

Microbial community structure and activity in agricultural soils under different management

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Summary - Zusammenfassung

For the development of management strategies in sustainable agriculture it is necessary to describe and predict the role of soil microbes in different management systems. The classical approach uses the microbial biomass as the key parameter for the entire system, but for ecological purposes the variability of biotic parameters in time and space has to be better described. Moreover, the biomass active in the total soil profile or its most active zones should be used as a basis for the assessment of soil activity. The sum of adenylates was found to be more closely related to the microbial biomass than was ATP, which however appeared to be a better indicator for the microbial activity. Fatty acids from phospholipids were highly correlated with the soil microbial biomass. The pattern of fatty acids from soils under different long-term management indicated a high potential to typify the microbial community in soils and special organism populations. To overcome the problem, that only a small portion of the soil inhabiting microbes can be cultivated, first steps to use serological and genetical methods to directly identify or localize specific populations in the rhizosphere are shown.

Mikrobielle Populationsstruktur und Aktivität in landwirtschaftlich genutzten Böden mit unterschiedlicher Bewirtschaftung

Um Strategien zur Bodenbewirtschaftung in der nachhaltigen Landwirtschaft entwickeln zu können, muß die Bedeutung der Bodenmikroorganismen in diesen Prozessen besser bekannt und prognostizierbar sein. Der klassische Ansatz benutzt die "mikrobielle Biomasse" als Schlüsselparameter für das Gesamtsystem Boden, doch für das Verständnis von ökologischen Zusammenhängen sollte die Variabilität der biotischen Parameter in Ort und Zeit besser bekannt sein. Es ist wichtig, die Biomasse im gesamten Bodenprofil oder der Zone mit der höchsten Aktivität für die Beurteilung der Bodenaktivität heranzuziehen. Es wurde gefunden, daß der Gesamtadenylatgehalt enger mit der "mikrobiellen Biomasse" korreliert als der ATP-Gehalt, welcher jedoch ein besserer Indikator für die aktuelle mikrobielle Aktivität ist. Der Fettsäuregehalt der Phospholipidfraktion von Bodenlipidextrakten war sehr hoch mit der "mikrobiellen Biomasse" des Bodens korreliert. Die klar unterschiedlichen Spektren der Phospholipidfettsäuren aus Böden, welche über lange Zeit unterschiedlich bewirtschaftet wurden, lassen ein hohes Potential für eine Typisierung von mikrobiellen Gemeinschaften in Böden sowie von spezifischen Organismengemeinschaften erkennen. Durch die systematische Anwendung von serologischen und molekulargenetischen Methoden zur direkten Identifizierung und Lokalisierung von mikrobiellen Populationen scheint das Problem der nur partiellen Kultivierbarkeit von Bodenmikroorganismen lösbar zu sein. Erste Ansätze zu einer *In situ*-Charakterisierung von spezifischen Populationen in der Rhizosphäre werden vorgestellt.

Introduction

Proper soil management needs synchronization of the decomposition and the uptake of nutrients mineralized by plants, to avoid leaching losses and to reduce harmful trace gas emissions like N₂O or CH₄. According to Swift et al. (1979), decomposition is governed by three factors: the physical and chemical environment, the quality of the organic matter (substrate) and the community of the decomposers present in the soil. Not much work has been done to relate the functions of soil microorganisms to parameters characterizing their community structure. The reason for

this lack of information can be seen in the fact that the population structure parameters needed are not easily obtained or are not sensitive enough to be purposely used.

Figure 1 shows the different approaches pursued by us, to characterize the structure and function of the microbial community or populations in soils, using parameters at different levels of aggregation of the biosystems. In a first step the characterization of the microbial community as a whole will be regarded. Studies at this level are mainly based on the determination of the microbial biomass or are linked to the biomass. There are several methods available for the quantitative estimation of the microbial biomass of soils as

