Title: **Microbial activity in a sandy arable soil is governed by the fertilization regime**

Running title: **Microbial activity in a sandy arable soil**

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Abstract

The aim of this study was to compare the influence of two different long-term organic fertilization regimes at an arable site on a nutrient-poor, sandy soil with respect to soil microbial biomass contents and microbial activities. The investigation was performed on a long-term experimental arable field site, located in the semi-continental climate of Central Europe (IOSDV, Berlin-Dahlem, Germany). Soil microbial biomass and dehydrogenase activity were most clearly increased by combined straw and green manure treatment. In comparison, farmyard manure had a weaker effect, explained by less frequent applications and different quality of organic materials incorporated into soil. The mineral N-fertilization did not significantly effect microbial biomass content or dehydrogenase activity of the soil under study. The cellulase activity was increased markedly by straw and green manure treatment, but was increased only slightly by mineral fertilization. Organic manuring with plant residues had a stronger impact on soil microbial activity as compared to different soil sampling dates. We conclude that soil microbial activity was governed most clearly by the fertilization regime under the conditions of the investigated field experiment. Furthermore, manuring with plant residues has the most beneficial effects on soil quality among the investigated types of fertilisation.

Keywords

soil microbial biomass / soil basal respiration activity / dehydrogenase activity / cellulase activity / organic manuring / nitrogen fertilization / long-term field experiment

1. Introduction

Routine applications of organic and mineral fertilizers are an essential component of soil management in arable crop production systems. Organic matter inputs, such as farmyard manure, green manure, or straw, either alone or in combination with mineral fertilizers, hold the potential to sustain or improve the soil ecological basis of crop production such as nutrient availability, water holding capacity, and soil structure, which are critical aspects in coarse-textured, sandy soils [3]. Organic manuring can furthermore improve water infiltration rates and drainage or air infiltration, and moreover, soil microbial communities and microbial activities are effected [14,12]. Soil microorganisms are the major protagonists of organic matter decomposition and nutrient turnover in arable soils. It has been frequently reported that soil microbial activity is an important aspect of soil quality [31], i.e., concerning soil to function within ecosystem and land use boundaries in order to sustain plant productivity. Thus, various soil microbiological parameters were studied and proved to be suitable for the assessment and evaluation of sustainable soil management practices [10], or as indicators for changes in soil organic matter composition [23]. The size and the activity of the soil microflora is determined by physical and chemical properties of soil, as well as by external factors such as temperature and humidity [e.g. 27,15]. The strong impact of cropping practices such as farmyard manure application and fertilizers on soil microbial activity has been shown for various soil types and climatic zones [12,16,34]. In Central European agricultural practice, the importance of farmyard manure is gradually decreasing in recent years due to new techniques in animal production. On the other hand, green manure or straw residues are still significant sources or organic matter inputs to arable soils in modern cultivation systems. We hypothesized that changes in

the fertilization regime have considerable effects on soil microbial activity especially in sandy soils, which are known to be susceptible to the input of organic material. Furthermore, the activity of soil organisms in sandy soils is supposed to be effected by management practices to a higher extent as compared to loamy soils [8]. The aim of this study was to compare the influence of two different long-term organic fertilization regimes at an arable site on a nutrient-poor, sandy soil (farmyard manure vs. manure with plant residues) on microbial biomass contents and microbial activities, as measured by basal and specific respiration activity, dehydrogenase and cellulase activities.

2. Materials & Methods

2.1 Characteristics of the field experiment

The investigation took place in the International Long-term Fertilization Experiment (IOSDV) located in Berlin-Dahlem, Germany (52° 28' N, 13° 18' E) at 51 m above sea level. The site is situated in the intermediate zone between oceanic and continental climate in Central Europe. The soil texture is classified as a silty sand (74.8 % sand, 20.1 % silt, 5.1 % clay). According to the FAO-classification, the soil type is an *Albic Luvisol*. A summary of site, climatic and soil chemical properties is presented in Table 1.

The field experiment was started in 1984 and is part of the summary list of long-term field experiments throughout the world [6]. The crop rotation consists of potato, winter wheat and spring barley. Three different regimes of organic manuring have been sampled in combination with additional variants with or without additional mineral N-fertilization (Tab. 2). In the "farmyard manure" treatment, the average input of organic dry matter was 84 dt ha^{-1} a^{-1} and 277.7 kg N ha^{-1} a^{-1} within a period of ten years. For the "straw- and green-manure" treatment, the average organic input was 171 dt ha^{-1} a^{-1} and 357 kg N ha^{-1} a^{-1}. As basic fertilization, 26 kg ha^{-1} P and 149 kg ha^{-1} K were given to potato as well as 26 kg ha^{-1} P and 100 kg ha^{-1} K to cereals, respectively. More information about the field experiment is given by Köhn et al. [21]. Monitoring of the soil climatical conditions revealed that in all years of investigation the temperature was generally higher in summer than in spring, whereas soil humidity was higher in spring than in summer (Tab. 3).

