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Temporal changes in soil microbial biomass carbon in an arable soil. Consequences for soil sampling

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Abstract

Sugar beet, winter wheat and winter barley were planted within a crop rotation on an arable soil with conventional soil management. Soil samples were taken monthly from different depths of the whole plough layer (0–10, 10–20 and 20–30 cm) during a 56 month period. The samples were analysed for microbial biomass carbon using the substrate-induced respiration technique.

Temporal changes in the amount of microbial biomass carbon were observed. Within a year, microbial biomass-C varied from low values (−15% of total mean) in winter to high values (+15% of total mean) in summer. Relative deviations from the annual means were calculated for each month in the year to demonstrate these fluctuations.

Temporal changes in microbial biomass-C depended on the sources of sample variation (5 years, 3 crops, 3 sampling depths). The highest relative deviation from the annual mean microbial biomass-C was attributable to the factor "year". Less variations were caused by "crops" and "sampling depth".

Soil microbial biomass-C remained constant during frost periods. From the observed temporal changes, recommendations for a suitable date for soil sampling are given, which allows a representative estimation of the mean annual microbial biomass-C content in arable soils.

Introduction

Soil microbial biomass is of crucial importance for long-term fertility of soils. The microbial biomass-C (C_{mic}) represents an important reservoir of nutrients in soil. It acts as an early and sensitive indicator for the suitability of management practices. such as crop rotation (Anderson and Domsch, 1989, 1990; Granatstein et al., 1987; Kaiser and Heinemeyer, 1993), fertilization practices (Insam et al., 1991), tillage (Carter, 1986, 1991; Linn and Doran, 1984), soil compaction (Heisler and Kaiser, 1994; Kaiser et al., 1991) and weed management (Buchanan and King, 1992; Wardle et al., 1993). For the investigation of effects of long-term treatments on soil microorganisms it is necessary to minimize the influence of environmental parameters between the experimental years. Selection of the most appropriate sampling time is needed to solve this problem.

In the past time, courses in microbial biomass, based on 6–11 sampling times throughout one year or vegetation period, were presented. However, the reports are contradictory. In some instances, little or no change in microbial biomass was observed during a growing season (Lynch and Panting, 1980b; Patra et al., 1990; Ritz and Robinson, 1988; Ritz et al., 1992). On the other hand, a decrease (Bremer and van Kessel, 1992) as well as an increase (Lynch and Panting, 1980a, 1982) in C_{mic} were reported.

The purpose of our study was to investigate annual dynamics of soil microbial biomass carbon in an arable soil for 5 consecutive years. From the observed temporal changes throughout each year, we evaluated conditions indicative of a soil sampling date which gives a representative estimation of the mean annual microbial biomass-C content in arable soils.

The data for this study were collected from a field experiment, which was carried out from May 1988 to

November 1992 by an interdisciplinary research group investigation the effects of agricultural traffic on the chemical, physical and biological characteristics of soil.

Materials and methods

Soil and site

In 1988 the field experiment was laid out at Timmerlah near Braunschweig, Lower Saxony, Germany. The soil, a luvisol from loess, (loamy silt, pH 7.1. Kaiser et al., 1992) is frequently used for intensive crop production in this area. In the trial year preceding, the experimental area (4 ha) was limed (5000 kg ha^{-1}) and divided into three sites (Field 1, 2 and 3). During this pre-experimental year fields 1, 2 and 3 were sown with sugar beet, spring wheat and spring wheat respectively. After this preliminary period, the experimental crop rotation started (sugar beet 'SB' – winter wheat 'WW' – winter barley 'WB', Fig. 1). During the first experimental cropping season, winter cereals were substituted by spring wheat, because the start of the experiment in May 1988 was later than the appropriate sowing period of the winter cereals.

Triplicates of 8 compactive treatments were laid out on each field. In accordance with conventional soil management, plant residues (chopped straw) were incorporated by mouldboard ploughing (30 cm tillage depth 'P') after chiseling (10 cm tillage depth 'S') the stubble. Disc drilling was used for sowing.