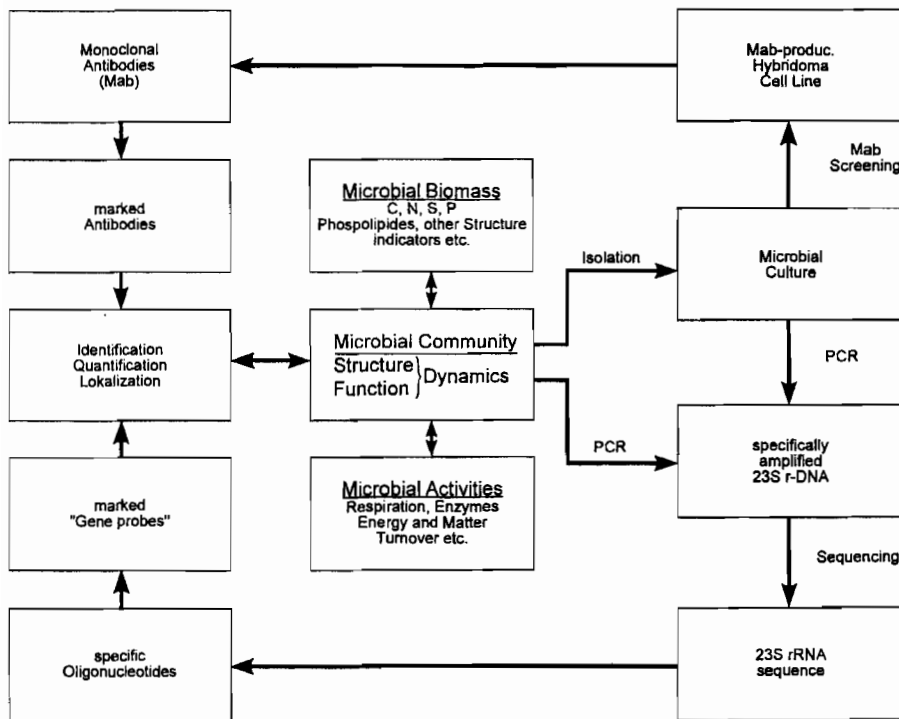


Figure 1: Overview of different approaches to study microbial community structure and activity in agricultural soils

Abbildung 1: Übersicht verschiedener Ansätze zur Untersuchung mikrobieller Populationsstrukturen und Aktivitäten in landwirtschaftlichen Böden

was summarized by Sparling (1985) and Jenkinson (1988). The determination of soil microbial biomass often is combined with a characterization of the physiological status of the microorganism community. Among others the relation to respiration was studied by Anderson and Domsch (1990). The relation to the energetic state was investigated by Brookes et al. (1987), Bai et al. (1989) and others and the relation to soil nutrients was studied among others by Jenkinson (1988) and Ocio et al. (1991).

The biomass parameters, however, cannot be used to describe changes in the microbial communities, in the diversity of the community or in the activities of single species and physiological groups. In these cases specific biochemical constituents, 'signature chemicals' which are restricted to certain species or groups, can be used. Fatty acids from phospholipids e.g. have been found as specific biomarkers for some microorganisms (Ratledge and Wilkinson, 1988; Tunlid and White, 1992). This approach was improved by Zelles et al. (1992) and is now used to determine patterns of phospholipid derived fatty acids in soils. In a further step, serological and genetical methods are used to identify, quantify and localize specific microorganisms in soils.

In the serological approach polyclonal as well as monoclonal antibodies are used to investigate population dynamics of microbes in soil. The application of immunofluorescence labelling with polyclonal antisera started already two decades ago (Bohlool and Schmidt, 1980). As compared to polyclonal antisera, monoclonal antibodies offer the lowest level of cross-reaction to non-target organisms, which is very important in complex systems. After labelling the antibodies either directly or in a second antibody reaction with

fluorescent dyes, enzymes or gold particles a direct identification, localization and quantification *in situ* is possible (Figure 1).

The molecular biological approach uses genes specific for a particular group of microbes or phylogenetic relevant stretches of the ribosomal RNA. The 16S and 23S rRNA offers highly specific sequences at the class, genus and species level (Manz et al., 1992) with rRNA targeted, fluorescently labelled oligonucleotide gene probes. A direct phylogenetic staining of bacteria is possible, since the rRNA is present in high amounts in physiologically active cells (De Long et al., 1989) (Figure 1).

Based on these different approaches to describe the structural and functional status of the organism community in soils, the chance to investigate the microorganisms as dynamic regulators in the decomposition of organic matter and related processes in soils could be improved.

2 Material and Methods

2.1 Soil Samples

Soil samples were taken from plough layers of soils at two locations in the surroundings of Munich in southern Germany. The first area (Tertiary hill slopes north of Munich, location "Scheyern") consists of sandy loam soils derived from loess and molasse sediments and of a silt-loam soil in the valley bottoms. The sandy loam soils were many years under the normal rotation used in Bavaria, including winter wheat, winter barley and rape seed. Besides texture soils differ in pH, total C- and N-contents and C/N ratio. The silt loam soils which cover the valley bottoms and the slopes show low C/N ratios; this is generally combined with deeper plough layers. Table 1 gives an overview of the site characteristics.

Table 1: Site characteristics at "Scheyern"**Tabelle 1:** Charakteristika der Versuchsflächen in "Scheyern"

Site	B1	B2	B3	R4	W5	W6	W7
Crop	W-Barley	W-Barley	W-Barley	Rape Seed	W-Wheat	W-Wheat	W-Wheat
Texture	silt loam	silt loam	sandy loam	sandy loam	sandy loam	silt loam	silt loam
Bulk density ¹⁾ (g · cm ⁻¹)	1.49 ± 0.02	1.48 ± 0.06	1.60 ± 0.09	1.57 ± 0.09	1.51 ± 0.12	1.38 ± 0.11	1.28 ± 0.10
Depth of plough layer (cm)	30	21	21	22	22	23	27
Mass of plough layer (Mg ha ⁻¹)	4500	3150	2856	2992	2805	3174	3456
total C (%) ¹⁾	1.10 ± 0.09	1.33 ± 0.09	1.38 ± 0.18	1.39 ± 0.13	1.51 ± 0.13	1.49 ± 0.12	1.40 ± 0.11
total N (%) ¹⁾	0.125 ± 0.009	0.146 ± 0.008	0.147 ± 0.017	0.147 ± 0.012	0.161 ± 0.013	0.164 ± 0.009	0.157 ± 0.010
C/N	8.8	9.1	9.4	9.5	9.4	9.1	8.9
pH ¹⁾	6.2 ± 0.1	6.0 ± 0.1	6.2 ± 0.2	6.2 ± 0.2	6.5 ± 0.2	6.6 ± 0.1	6.5 ± 0.2

