activity (Ross, 1987). Crop growth often stimulates an increase in the size of microbial biomass during the growing season (Lynch and Panting, 1982; McGill et al., 1986; Fraser et al., 1988; Kaiser and Heinemeyer, 1993). Granatstein et al. (1987) observed a significant increase in microbial biomass after harvest. Patra et al. (1990) argued that biomass C concentration remained fairly constant during the year due to a relatively even distribution of litter with time. In this study, however, the peak in microbial biomass C concentration occurred in early May 1995 when no crop growth had yet taken place (Fig. 2(a)). The autumn (October 1995) increase in biomass C concentrations coincided with the onset of rain (Fig. 1) and with the addition of new crop residues. Moisture probably became a limiting factor during the summer 1995 (Fig. 1), with the lowest biomass levels occurring in July (Fig. 2(a)). In contrast, a significant increase in microbial biomass was observed in July 1996 during the period of lowest soil moisture. In many studies, the temporal variations of microbial biomass were attributed to variations in soil water content. Wetting and drying of soil has both been shown to increase (Ross, 1989; Joergensen et al., 1994), lower (Kieft et al., 1987; van Gestel et al., 1992) or to have no effect on soil microbial biomass levels (Ross, 1989; van Gestel et al., 1992).

Enzyme activity displayed different temporal patterns of the cellulase complex of enzymes as revealed in different assays (Fig. 2(b)–(d)). According to Bolton et al. (1985), Martens et al. (1992) and Dick (1994) some cellulases are closely related to inputs of fresh organic materials, plant growth and plant residues, while others appear to be more sensitive to soil temperature and moisture. Temporal variations in community structure and activity, induced by manure.
decomposition and crop residue decomposition, may modulate microbial biomass and the extracellular enzymes that are released into the soil by the microbial biomass (Fauci and Dick, 1994; Ritz et al., 1997; Bossio et al., 1998).

Temporal variations in the size of microbial biomass C concentration and extracellular enzyme activities can be differentiated from that representing long-term changes in the biomass and enzyme activity in response to inorganic fertiliser, organic manure, straw incorporation or crop rotation with the statistical approaches used in this study. Cellobiohydrolase and endocellulase activity over two years showed a consistent trend, i.e. a steady increase in the enzyme activity in both high and low-OM treatments, but such an increase did not appear in biomass C and β-glucosidase. An increase in cellobiohydrolase and endocellulase suggested that there were fundamental differences in C-supplying mechanisms to the microbial community in both treatments in addition to current-season manure or crop-derived inputs (Ritz et al., 1997). These results suggest that a new equilibrium had not yet been attained after the change in management. Following a sustained management change such as the addition of organic fertiliser, crop residues and crop rotation, it may take many years for the soil organic matter to reach a new equilibrium (van der Linden et al., 1987).

4.2. Soil quality and fertility

With the increasing emphasis on sustainable agriculture and soil quality it is important to determine the effect of different organic inputs on soil organic matter (Christensen and Johnston, 1997). Changes in the quality and quantity of soil organic matter occur slowly. These changes are difficult to quantify in short-term studies because they are small in relation to the large background of organic matter and the natural soil variability (Bosatta and Ågren, 1991). Due to their dynamic nature, soil microbial biomass and soil enzymes respond quickly to changes in organic matter input (Powlson and Jenkinson, 1981; Powlson et al., 1987; Dick, 1994).

In this study, no changes in total carbon levels in soils were observed after eight years (Table 1). The increased levels of microbial biomass C concentration and cellulolytic enzyme activity in the high-OM treatment (Table 2) reflect high annual inputs of organic matter (6477 kg ha⁻¹) in the form of pig slurry, straw residue and winter cover crop. Several studies have looked at the shifts in microbial numbers and activity in soil as related to changes in C inputs. In some studies they were attributed to amount and diversity of organic inputs (Fraser et al., 1988; Knudsen et al., 1999; Martyniuk and Wagner, 1978; Powlson et al., 1987). The importance of microbial biomass and extracellular enzyme activity in assessment of soil quality is established by the essential role of soil microorganisms in nutrient cycling within agricultural ecosystems (Rice et al., 1986). Of particular importance are free enzymes in the extracellular space, for example for the initial depolymerization steps of cellulose (Burns, 1982).

5. Conclusions

Large temporal variation in microbial biomass C concentration and extracellular enzyme activity were observed, which could be related to environmental factors (e.g., temperature and moisture) and crop growth but not differences in organic matter input. Microbial biomass C concentrations and the activity of extracellular cellulases appeared to be very good indicators of early changes in soil biological status. Biomass C concentration and extracellular enzyme activity increased (≈30%) as a consequence of changing the cultivation system from one of low to one of high organic matter input after eight years. In addition, our results demonstrated that due to temporal variability repeated sampling is required to calculate annual estimates of the size of microbial biomass C concentration and cellulolytic enzyme activity for the purpose of comparative ecosystem study.

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