2.2 Sampling

Soil samples were taken from three spring barley plots in spring (before seeding) and in summer (after harvesting) in three consecutive years (2001-2003), respectively. Samples were collected at 20 sampling points per plot using a drill corer (0 – 15 cm depth) and pooled, finally sieved (2 mm mesh size) and stored at 4 °C until investigation.

2.3 Soil microbial biomass, basal soil respiration and metabolic quotient

For the determination of substrate induced respiration we used an automated system that allows the parallel measurement of CO_2 production of 24 soil samples by infrared gas analysis [11]. According to the method described by Anderson and Domsch [1], soil samples were equilibrated (2 d, 20 ºC), mixed with glucose (3 mg g^{-1} soil) and incubated for up to 24 hours at 22 °C in three replicates. Soil microbial biomass was calculated at the maximum initially respiration response as C_{mic} (µg g^{-1} soil) = (µl CO_2 g^{-1} soil h^{-1}) 40.04 + 0.37. The soil basal CO_2 respiration was measured continuously without the addition of substrate for up to 24 hours at 22 °C in three replicates. The metabolic quotient was calculated as the ratio between basal respiration and the substrate induced respiration and expressed in ng CO_2-C μg^{-1} C_{mic} h^{-1}.

2.4 Enzymatic activities

Dehydrogenase activity

Dehydrogenase activity was determined according to the method described by Thalmann [33]. The soil samples (5 g) were incubated in triplicate for 24 hours with 2,3,5–triphenyl-tetrazoliumchloride (TTC, 3 mg ml^{-1}) at 27 °C, pH 7.8. The produced

triphenylformazan (TPF) was extracted with acetone and measured photometrically at 546 nm. Dehydrogenase activity was expressed as μg TPF g^{-1} soil 24 h^{-1}.

Cellulase activity

According to the method published by Schinner and von Mersi [30], cellulase activity was determined by incubation of soil samples with water-soluble carboxymethylcellulose (Fluka BioChemika, Germany; 7 mg ml^{-1}) for 24 hours at 50°C, pH 5.5. Low-molecular products and sugars resulting from the enzymatic degradation of carboxymethylcellulose are used for the quantitative reduction of potassium hexacyanoferrate II to potassium hexacyanoferrate III, which reacts with Fe (III) ammoniumsulfate to form a complex known as "Prussian Blue", which is determined photometrically at 690 nm. Cellulase activity is expressed as μg glucose g^{-1} soil 24 h^{-1}.

2.5 Statistical analysis

The significance of differences between treatments was estimated by one-way ANOVA (Tukey test), using the software package SPSS version 10.0 (SPSS Inc., Chicago, USA).

3. Results

3.1 Microbial biomass, basal respiration and metabolic quotient

The highest soil microbial biomass contents (range: 94.8 - 324.8 C_{mic} g^{-1}) were generally found in the treatments with straw- and green-manure (Fig. 1). This effect was evident in all three years under study. The long-term farmyard manure applications never increased microbial biomass contents when given without additional application of mineral N-fertilizer, whereas the combined farmyard manure and mineral N-fertilization had a significant effect on microbial biomass in summer 2001 and 2003. The treatment with mineral N-fertilization but without organic manure did not show any significant differences from the control, or even tended to decrease microbial biomass in spring 2001 and 2002. In 2002 and 2003, microbial biomass contents in the treatments were significantly higher in summer than in spring (P <0.01), whereas in 2001 no seasonal effect was observed.

Basal respiration activity ranged from 0.15 µg CO_2-C g^{-1} soil h^{-1} ("without-N 0", summer 2001) to 0.41 µg CO_2-C g^{-1} soil h^{-1} ("straw-/green-N 0", spring 2002). Throughout the different sampling dates, the basal respiration was clearly highest in the treatments with straw and green manure (Fig. 2). Farmyard manure application and fertilization with mineral N did not result in increased respiration activities. The metabolic quotient qCO_2 as a measure of the specific respiration fluctuated considerably over the different sampling dates and investigated treatments (Tab. 4). The values ranged from 0.86 ng CO_2-C μg^{-1} C_{mic} h^{-1} in the "Straw-/Green-N 3"-treatment (summer 2003) to 2.55 ng CO_2-C μg^{-1} C_{mic} h^{-1} in the "without-N 3"-treatment (spring 2002). The qCO_2 was rarely increased by treatments, e.g., sole N fertilizer applications in spring and summer 2002, or green manuring in spring 2002.