¹⁾ Values are means with standard deviation (n = 16)

¹⁾ Mittelwerte und Standardabweichung (n = 16)

Soil samples were taken at seven sites at the end of August, four to six weeks after harvest. Directly after harvest the soil was chiselled to incorporate the straw. An area of 1 hectare per soil unit, was subdivided into 16 subplots. In the middle of each subplot three samples, each 10 cm apart, were taken with an auger 3 cm in diameter, down to the bottom of the plough pan and then combined to one sample and homogenized. From each of the seven 1 hectare sites, 16 samples were taken and analyzed separately. In the middle of each site nine samples were taken within a microplot of 1 m² and treated in the same way as described before.

The second soil at location "Puch" is a luvisol with silt loam soils, derived from loess over Riss morain. The plots of the silt loam soils at "Puch" did not differ in texture (20 % clay, 63 % silt and 17 % sand). The tested 10 plots, however, showed large differences in the total C- and N-content because of an extreme different continuous management over 38 years including bare fallow, monoculture of potatoes and cereals, rotation systems and grassland (Table 2).

The soil samples from "Puch" were taken in early spring before the application of mineral fertilizers. To get representative soil samples from each plot, samples were taken with an auger (2.5 cm i.d.) from the surface horizon (0-10 cm) and at least 50 single soil samples were mixed.

2.2 Methods

For the investigation of basic microbial properties in the soils of the location "Scheyern", the following parameters were determined: C and N by dry combustion; microbial biomass by substrate induced respiration (SIR) (Anderson and Domsch, 1978); adenylate-content and adenylate energy charge (Bai et al., 1989); basal respiration as oxygen-consumption and calculation of the metabolic quotient (Anderson and Domsch, 1990).

With the soil samples from the experimental field at "Puch", the influence of long-term management history on microbial properties was tested. The following parameters were determined: microbial biomass (Anderson and Domsch, 1978); enzymatic activities (Schinner et al., 1993); lipid phosphate (Carter and Karl, 1982).

Microbial biomass was determined by the SIR-method and obtained with an automated IR-CO₂ gas analysis equipment (Heinemeyer et al.,

Table 2: Vegetation, agricultural management and chemical characteristics in eight selected soils from the long-term management experimental field at "Puch"**Tabelle 2:** Charakteristika der Vegetation, landwirtschaftlichen Bewirtschaftung und chemischen Parameter der acht Böden des Langzeitversuchs mit unterschiedlicher Bewirtschaftung in "Puch"

Soil	cropping history	manure ¹⁾ or straw residue	organic-C (%)	pH
1	bare fallow (no vegetation)	-	0.66	5.0
2	green fallow (natural vegetation)	-	0.95	5.7
3	potatoes (monoculture)	-	0.89	5.9
4	potatoes (monoculture)	9 x 10 ³	0.99	6.2
5	w. wheat-s. wheat (monoculture)	straw residues	1.19	6.2
6	w. wheat-s. wheat (monoculture)	straw residues vetch after winter wheat	1.14	6.2
7	w. wheat, crop-rotation (1 x red clover each 6th year)	15 x 10 ³	1.26	6.3
8	grassland	-	1.76	5.3

¹⁾ kg ha⁻¹ yr⁻¹

Soils 3-7 were fertilized with mineral fertilizer (normal practice levels)

1989). The incubation temperature was 22 °C. Soil enzyme activity was tested for cell bound reductases, catalase, DMSO-reductase, amino acid desaminase as well as for cell free hydrolases, β-glucosidase and protease as described by Beck (1984).

An improved method for the extraction of lipids and the separation of fatty acids was described by Zelles and Bai (1993). Lipids were subdivided

into neutral, glyco- and phospholipids. In further treatments four different fractions were obtained: a) saturated, b) monounsaturated, c) polyunsaturated and d) hydroxy fatty acids. The quantitative measurement of the fatty acids of each fraction was achieved by GC-MS as described by Zelles and Bai (1993). Besides the determination of the fatty acids of phospholipids the fatty acids of the lipopolysaccharides were extracted and measured by GC-MS.

The quantification of specific bacteria in the rhizosphere with monoclonal antibodies was performed using a sensitive chemoluminescence-ELISA according to Schloter et al. (1992). For a direct *in situ*-identification and localization confocal scanning laser microscopy was used (Schloter et al., 1993). The labelling of bacteria with oligonucleotide gene probes followed the procedure of Amann et al. (1990). Specific staining was performed using a fluorescein labelled eubacterial oligonucleotide probe. A wheat seedling was grown in sandy soil in a little flow cell (7.5 x 2.5 x 0.5 cm) which allows *in situ*-fixation and hybridisation treatments. After covering with a thin glass slide, the root underneath was directly studied with the BioRad MRC600 confocal scanning laser microscope.

Statistical significance was calculated with the least significant difference test (LSD-test) at the 95 % confidence level.