Sampling and storage

From 4 treatments of each replication of each field, 15 soil cores were taken monthly from the plough layer and sectioned into 0–10, 10–20 and 20–30 cm increments. The bulked samples (800 g) of each increment were brought to the laboratory within one day. In accordance with Anderson and Domsch (1978) the samples were sieved (< 2 mm) and moisture adjusted to a water content in the range of 0.13–0.20 g H_2O g^{-1} dry soil. This is equivalent to 30–46% of the water-holding capacity (sieved soil). In a preliminary experiment, this soil moisture content was found to be optimal for the respiration measurements conducted on the Timmerlah soil. Subsequently, the soil samples were stored in the dark for up to 6 weeks at 4°C in polyethylene bags closed with cotton wool plugs to allow gas exchange.

After a preincubation at 22°C for 3 days, analyses of soil microbial respiration was carried out on 4 replicates (50 g oven-dry soil).

Determination of microbial-C

The substrate-induced respiration method (SIR, Anderson and Domsch, 1978) was applied to estimate soil microbial biomass-C (C_{mic}). The CO_2 production rate was measured hourly with equipment developed by Heinemeyer et al. (1989, MarCo Analytic, Hildesheim, Germany). Soil microbial biomass-C was calculated using the equation C_{mic} [μg C g^{-1} soil] = 30.0 μL CO_2 g^{-1} soil h^{-1}, proposed by Kaiser et al. (1992).

The SIR-method is known to overestimate biomass when used on soils containing recently incorporated residues (e.g. C_{mic}-'peaks' after harvesting the sugar beet or chiseling the stubble, Fig. 1). According to Ocio and Brookes (1990), only the chloroform fumigation extraction (CFE-) method is suitable for microbial biomass-C estimation during the early stages of straw decomposition.

However, it has been shown that these anomalous conditions for the application of the SIR method last for only four weeks (Kaiser and Heinemeyer, 1993).

If these limitations of the method are considered, the SIR-method gives reliable data (Anderson and Domsch, 1978; Kaiser et al., 1992).

Results and discussion

Soil microbial biomass-C

The time courses of soil microbial biomass-C (C_{mic}) during the experimental period from May 1988 to November 1992 are presented in Figure 1. Information about the period under each crop and times for soil tillage is given for each field. The missing values in Figure 1 are caused by restrictions on soil sampling due to soil freezing in winter 1991 and 1992 (Fig. 2).

The microbial biomass-C were normal distributed, therefore ANOVA (analysis of variance) was applied for statistical analysis. All main effects but 'replicates' had a significant influence on microbial biomass-C. The influence of the factor 'treatment' on microbial biomass-C was described by Heisler and Kaiser (1994) and Kaiser et al. (1991).

From the comparison of the sum of squares (Table 1), the highest influence on C_{mic} was attributed to the factor 'month', reflecting seasonal variability. This