3.2 Dehydrogenase activity

The highest dehydrogenase activities (range: 8.4 – 37.2 µg TPF g^{-1} soil 24 h^{-1}) were clearly observed in the treatments with straw- and green manure (Fig. 3). In 2002 and 2003, the effects of straw- and green-manure tended to be stronger when applicated without additional mineral N-fertilization. An effect of farmyard manuring on dehydrogenase activity was observed only in spring 2002. The combination of farmyard manure and mineral N-fertilization, as well as mineral N-fertilization alone did not result in any significant differences from the control. In all years of investigation and in mostly all treatments, the dehydrogenase activity was about twice as high in summer than in spring. This effect was significant for 2001 and 2003 (P <0.001).

3.3 Cellulase activity

Cellulase activity ranged from 177.14 µg glucose g^{-1} soil 24 h^{-1} ("without-N 0", summer 2003) to 488.03 µg glucose g^{-1} soil 24 h^{-1} ("straw-/green-N 3", spring 2001). The treatments with straw- and green-manure generally led to the highest cellulase activities detected (Fig. 4). No effect of farmyard manure was observed except for the sampling in summer 2003, when cellulase activity was significantly increased as compared to the control. Mineral N-fertilization alone as well as in combination with both types of organic manuring tended to increase cellulase activity, but no significant effects were observed. No consistent seasonal impact was detected, comparing spring versus summer samplings across all treatments.

4. Discussion

The soil microbial biomass contents as a measure of the active part of the soil microflora ranged from 90 to 330 µg g^{-1} soil in the Berlin-Dahlem field experiment, which was in accordance with our expectations. Insam [13] reported in an investigation of arable soils from different climatical zones that soil microbial biomass ranged from 41 to 562 µg g^{-1} soil and was the lowest in soils with a low clay content. Correspondingly, Wirth [35] found contents of C_{mic} between 169 and 967 µg g^{-1} soil in an investigation of soils at 89 arable sites across the northeastern German lowland, which is characterized by nutrient-poor sandy to loamy soils. Among the investigated fertilizer treatments, the combination of straw and green manure had the strongest impact on the microbial biomass and on the basal respiration activity. Obviously, this effect is mainly due to the input of straw manure as an organic carbon source, applied immediately to spring barley. An increasing effect of straw manure on the microbial biomass is well known from literature. Lynch and Panting [24] reported that eight months after application of straw manure to a loamy arable soil the microbial biomass was almost twice as high as compared to a control. In long-term field experiments in Denmark, Powlson et al. [28] showed that straw manure could increase soil microbial biomass up to 45 %. These findings correspond with our results from the Berlin-Dahlem field experiment, where soil microbial biomass was on average 69 % higher in the treatment “Straw-/Green-N 0” as compared to the unfertilized control. The comparably weak effects of farmyard manure on soil microbial biomass in the Berlin-Dahlem field experiment were unexpected and in contrast to the results of other investigations. Kandeler et al. [18] found, that farmyard manure (30 t ha^{-1}, applicated every second year) doubled microbial biomass in a Haplic Phaeocem under spring barley.

Kanchikerimath and Singh [16] reported an increase of microbial biomass by factor three after a combined application of farmyard manure and mineral N-fertilizer in a semiarid Cambisol. Nevertheless, our results seem to be plausible, because in the Berlin-Dahlem field experiment the farmyard manure was already applied to potato, whereas we performed our investigations about two and a half years after application of manure under spring barley. We assume, that the farmyard manure was mostly decomposed at the time of investigation, thus, no direct impact on soil microbial biomass was detected. Weak or slightly depressive effects of mineral N-fertilization on soil microbial biomass have been reported by other authors [4,22] and may be explained by a lack of available C-sources after an initial boost of mineralization activities [7]. Ritz and Robinson [29] found no consistent effect of mineral N-fertilization on microbial biomass in a field experiment under spring barley and assumed that the microflora in the investigated soil was not limited by nitrogen supply. Svensson and Pell [32] found in three Swedish long-term field experiments that the microbial biomass was not influenced by mineral N-fertilization, concluding that substrate induced respiration depends on available N-sources as well as on available soil carbon sources. The metabolic quotient (qCO_2) as a measure of specific soil microbial respiration activity indicates higher specific activities per unit biomass by lower qCO_2 values. For arable soils, Kandeler et al. [17] reported metabolic quotients in the range of 0.5 to 3.0 ng CO_2-C μg^{-1} C_{mic} h^{-1}. In our field experiment, the qCO_2 ranged from 0.86 to 2.55 ng CO_2-C μg^{-1} C_{mic} h^{-1}. In 2002 and 2003, we found that the qCO_2 was generally decreased comparing spring versus summer samplings, indicating a higher carbon efficiency at summer and more favourable conditions for microbial growth. The investigated

treatments of organic-mineral fertilization had no consistent or significant effect on the metabolic quotient.