3 Results and Discussion

3.1 Variability of basic microbial parameters of soils under normal rotation at Scheyern

Total carbon, total nitrogen and pH (Table 1) differed significantly between the first four sites (B1-B3, R4) and the last three sites (W5-W7). These differences are not influenced by texture or the last crop in the rotation. The C/N ratios of the sandy loams were significantly ($P = 95\%$) different from the silt loams. In characterizing soil properties, one not only has to look at the concentrations of elements or compounds but also at the mass within comparable "reaction vessels" like the plough layer of arable land. If one includes the bulk densities, the depth of the plough layer and the volume of stones, the picture may change drastically. Figure 2 shows the amounts of total C and total N stored in the plough layer of one hectare at the different

sites. Clear differences can be seen between the two textures, with lower total C and total N reservoirs in the sandy loam. This example demonstrates that if one only looks at the concentrations, important ecological results may be masked. The coefficients of variation (CV %), for pH, total C and total N were 3 %, 9 % and 8 % respectively, when the 1 hectare plots were regarded. The values for the 1 m² plots were lower (1 %, 4 % and 3 % resp.) indicating that the soils are well homogenized with respect to total C and N, and well buffered.

For the biological parameters, the sandy loams exhibit the highest concentrations of microbial biomass, whereas the silt loams in the valley bottoms with deep plough layer had significant ($P = 95\%$) lower values (Figure 3). In all cases the variability is much higher than for total C and total N, indicating that the microbial biomass is linked to coarse and fresh organic substrates, unevenly distributed in the soils by management. This assumption is supported by the finding, that in average the CV % was basically the same at a 1 hectare or a 1 m² plot (32 % and 34 % respectively). From the data obtained from the m² plots, however, it was not possible to predict the mean values of the hectare plots with a probability of 95 %. In Figure 4 the amounts of microbial biomass-C in the plough layers of 1 hectare are shown. With two exceptions (Sites B1 and W7) the total amounts were the same at a 95% probability range. Assuming that the annual input of plant material is the same at all sites, one would expect comparable microbial respiration activities. Based on a gram of soil, no significant differences were found between the basal respiration of the different sites (data not shown). However, if the respiration activity is related to the microbial biomass - this is the metabolic quotient - a reverse pattern compared to the microbial biomass is obtained (Figure 5). Santruckova and Straskraba (1991) also found a reverse relation between the microbial biomass and the metabolic quotient for a large number of soils. But without taking into account the total amount of microbial biomass, the results may be misleading from an

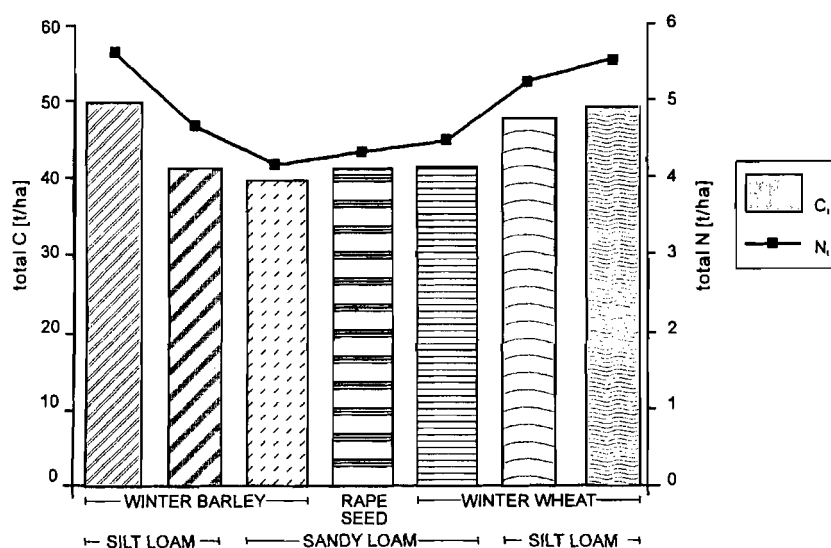


Figure 2: Amounts of total C and total N in the plough layer of different sites on a hectare basis
Abbildung 2: C_t und N_t-Gehalt der Pflughorizonte verschiedener Versuchsflächen auf ha-Basis

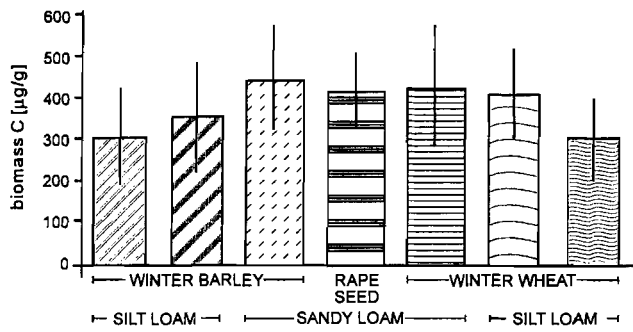


Figure 3: Microbial biomass carbon at the seven 1 hectare sites studied. Mean values and standard deviations, n = 16

Abbildung 3: Mikrobieller Biomassekohlenstoff von sieben Hektarflächen. Mittelwerte und Standardabweichungen, n = 16