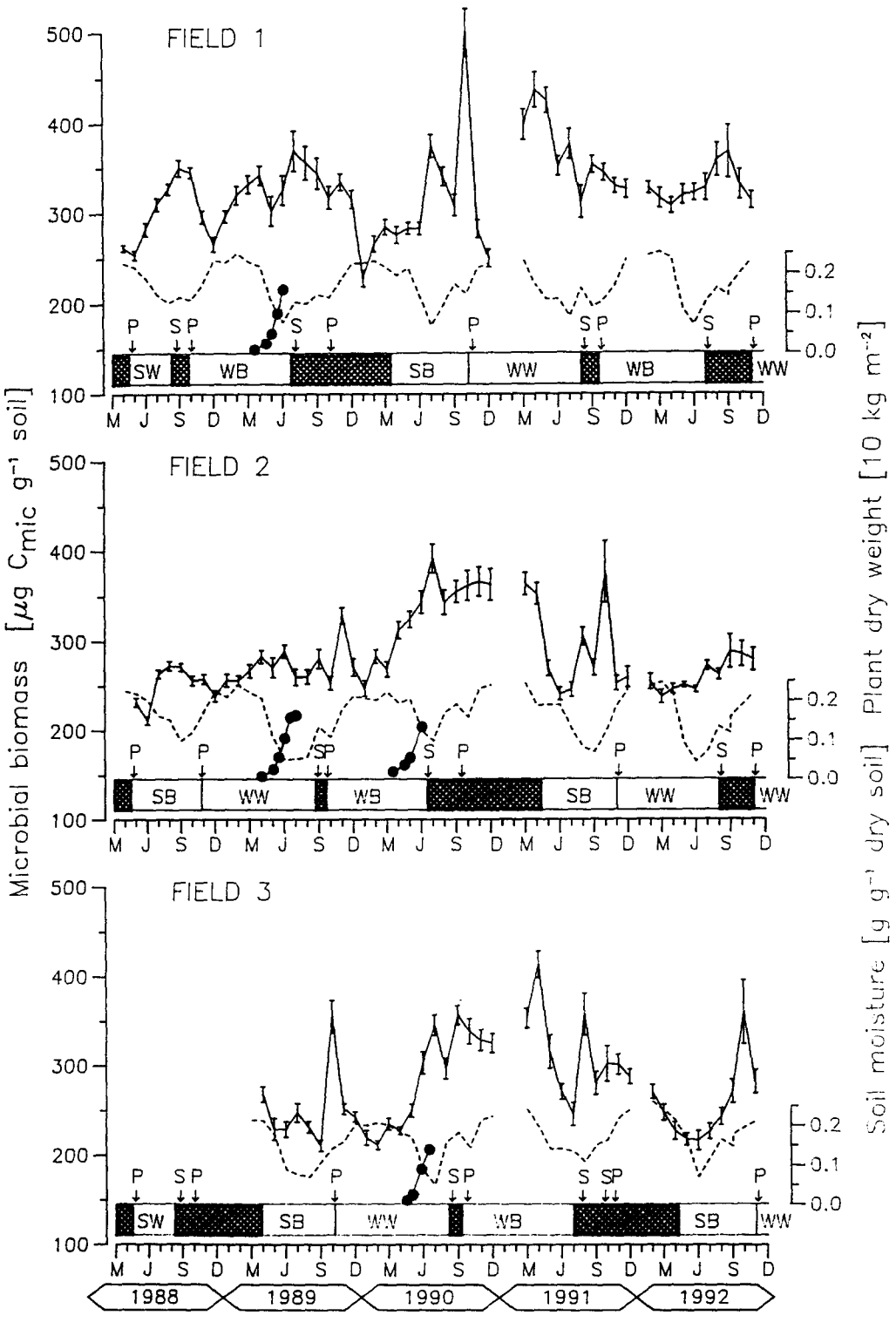

Fig. 1. Microbial biomass-C (n = 144, confidence level $p \leq 5$, solid line), soil moisture (n = 36, dotted line) in the plough layer (0–30 cm) and plant dry weight (points, data kindly supplied by J. Bartels) of Field 1, 2 and 3 (■ = fallow, arrows = time of tillage, S = chiseling the stubble (0–10 cm), P = plouging (0–30 cm), SW = spring wheat, WW = winter wheat, WB = winter barley, SB = sugar beet).