The dehydrogenase activity as a measure of over-all, intracellular microbial activity was increased by straw and green manure applications as compared to farmyard manure treatments, which reflects our results from substrate induced respiration assays. The total annual input of organic matter in the “straw and green manure” application is about twice as high as in the “farmyard manure” treatment, but this alone does not sufficiently explain the marked differences in the dehydrogenase activity. According to Pancholy and Rice [26], dehydrogenase activity is influenced rather by the quality than by the quantity of organic matter incorporated into soil. Thus, the stronger effects of straw and green manure on dehydrogenase activity might be due to the more easily decomposable components of crop residues on the metabolism of soil microorganisms. The observation that dehydrogenase activity is poorly influenced by mineral nitrogen fertilization is consistent with our results. Marinari et al. [25] showed, that in a sandy loam under maize mineral nitrogen fertilization has weaker effects on dehydrogenase activity as compared to organic manuring. The authors concluded, that mineral nitrogen additions are rapidly dispersed into the soil organic matter, the plant biomass, or are lost by leachates without effecting soil biological properties. The finding that cellulase activity is strongly increased by vegetal residues and tends to be higher due to mineral N-fertilization is in accordance with data from the literature. In an investigation of a winter wheat monoculture, Bandick and Dick [2] found similar results for the β–glucosidase, that is known as one component of a cellulose decomposing enzyme complex, including endo- and exo-acting enzymes essential for a complete degradation of native cellulose [36]. This finding is supported by the results of Debosz et al. [5],

who reported that the activities of β–glucosidase, cellobiohydrolase, and endo-cellulase in a fertilization system with slurry, straw and green manure were significantly higher as compared to a system with exclusive mineral nitrogen fertilization. The activities of xylanase, which is another indicator of litter or plant cell wall decomposition, is closely associated with cellulase activity. Kandeler et al. [18] found that xylanase activity was doubled after farmyard manure application and increased by the factor 2.5 under the influence of mineral nitrogen fertilization alone. It is assumed, that the strong stimulating effect of mineral nitrogen fertilization on cellulase activity is indirectly caused by the higher amounts of available crop and root remnants [19], which are frequently reported from plots fertilized with mineral nitrogen [9]. Moreover, nitrogen sources are essential for the build-up of microbial biomass and the production or synthesis of enzymes.

Concerning temporal variability, substrate induced respiration and dehydrogenase activity were generally higher in the summer samplings as compared to the spring samplings. This can obviously be explained by the input of plant residues after harvest and by higher soil temperatures prevalent in summer. Reduced soil humidity in summer samplings obviously had no detrimental effect on soil microbial activities. In contrast to substrate induced respiration and dehydrogenase activity, which are non-specific, over-all indicators for microbial activity, specific enzyme activity, i.e., cellulase activity was not clearly effected by season. We assume, that the effect of temperature on cellulase activity was overshadowed by other factors, e.g., the C:N-ratio.

Conclusions

The comparison of two different regimes of organic manuring on a sandy arable soil revealed that annual straw-, green- and sugar beet leaf manure increased soil microbial

activity stronger than farmyard manure applied every three years only. We assume, that the replacement of farmyard manure by manuring with plant residues has stimulating effects on decomposing potentials of soil microorganisms and nutrient turn-over, due to the quantity and quality of the organic materials incorporated into soil. The effect of mineral nitrogen fertilization on substrate induced respiration and enzyme activities - either applied alone or in combination with any type of organic manuring - was negligible and thus no increased organic matter turn-over may be expected. Since it is generally accepted that soil functioning and maintenance of soil fertility depends on the activity of soil microorganisms we conclude that manuring with plant residues provides the most beneficial effects for sustaining or enhancing soil quality among the investigated types of fertilisation on sandy soils. Furthermore, organic manuring with plant residues had a stronger impact on soil microbial activity as compared to spring versus summer sampling dates. Thus, it can be concluded that soil microbial activity was clearly governed by the fertilization regime under the conditions of the investigated field experiment.

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Table 1: Climate (1971-2000) and soil chemical properties [20] at the study site in Berlin-Dahlem

Parameter	**Mean**
annual air temperature (°C)	9.6
annual precipitation (mm)	540.1
C_{org} content (mg 100 g^{-1} soil)	656
total N content (mg 100 g^{-1} soil)	62
C/N-ratio	10.6
total P content (mg 100 g^{-1} soil)	11.8
total K content (mg 100 g^{-1} soil)	11.6
pH	5.4

Table 2: Fertilization regimes under study (fresh matter input by fertilizers per hectare and year)