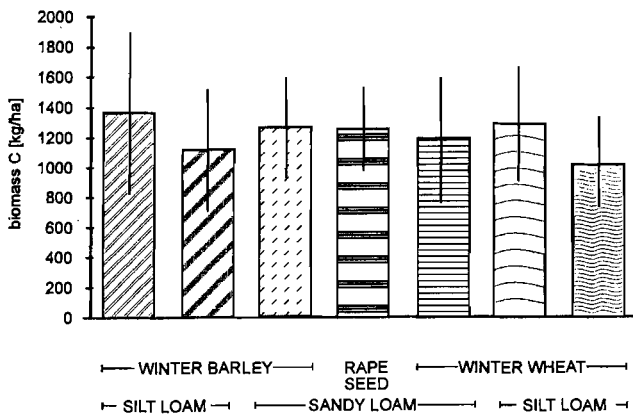


Figure 4: Amounts of microbial biomass C in the plough layers of different sites on a hectare basis

Abbildung 4: Mikrobieller Biomassekohlenstoff in den Pflughorizonten auf verschiedenen Flächen auf ha-Basis

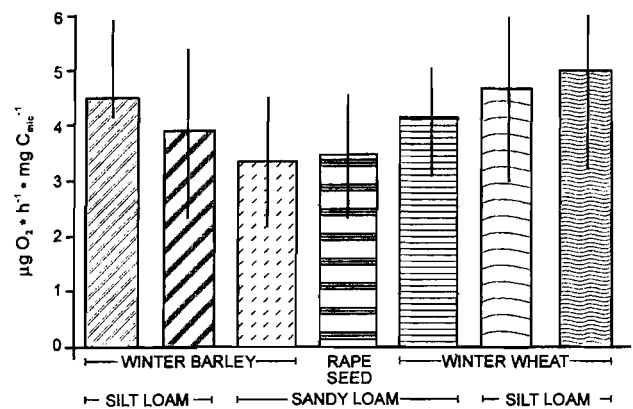


Figure 5: Metabolic quotient at the seven 1 hectare sites studied. Mean values and standard deviations, n = 16

Abbildung 5: Metabolischer Quotient von sieben 1 ha Flächen. Mittelwerte und Standardabweichungen, n = 16

ecological point of view. The results also show that different activities of the microorganism communities occur and that these differences may be due to a different physiological status of similar communities or different structures of the communities.

Like other biotic parameters, the concentrations of adenylates are significantly ($P \geq 95\%$) higher in the sandy loam soils on top of the hills and lower in the valley bottom soils. An analysis of variance did not show significant differences with respect to the preceding crop. The ATP fraction was 63 to 70 % of the sum of adenylates. The differences in ATP of the barley plots ($< 1.5 \mu\text{g ATP g}^{-1}$ soil) were however significantly lower as compared to rape seed and wheat plots ($> 1.7 \mu\text{g ATP g}^{-1}$ soil), which were harvested later. The energy charge ($EC = [ATP] + 0.5 \cdot [ADP] / [AMP] + [ADP] + [ATP]$) of the microorganisms were found to be in a very narrow range of 0.75 to 0.79. This was in the same range as described by *Brookes et al. (1987)* for different soils. The small differences between the barley plots ($EC < 0.76$) and the rape seed and wheat plots ($EC > 0.77$) were significant ($P = 95\%$). As mentioned above, this might be caused by the time since the decomposition started and may have resulted in a decrease of ATP formation at a constant level of total adenylates. Using soils with a wide range of microbial biomass, the contents of ATP and total adenylates were examined. As shown in Figure 6 and Figure 7 the correlation between the adenylates to the microbial biomass is closer compared with the correlation to ATP. This shows that ATP reacts much quicker on changes of the environment than the adenylates as a whole. ATP is a more sensitive indicator for the physiological status of the microorganisms, whereas the total adenylates are better indicators for the microbial biomass.

The coefficients of variation for ATP and adenylates at the 1 hectare sites were 15 % and 13 % respectively. Table 3 shows the numbers of samples needed for the assessment of the different soil parameters, if the confidence interval is 10 % and 5 %, respectively. For soil microbial parameters and the 10 % interval 10 to 30 samples have to be taken within a homogeneous mapping unit of 1 hectare size. For total C and total N less than five were sufficient. To reduce the confidence interval to 5 %, a tremendous increase in sampling is needed. This has to be taken into account when small differences in the microbial biomass or in the physiological status of soil organisms have to be interpreted.

3.2 Influence of different long-term management on fatty acids and microbial biomass parameters (experimental field at "Puch")

The biomass contents of soils with different management conditions follow generally the organic C contents of the respective soils (Table 4). Scale straddling of the biomass contents and the activity of enzymes is, however, higher than that of the humus contents (Table 2). This observation is in agreement with the results of *Powlson et al. (1987)*, that measurements of soil microbial biomass provide an early indication of changes in total organic matter due to the management practice. In the plots with different management not only microbial biomass differed considerably, there seemed to be an alteration in the quality of biomass.