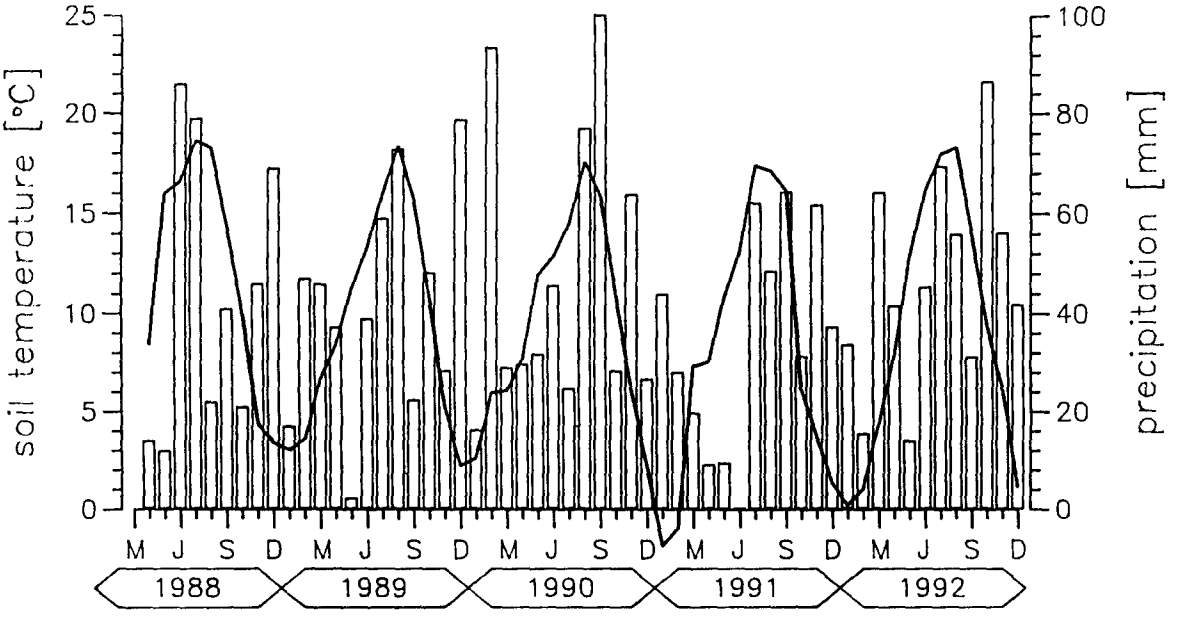

Fig. 2. Precipitation (bars) and soil temperature (curve) during the experimental period.

Table 1. ANOVA on microbial biomass-C (n = 21159)

Source of variation	Sum of squares	d.f.	Mean squares	F-ratio	Sig. level
Main effects					
Month	15428781.9	11	1402616.5	538.28	0.000
Year	1972559.5	4	493139.9	189.25	0.000
Crop	8193903.4	2	4069951.7	1572.27	0.000
Depth	7148839.7	2	3574419.9	1371.74	0.000
Field replication	7727107.4	2	3863553.7	1482.70	0.000
Treatment	3255973.6	3	1085324.5	416.51	0.000
Replicates	5526.0	3	1845.3	0.71	0.579
2-factor interactions					
Month × year	25291243.1	36	702534.5	269.61	0.000
Month × crop	13301616.4	8	604618.9	232.03	0.000
Month × depth	9027324.9	8	410333.0	147.54	0.000
Within cells	23451.9	9	2605.8		

d.f. = degrees of freedom.

temporal changes in microbial biomass-C were influenced by 'year', 'crop' and 'depth', indicated by significant interactions (Table 1). The influence of theses factors decreased in the order year, crop and depth. This was deduced from the sum of squares calculated for the 2-factor interactions (Table 1).

To demonstrate these interactions, relative temporal changes in microbial biomass-C in the plough layer (0–30 cm) could be established (Fig. 3) when the quotients of the annual mean (Table 2, set to be 1) and the corresponding mean of each month were calculated from the biomass-C data obtained over the whole experimental period from all 3 fields (Fig. 1).