	Treatment	potato	winter wheat	spring barley
A	**"without-N 0"**	-	-	-
B	**"without-N 3"**	150 kg mineral N	160 kg mineral N	120 kg mineral N
C	**"FYM-N 0"**	300 dt FYM	-	-
D	**"FYM-N 3"**	300 dt FYM 150 kg mineral N	160 kg mineral N	120 kg mineral N
E	**"Straw-/Green-N 0"**	60 dt straw[1] Green manuring[2]	250 dt beet leaf	60 dt straw[1]
F	**"Straw-/Green-N 3"**	60 dt straw[1] Green manuring[2] 150 kg mineral N	250 dt beet leaf 160 kg mineral N	60 dt straw[1] 120 kg mineral N

[1] with additional 60 kg mineral-N, [2] green manuring with oil radish

Table 3: Temperature and soil humidity at sampling dates (daily means)

Sampling date	air temperature (°C)	soil temperature 5 cm (°C)	soil humidity 0-20 cm (%)
05.04.01	7.9	10.2	18.4
31.07.01	19.4	23.9	13.4
25.03.02	1.5	3.0	21.8
29.07.02	26.4	26.7	12.6
24.03.03	7.8	7.5	17.3
18.07.03	20.3	24.1	10.5

Table 4: Metabolic quotient (qCO_2, ng CO_2-C μg^{-1} C_{mic} h^{-1}) in upper soil material (0-15 cm) at the different sampling dates

Treatment	summer 2001	spring 2002	summer 2002	spring 2003	summer 2003
without-N 0	1.05	1.60	1.33	1.57	0.93
without-N 3	1.55	2.55	2.25	1.32	0.96
FYM-N 0	1.44	1.44	1.39	1.42	0.87
FYM-N 3	1.37	1.58	1.11	1.92	0.98
Straw-/Green-N 0	1.47	2.41	1.26	1.36	0.87
Straw-/Green-N 3	1.57	2.50	1.09	1.33	0.86