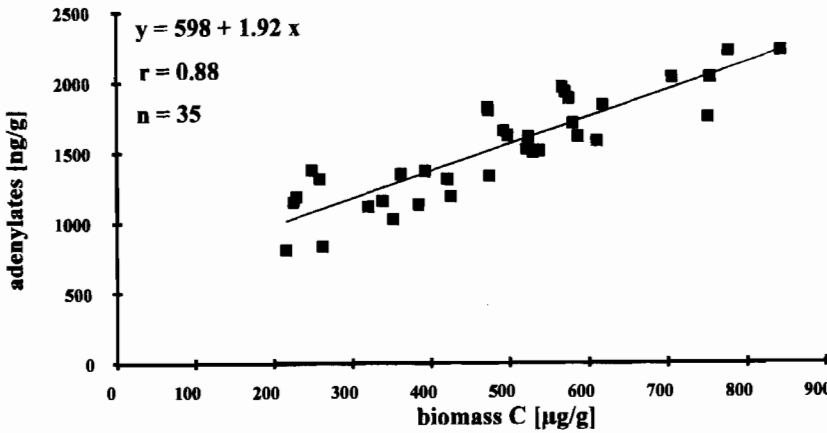


Figure 6: Relation between the microbial biomass carbon and the sum of adenylates AMP + ADP + ATP

Abbildung 6: Beziehung zwischen mikrobiellem Biomassekohlenstoff und der Summe der Adenylate AMP + ADP + ATP

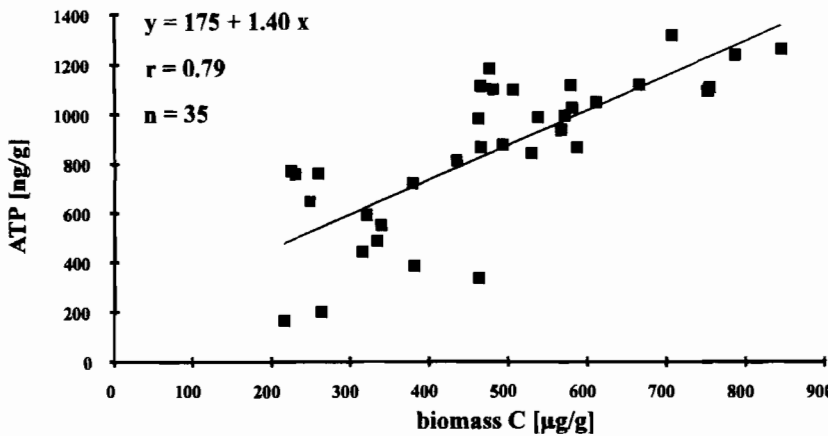


Figure 7: Relation between the microbial biomass carbon and ATP

Abbildung 7: Beziehung zwischen mikrobiellem Biomasse-C und ATP

Table 3: Number of samples necessary for confidence intervals of 10 % and 5 %

Tabelle 3: Anzahl der Proben, die zum Erreichen der Vertrauensintervalle von 10 % bzw. 5 % notwendig wäre

	CV %	n for f = 0.1	n for f = 0.05
biomass C	32	27	111
ATP	15	6	24
total C	9	2	9
total N	8	2	7

This was also indicated in incubation experiments testing mineralization and assimilation of ¹⁴C from uniformly ¹⁴C-labelled wheat straw (Haider et al., 1991). While 15-23 % of the metabolized ¹⁴C was assimilated by the biomass of the grassland or crop rotation plots, it amounted to only 7-8 % for monocultures or the bare fallow.

Best correlations were found between microbial biomass and the activities of cellular reductases, like catalase and DMSO-reductase (Table 4). The reason for the less stringent correlation of microbial biomass to dehydrogenase measurements could be the influence of the different soil pH-values. The correlation of extracellular hydrolases, like β-glucosidase and protease, to microbial biomass shows an even lower relationship, because these enzymes accumulate in soils when specific substrates had been applied.

The data in Table 4 demonstrate that the sum of the fatty acids (FAs) of the phospholipid fraction is closely related to the soil microbial biomass carbon and to other enzymatic parameters which can be used as general indicators of the microbial community. F-tests indicated that all correlation coefficients with the exception of lipid phosphate were significant (P = 99 %). This is an essential prerequisite for taking the pattern of the phospholipid fatty acids to characterize the microbial community.

The soils studied also show distinct differences in the contents of the cumulated FAs. In general the number of various fatty acids was positively related to the amount of fatty acids. With respect to the total number about 67 % to 78 % of the fatty acids were found in the PL-fraction. These data already show, that a different management of the same soil type is changing the pattern of the phospholipid fatty acids and therefore also the composition of the microbial community.

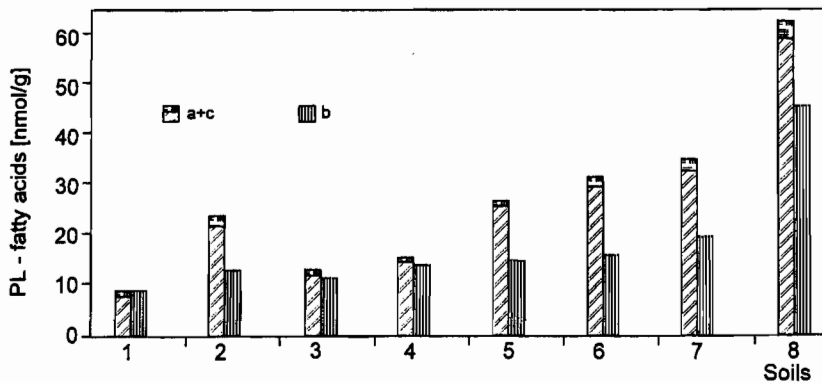
In a further step the PLFAs were subdivided in their monoenoic (a), saturated and polyenoic (b) and hydroxy (c) fractions. The results are shown in Figure 8. In most cases the fraction (a) exhibited the highest amounts followed by fraction (b) and fraction (c). As expected the lowest values were found in the bare fallow soil and the highest in the grassland soil. The fractions as shown in Table 5 are site specific and can be used to typify the organism community with regard to their phospholipid FAs. This approach can