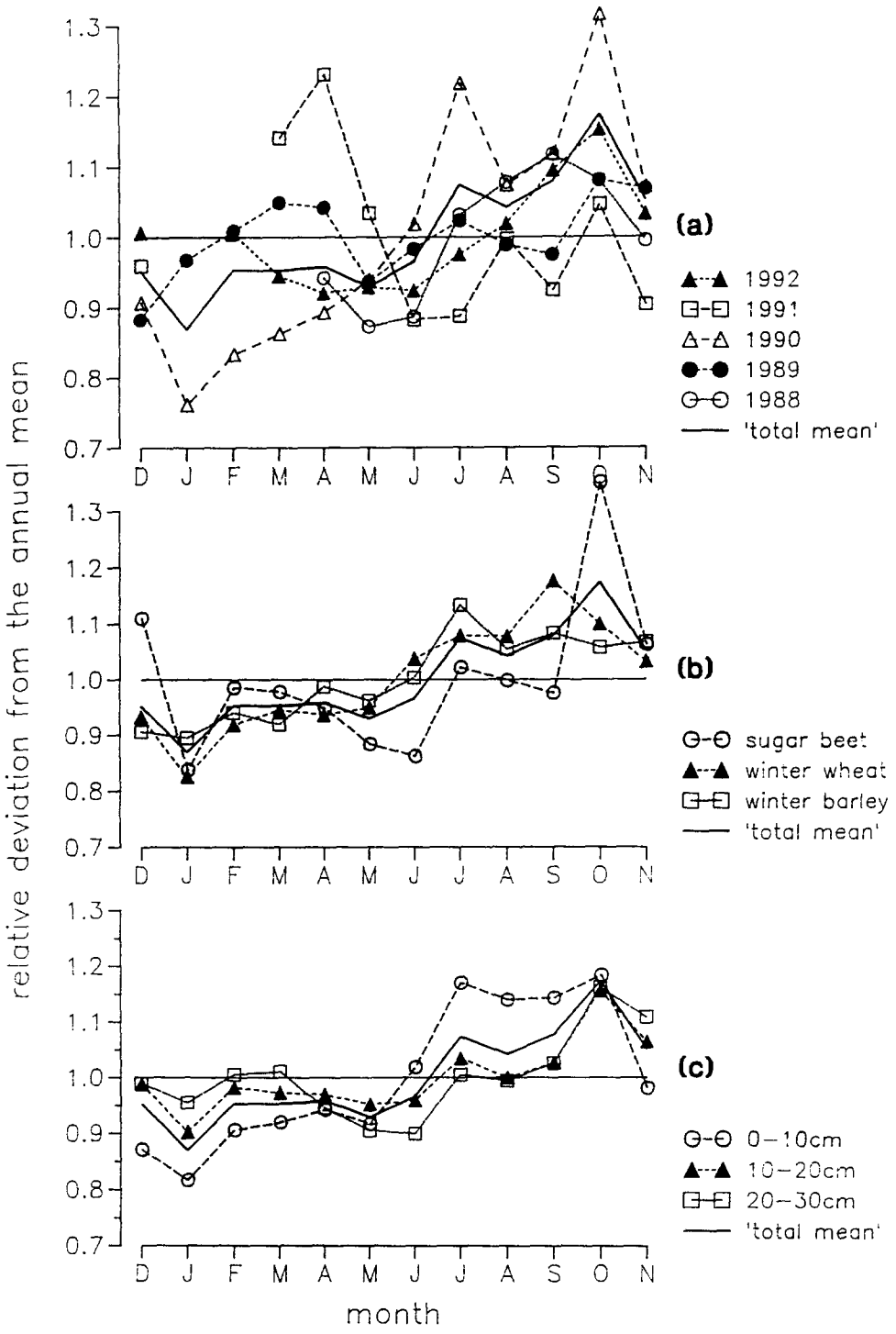

Fig. 3. Relative deviation from the annual mean of soil microbial biomass-C (solid line) as affected by different years, crops and soil depth within the plough layer (no data from 1991 were included in 'total', original data see Fig. 1).

Table 2. Annual means and the coefficients of variation of microbial biomass-C [μg C g^{-1} soil] (no data from 1991 were included in 'total')

Source of variation	n	Microbial biomass-C		Source of variation	n	Microbial biomass-C		Source of variation	n	Microbial biomass-C	
Year				crop				depth			
1988	2304	278[a]	0.24	sugar beet	5613	275[a]	0.37	0–10 cm	5616	302[a]	0.43
1989	4605	285[a]	0.20	winter wheat	6191	287[b]	0.26	10–20 cm	5612	305[a]	0.18
1990	5184	305[b]	0.47	winter barley	5040	319[c]	0.20	20–30 cm	5616	270[b]	0.15
1991	4315	326[c]	0.30								
1992	4751	282[a]	0.18								
total	16844	294		total	16844	294		total	16844	294	

For each column values not marked with the same letter are significantly different ($p \leq 5\%$).