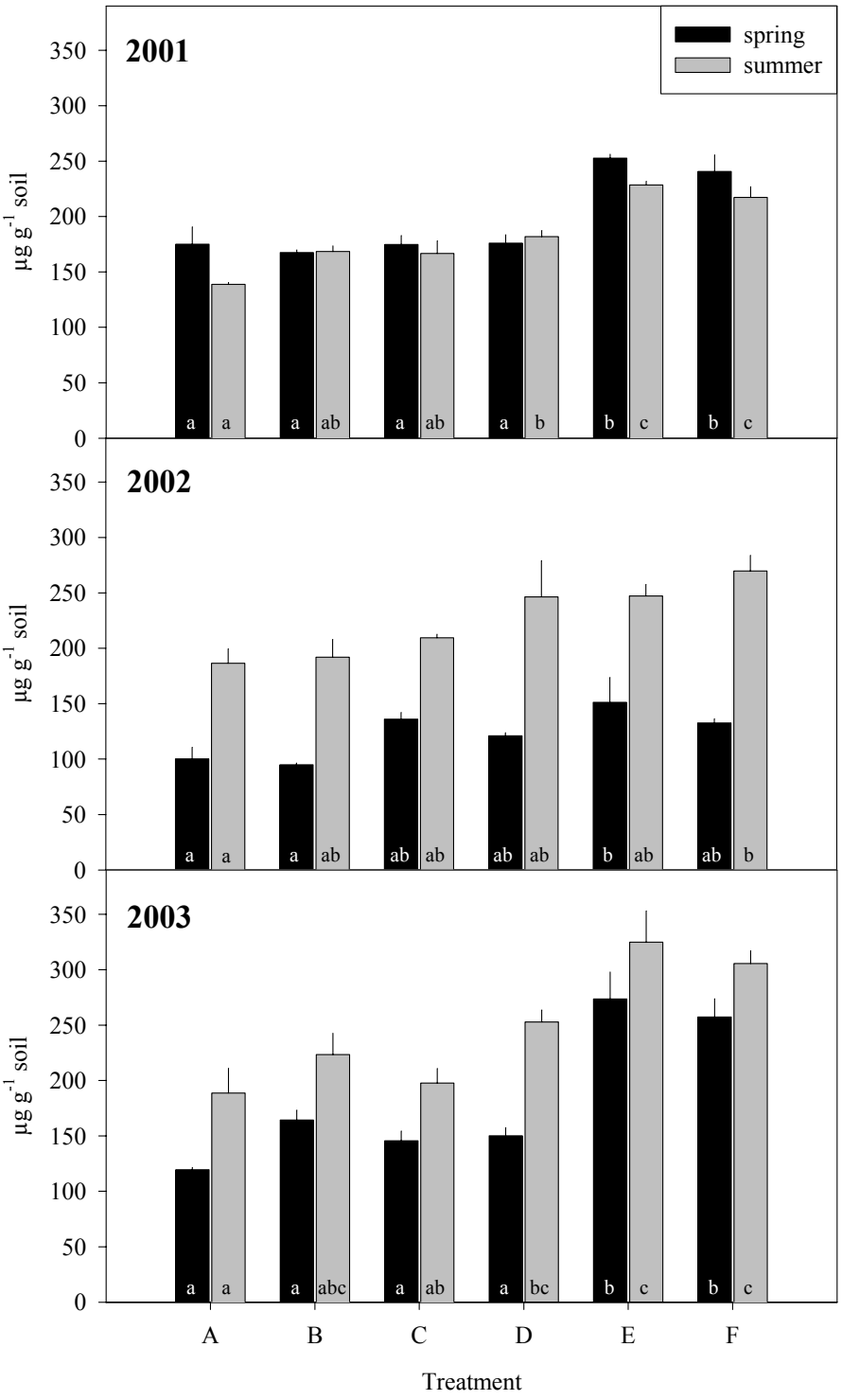

Figure 1: Soil microbial biomass contents in a sandy arable soil (0-15 cm) at spring and summer sampling dates (2001-2003), as influenced by different long-term fertilizer regimes. A: "without-N 0", B: "without-N 3", C: "FYM-N 0", D: "FYM-N 3", E: "Straw-/Green-N 0", F: "Straw-/Green-N 3". Abbreviations are explained in Table 2. Error bars represent standard errors. Treatments with the same letter at one date are not significantly different ($p < 0.05$).