Table 4: Comparison and correlation coefficients (r) between phospholipid fatty acids (nM g⁻¹), microbial biomass C, lipid phosphate and several enzymatic parameters of soils under different long-term agricultural management (see Table 2, field experiment "Puch")**Tabelle 4:** Vergleich und Korrelationskoeffizienten (r) zwischen Phospholipidfettsäuren (nM g⁻¹), mikrobieller Biomasse C, Lipidphosphat und einigen enzymatischen Parametern in Böden unter verschiedenen Langzeit-Bewirtschaftungsmaßnahmen (siehe Tabelle 2, Feldexperiment "Puch")

	1	2	3	4	5	6	7	8	r ⁺	r ²
PL-FAs (nM g ⁻¹)	16.88	35.68	23.87	29.06	41.54	47.61	55.43	108.04		
Lipid phosphate (g g ⁻¹)	0.039	0.046	0.044	0.043	0.040	0.050	0.043	0.049	0.4156	0.1727
Microbial biomass C (g g ⁻¹)	135	399	281	326	449	438	514	1298	0.9545	0.9111
Organic C (%)	0.66	0.95	0.89	0.99	1.19	1.14	1.26	1.76	0.9400	0.8836
β-Glucosidase (g saligenine)	26	58	61.6	69.2	78.6	81.8	105.6	198	0.9158	0.8387
Protease (g tyrosine)	40	182	134	132	325	303	265	720	0.9400	0.8836
AA* (g NH ₄ ⁺ -N g ⁻¹ h ⁻¹)	1.20	3.72	2.15	2.54	3.78	3.63	4.87	13.0	0.9633	0.9279
DMSO (g DMS g ⁻¹ h ⁻¹)	1.19	3.08	1.96	2.18	3.56	3.44	3.90	9.36	0.9665	0.9341
Catalase (arbitrary unit)	3.20	6.80	5.06	6.36	7.35	7.81	8.82	22.1	0.9657	0.9326
Dehydrogenase (g TPF g ⁻¹)	5.7	55.6	35.4	41.3	61.0	53.7	66.3	198.0	0.8966	0.8039

* Arginine ammonification

+ Correlation coefficient of soils without soil 8

**Figure 8:** Distribution of phospholipid fatty acids in different soils Monoenoic (a), saturated and polyenoic (b) and hydroxy (c) FAs**Abbildung 8:** Verteilung von Phospholipid-Fettsäuren in verschiedenen Böden Monoenoische (a), gesättigte und polyenoische (b) und Hydroxy (c)-Fettsäuren**Table 5:** Distributions of phospholipid fatty acids in eight selected soils (see Table 2)**Tabelle 5:** Verteilung von Phospholipidfettsäuren in acht ausgesuchten Böden (siehe Tabelle 2)

Soil	1	2	3	4	5	6	7	8
Total PL-FAs in nM g ⁻¹	16.88	35.68	23.87	29.06	41.54	47.61	55.43	108.04
% of total FA mass	94.7	95.9	94.5	93.6	95.1	96.2	96.9	96.1
Pattern (M/N/O)*	18/45/38	20/50/42	17/46/38	20/40/40	22/58/42	19/54/44	23/64/45	19/47/65
different types of FAs	101	112	101	100	122	117	132	131
% of total FA types	73	76	73	70	76	73	78	67

* different types of monoenoic-, saturated and polyenoic and OH-FAs

be refined by using the cis- or trans-isomers of the FAs, the straight chains, branched chains, the even or odd numbers and so on. More details of this method can be found in the papers of Zelles et al. (1992) and Zelles and Bai (1993). The classification can give a relatively simple and fast survey of the diversity of fatty acids in soils. In a further step this has to be linked to the diversity of the organism community. Genetic and serological methods could help to get a closer insight into microbial community structures.

3.3 Application of serological techniques to study bacterial populations in soil

Using classical microbiological methods, the study of the population of soil microbes is dependent on the cultivation techniques used in the laboratory. The microbes need to grow in more or less selective media to be counted (MPN techniques) or studied further. The direct staining techniques with e.g. acridine orange or fluorescein diacetate are non-selective. However, antibodies developed against specific organisms offer a direct and specific identification (Bohlool and Schmidt, 1980). We chose the most active soil habitat and the habitat which is changed most drastically by management practices, the rhizosphere, to apply serological techniques for a specific *in situ*-localization and quantification.