Temporal changes in microbial biomass-C, relative to the annual mean of each year, crop species and sampling depth are shown in Figure 3a, b and c, respectively.

The time period (Dec.–Nov., Fig. 3) reflects approximately the cropping period of winter wheat. Because of the cropping period of spring wheat, only 8 month were available for the first year (1988; Fig. 1, 3a).

Overall, the mean seasonal variation of C_{mic} ranged from −156% (winter) to +15% (summer) of the annual mean ('total', Fig. 3). However, when single growing seasons were investigated, contradictions were observed (Fig. 1). This is in accordance with those described by other authors (e.g. Bremer and van Kessel, 1992; Lynch and Panting, 1980a, b). For example for winter wheat (March–July), changes in spring ranged from a gradual increase in C_{mic} in 1989 and 1992 (Fig. 1b) to a large increase in 1990 (Fig. 1c) contrasting with an increase (March, April) and a decrease (May, June) in 1991 (Fig. 1a).

Differences in the environmental soil conditions (temperature and moisture) and crop growth should be discussed as possible reasons.

Influence of soil temperature

Changes in soil temperature during the experimental period are presented in Figure 2. The mean annual soil temperatures ranged from 8.0 to 9.5°C. Soil temperatures in summer and autumn were similar each year; however, yearly differences were found in winter and spring. In spring, as described above, remarkable C_{mic} variations were found between the years.

During the time of the experiment, frost periods occurred only in 1991 and 1992. In 1991, very extreme conditions were observed. After two months of permanent frost at the end of February, daily freezing and thawing cycles were observed, which were followed by a very warm period in March (+8°C increase, Fig. 2). In 1991, in contrast to all other years, the highest C_{mic} values were estimated in spring (March and April) immediately after the long frost period under winter wheat (Fig. 1a). This was less pronounced under winter barley (Fig. 1c). No increase in C_{mic} was detectable at the same time on bare soil (Fig. 1b). In 1992, no C_{mic} increase was observed after thawing on any fields (Fig. 1).

Scientific opinion varies on the impact of freezing on soil microorganisms. An increase in microbial activity was reported after freezing and thawing (Anderson and Domsch, 1994; Zelles et al., 1991). On the other hand, Ritz and Wheatly (1989) described freezing as a method to preserve samples for respiratory studies.

A conservation of C_{mic} was found throughout the frost period from December 1989 to March 1990 for sugar beet and throughout the frost period from December 1991 to February 1992 for cereals (Fig. 1). Therefore the higher values under winter cereals in 1991 may be caused by intense plant growth, induced by the rise in temperature (Fig. 2). Because of these extreme differences, the data from 1991 were not included in the calculation of the 'total' mean (Fig. 3).

The winter 1988/89 was mild (Fig. 2), therefore plant growth (winter cereals) started early in the year, accompanied by higher C_{mic}-values compared to all other years (Fig. 3).

Influence of soil moisture

Soil microbial biomass-C decreases with increased moisture stress and increases strongly after rewetting (Hassink et al., 1991). In our experiment, no obvious relation was found between soil moisture and the development of microbial biomass-C, despite large seasonal variations in total soil moisture content (Fig. 1).

In accordance with Bottner (1985), an influence of soil moisture was observed only under very dry conditions. For instance, in May 1989 soil moisture dropped to 0.05 g H_2O g^{-1} dry soil (pF < 4.2, Fig. 1a) after a period of low precipitation (Fig. 2), and C_{mic} decreased simultaneously. The parallel course of microbial biomass-C and cereal plant dry weight was interrupted at this time (Fig. 1a), which may be attributed to a high susceptibility of actively growing microbial populations to desiccation (van Gestel et al., 1993).