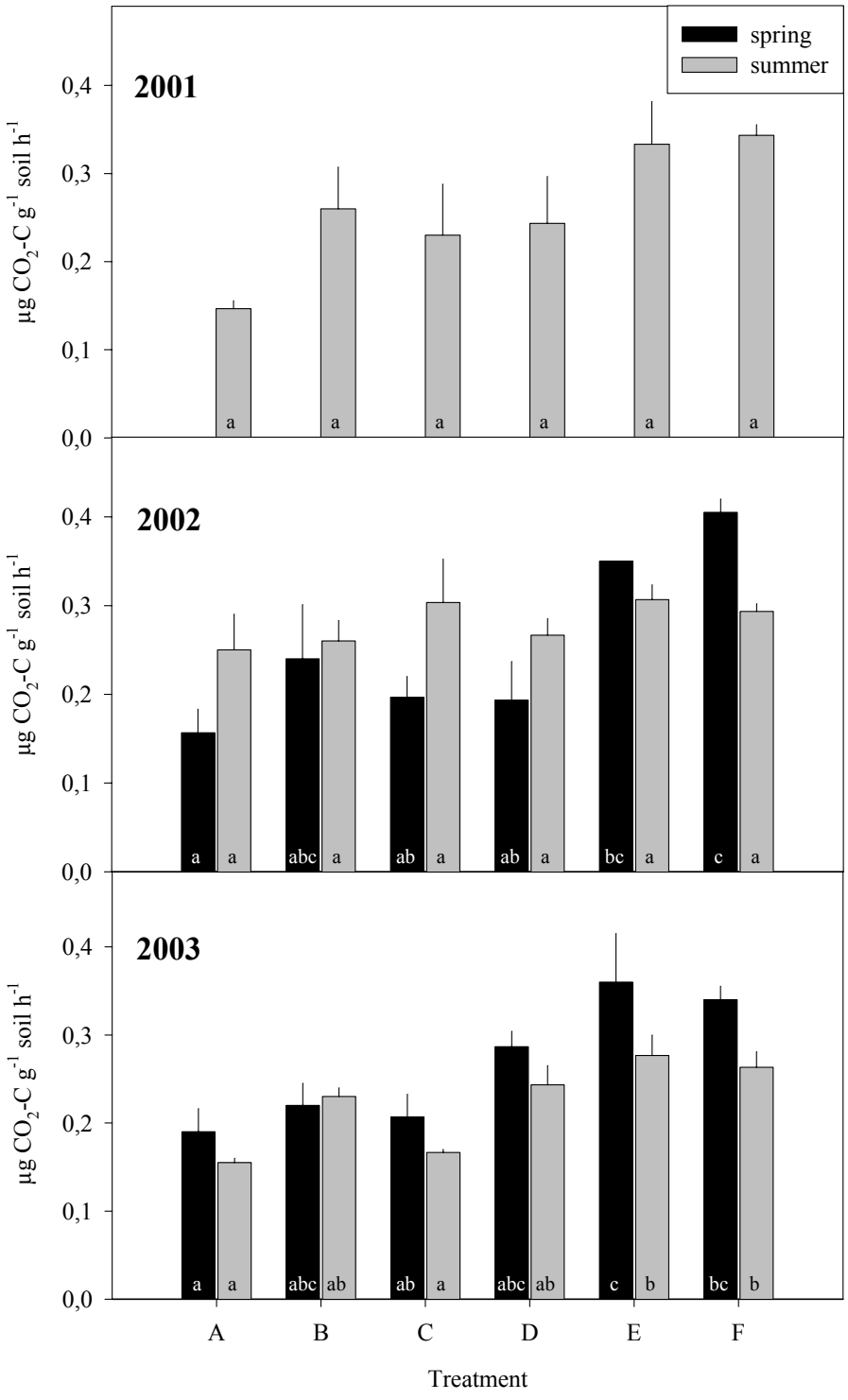

Figure 2: Basal respiration activity in a sandy arable soil (0-15 cm) at spring and summer sampling dates (2001-2003), as influenced by different long-term fertilizer regimes. A: "without-N 0", B: "without-N 3", C: "FYM-N 0", D: "FYM-N 3", E: "Straw-/Green-N 0", F: "Straw-/Green-N 3". Abbreviations are explained in Table 2. Analyses of basal respiration were included to the studies since summer 2001. Error bars represent standard errors. Treatments with the same letter at one date are not significantly different ($p < 0.05$).

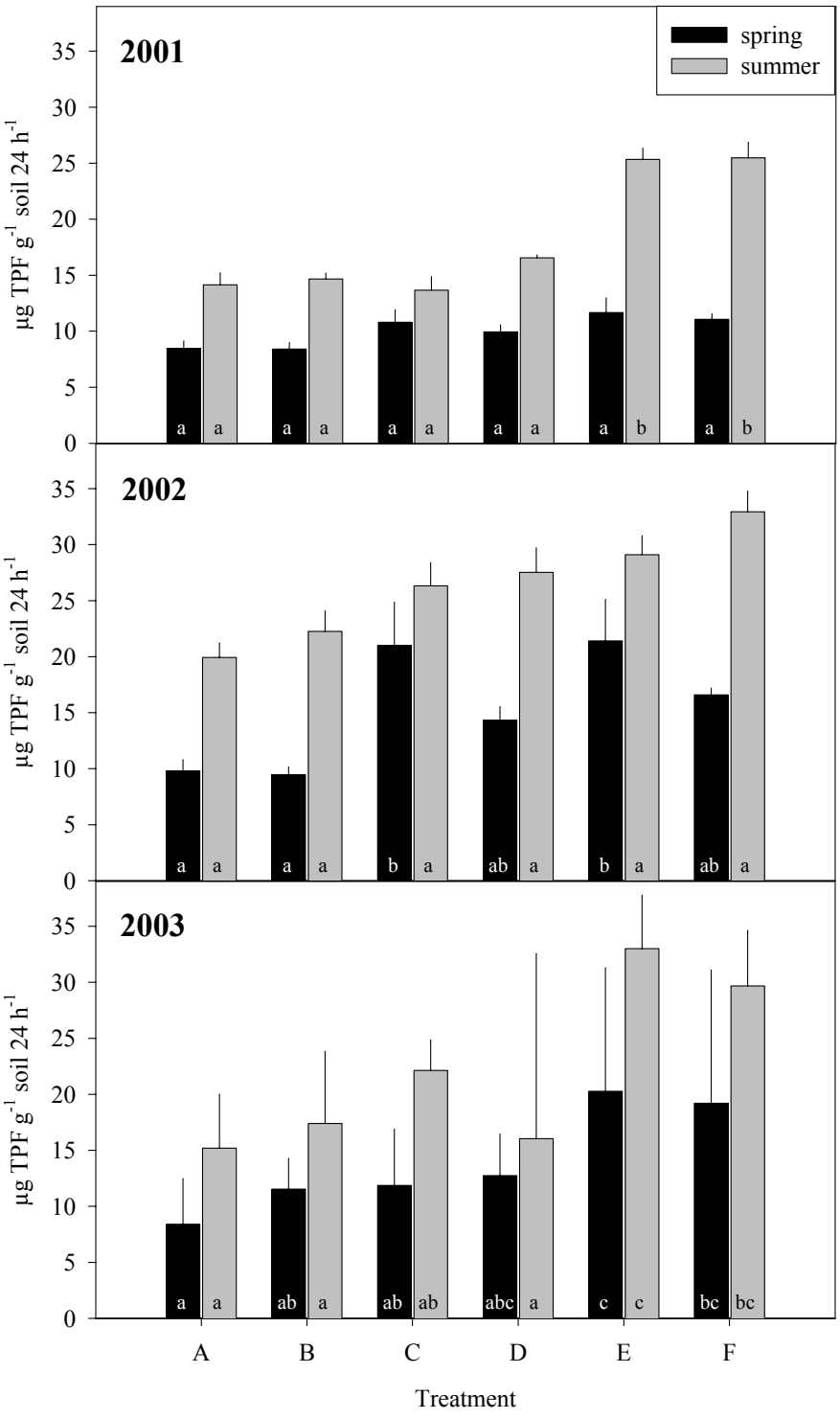

Figure 3: Dehydrogenase activity in a sandy arable soil (0-15 cm) at spring and summer sampling dates (2001-2003), as influenced by different long-term fertilizer regimes. A: "without-N 0", B: "without-N 3", C: "FYM-N 0", D: "FYM-N 3", E: "Straw-/Green-N 0", F: "Straw-/Green-N 3". Abbreviations are explained in Table 2. Error bars represent standard errors. Treatments with the same letter at one date are not significantly different ($p < 0.05$).