Monoclonal antibodies were raised against bacteria of the genus *Azospirillum brasilense*, diazotrophic bacteria, which exert plant growth promotion activity. We investigated the colonization potential of different strains of *A. brasilense* in the rhizosphere of wheat plants. Six weeks after inoculation the occurrence of these bacteria were quantified in different root zones using strain-specific monoclonal antibodies and the highly sensitive chemoluminescence-ELISA technique (Schloter et al., 1992). As Table 6 clearly shows, sustainable colonization of the roots was strictly dependent on the strain used and on its potential to penetrate the root cortex. In addition to the quantification of rhizosphere colonization a clear localization of bacteria was possible on the root surface using microscopic techniques. Due to the high autofluorescence of the root, a confocal scanning laser microscope, which produces optical sections, could resolve the localization on the root surface (Schloter et al., 1993). Presently monoclonal antibodies for autochthonous soil bacteria such

Table 6: *Azospirillum* counts in the rhizosphere 3 months after inoculation with the strains *Azospirillum brasilense* Sp7 or Sp245 (detection with chemoluminescence ELISA, Schloter et al., 1992)

Tabelle 6: *Azospirillum*-Zahlen in der Rhizosphäre 3 Monate nach Beimpfung mit den Stämmen *Azospirillum brasilense* Sp7 oder Sp245 (Nachweis mit Hilfe des Chemolumineszenz-ELISA, Schloter et al., 1992)

treatments	A. bras. Sp7 (bacteria/g root)	A. bras. Sp245 (bacteria/g root)
washed roots	$8 \cdot 10^4$	$1 \cdot 10^5$
Surface sterilized roots	$5 \cdot 10^3$	$1 \cdot 10^5$

as *Arthrobacter* spp. are used to study the distribution of these bacteria in active soil habitats like the rhizosphere, faeces of earthworms and mineralizing crop residues.

3.4 Application of genetic methods for soil microbial population analysis

While monoclonal antibodies allow the detection of bacteria with a resolution down to strain-specificity, the rRNA directed oligonucleotide gene probes offer the identification of bacteria at species, genus and higher group levels. Therefore, a realistic chance exists to investigate a major population of soil microbes using direct cell labelling with rRNA directed oligonucleotide probes. Root and soil samples were fixed and hybridized with a eubacterial probe according to Amann et al. (1990). Figure 9 shows the image of a wheat root hair surrounded by bacteria taken with a confocal scanning laser microscope. Bacteria are clearly visible in the vicinity of the root hair, which obviously are active enough to enrich sufficient labelled probe. The application of this technique should allow the detection of unculturable bacteria. This is most important, since the majority of soil bacteria is known as difficult or impossible to be grown in laboratory enrichment media and hence escape the classical microbiological analysis.

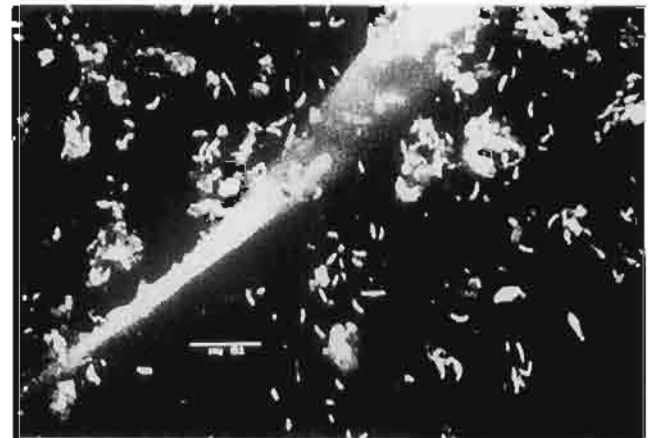


Figure 9: Confocal Scanning Laser Microscopic (CSLM) picture of a root hair of wheat surrounded by bacteria. The bacteria were labelled with a fluorescein-coupled eubacterial oligonucleotide gene probe (scale: 10 μ m).
Abbildung 9: Aufnahme eines Wurzelhaars von Weizen und umgebender Bakterien mit dem Confocal Scanning Laser Mikroskop (CSLM). Die Bakterien wurden mit einer Fluorescein-markierten Eubakterien-spezifischen Sonden markiert (Skalenlänge: 10 μ m).

4 Conclusions

To develop sustainable management strategies for agriculture, the function and structure of the soil microbial communities need to be better understood, quantified and predicted. The biomass concept allows to quantify nutrient element pools stored in the soil biota. This is essential to base nutrient dynamics or fluxes on the organism biomass and not on the soil mass. The temporal and spatial variability

ity of biotic parameters should be included for the quantitative description of the microbial processes in soils. These differ considerably under different management and site conditions. From the ecological point of view it might be useful to determine the inventory of the microbial biomass in soils or in different compartments of the soil. Relating the function of the organism community to the inventory may lead to other conclusions than relating them to concentrations. In this respect, other ecological parameters have to be measured and included like bulk densities, depth of the plough layer or rooting depth and the gradients of physical, chemical and biotic parameters in the soil profile. Activity indicators, like respiration, heat production, ATP concentration and enzyme activities then can be more successfully related to the biomass.

A promising approach to study soil microbial populations is the analysis of the pattern of fatty acids occurring in different fractions of microbial constituents. Our results indicate, that the crop rotation can significantly alter the pattern of phospholipid derived fatty acids. This approach has the potential to typify different microbial communities, which might be a further step to relate the structure of microbial communities to soil processes. To answer questions which are linked to specific groups of microbes, this approach is not specific enough. Serological or genetic tools can offer suitable methods to investigate the fine structure of microbial populations. There is no general method available to solve all questions. The problems which have to be solved dictate the tools to be used. Today, however, the variety of methods available brings the microbiologist in a much better position than a few years before.

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