Influence of crops

Except under the dry conditions described above, soil microbial biomass-C increased parallel to crop growth (Fig. 1). This observation was confirmed by other authors (Lynch and Panting, 1980a, 1982; Wildung et al., 1975). Due to the late sowing of sugar beet in April, an increase in C_{mic} was first observed after June, when the soil was completely covered with the leaves of the sugar beets. In accordance with Lynch and Panting (1980a, 1982), we concluded that microbial biomass-C content is primarily related to the availability of substrates derived from plant roots. This conclusion is supported by temporal changes in C_{mic} observed for crops with contrasting growing seasons. The high value for sugar beet in December (+ 10%, Fig. 3b) reflects the influence of the previous crop (winter barley, Table 2). Within the crop rotation, C_{mic} increased in the order sugar beet, winter wheat, winter barley (Table 2).

Temporal changes within the plough layer

Means of microbial biomass-C contents were presented in Fig. 3c from 3 depths (0–10, 10–20 and 20–30 cm) of the plough layer. As reported earlier by Kaiser and Heinemeyer (1993), significant differences in C_{mic} were found between the 3 investigated depth levels of the plough layer due to tillage and crop management. Highest seasonal changes were found within the surface layer (0–10 cm), which has been the primary subject of current investigations of arable soils (e.g. Anderson and Domsch, 1989, 1990; Witter et al., 1993). Within the plough layer, a decrease in seasonal fluctuation of C_{mic} was found with sampling depth (Fig. 3c). Significant differences were found between 0–20 cm and 20–30 cm ($p \leq 5\%$, Table 2). These differences were equalized out after ploughing, which resulted in a homogeneous depth distribution of C_{mic} until the beginning of the growing season in spring (Kaiser and Heinemeyer, 1993).

Representative soil sampling

Exploiting observations of the influence of crop growth, soil moisture and temperature reported here, recommendations on how soils may be sampled once a year to determine the mean annual soil microbial biomass-C content are given:

The period between the harvest of winter barley (July) and sugar beet (October) is not suitable because of very high values resulting from the proliferation of the microorganisms induced by the release of high amounts of available carbon.

Sampling in winter is also not suitable, because of possible restrictions due to soil freezing. Without freezing extreme low values were found (1989 and 1990, Fig. 3a).

The experimental period May to June was conditionally suitable, because of remarkable differences due to plant growth of winter cereals and sugar beet (−10%, Fig. 3b). The differences might be higher without the restrictions imposed by desiccation. Lynch and Panting (1982) found maximum C_{mic} concentrations in soil, because of maximum root growth of winter wheat at that time (May, June).

A small influence of the different crops on soil microbial biomass was detectable in spring (February, March, April, Fig. 3b). Therefore sampling in early spring, before the start of crop and soil management, seemed to be appropriate for the comparison of arable soils (Anderson and Domsch, 1989). On the other hand, the variations between the years were highest at that time (Fig. 3a).

For representative soil sampling, we propose a period in autumn four weeks after incorporation of plant residues. At this time, the influence of plant growth was least and incorporation of plant residues had no measurable influence. Additionally only a small variation between the years was detected (Fig. 3a). The latter was attributed to optimal soil moisture in the field (0.16–0.19 g H_2O g^{-1} dry soil) and a small influence of soil temperature. Temperature effects at this

time are minimal, because every year it was near 4°C (usual temperature of soil storage after sampling).

The proposed recommendations for representative sampling in arable soil are only valid for investigations of the whole plough layer.

The presented results from the entire investigation period (56 months) at Timmerlah confirm former conclusions based on the first 32 months of investigation (Kaiser and Heinemeyer, 1993).

Although our observations concern only one type of soil and one specific crop rotation, the observed sources of variations from the annual mean microbial biomass-C should be taken into consideration for representative soil sampling or for the comparison of results.

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