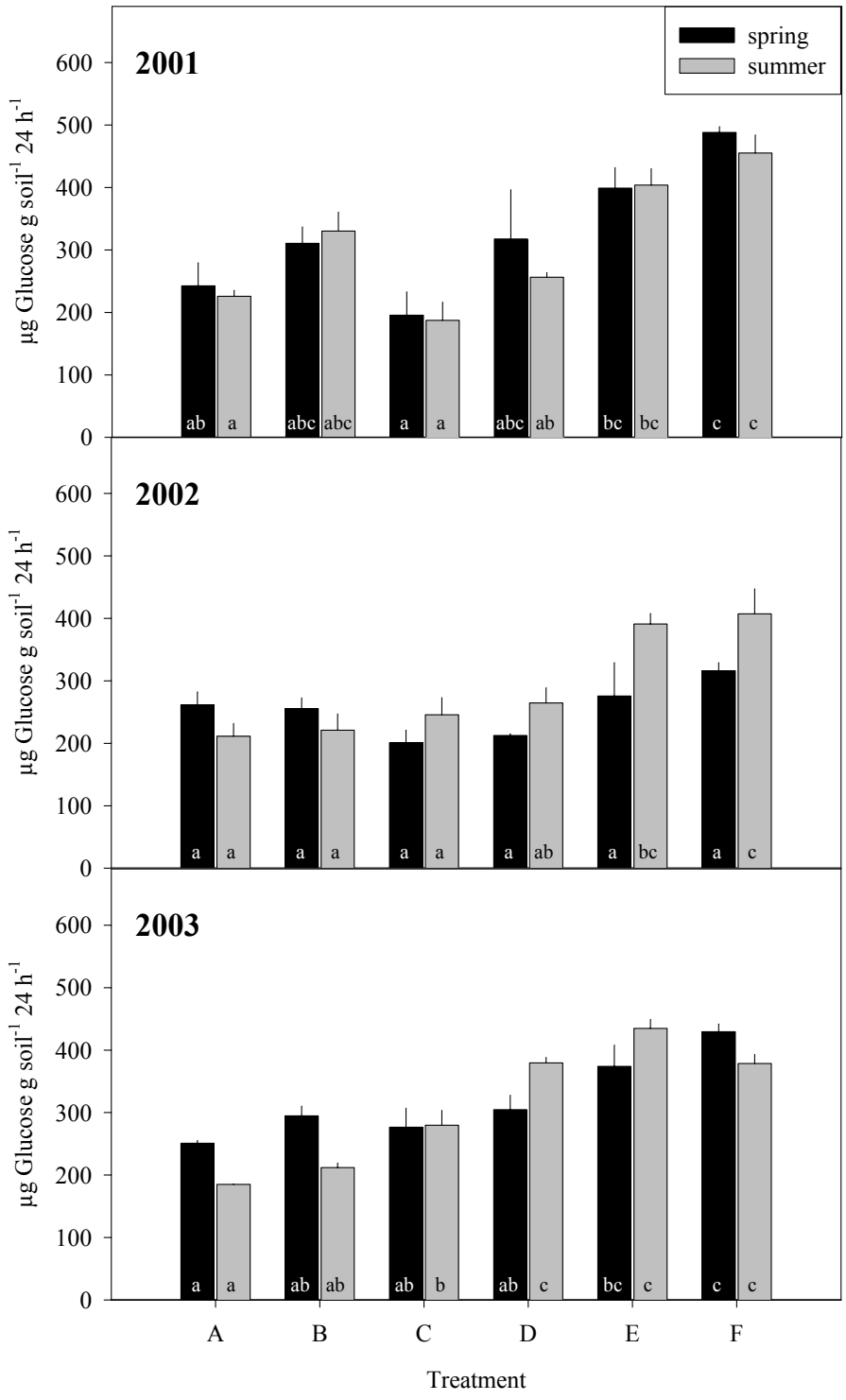

Figure 4: Cellulase activity in a sandy arable soil (0-15 cm) at spring and summer sampling dates (2001-2003), as influenced by different long-term fertilizer regimes. A: "without-N 0", B: "without-N 3", C: "FYM-N 0", D: "FYM-N 3", E: "Straw-/Green-N 0", F: "Straw-/Green-N 3". Abbreviations are explained in Table 2. Error bars represent standard errors. Treatments with the same letter at one date are not significantly different ($p < 0.05$).