Carbon Source Utilization by Microbial Communities in Soils under Organic and Conventional Farming Practice

Andreas Fließbach and Paul Mäder

Research Institute of Organic Agriculture (FiBL), Ackerstrasse, CH-5070 Frick,
E-mail: andreas.fliessbach@fibl.ch

Abstract. In a long-term field trial in which organic and conventional agricultural systems were compared since 1978 we analyzed soil microbial biomass, microbial activity and substrate utilization patterns by the Biolog GN microplates. Microbial biomass and the C_{mic}-to-C_{org} ratio was distinctly higher in organic plots whilst the metabolic quotient qCO_2 as an indicator of the energy requirement of soil microorganisms was lower. Substrate utilization profiles were affected by the different lon-term treatments, but also indicated differences of short-term effects like crop and soil management steps.

Keywords. Organic farming, microbial biomass, C_{mic}-to-C_{org} ratio, qCO_2, substrate utilization patterns, functional diversity

1. Introduction

Growing concern about the environmental and socio-economic effects of conventional agricultural practice has led farmers, consumers and recently politicians to seek alternative approaches, that are more sustainable in the view of environmental safety, soil protection, and the socio-economic security of the farmer. Especially in Western European countries, Australia, New Zealand and North America farmers developed organic farming systems to maintain soil fertility and profitability without the use of synthetic pesticides and fertilizers (Reganold 1995). Organic farming intends to provide practices that may keep the agricultural system close to natural systems.

Natural ecosystems are typically in a steady state as determined by environmental and geological factors. However agricultural systems, with crop and plant residue removal and intensive soil tillage will hardly reach this state. According to Odum's (1969) hypothesis climax communities support the highest possible biodiversity, with numerous species interactions, and a balanced biomass production to respiration ratio, as well as a low nutrient loss. The hypothesis was recently confirmed by Tilman *et al.* (1996), who found a reduced nitrate leaching potential of soils with a high plant diversity.

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For soil ecosystems, Odum's theory of bioenergetics in ecosystem development was confirmed by studies of Insam and Domsch (1988), who found a decreasing CO_2 release per unit microbial biomass (qCO_2) from a series of soils from young to mature sites. Anderson and Domsch (1989; 1990) found that the qCO_2 of the soil microbial biomass (C_{mic}) in soils under crop rotation was significantly lower than in monoculture soils and the ratio of C_{mic} to total soil organic C was significantly higher. It may thus be assumed that a diverse plant community will favour effective soil microbial communities by reducing their energy demand.

Under similar environmental conditions the only explanation for these qualitative differences between communities is a different species composition that may use the available C sources more efficiently. However, only a few researchers have attempted to link these two ecological domains of autecology and synecology (Insam *et al.* 1996).

The present paper addresses functional properties of microbial communities from soils of different agricultural systems. A recent method based on the utilization patterns of 95 different C sources on a microtitre plate was tested. It has already been applied to soils and was able to distinguish between different sites and treatments (Bossio and Scow 1995; Garland and Mills 1991; Winding 1993; Zak *et al.* 1994). It was the aim of our investigations to see if a) microbial populations from soils of different agricultural systems differ in their community energetics and b) if differences in community energetics can be correlated to the functional abilities of the soil microbial community.

2. Materials and Methods

2.1 Soils and field experiment

Investigations were carried out on soils from plots of a long-term field trial (DOC) at Therwil (CH), that was started in 1978 to compare different agricultural systems regarding system productivity, nutrient balance, energy efficiency, soil fertility, and in general their effect on the environment (Besson and Niggli 1991). The soil is a luvisol on deep deposits of alluvial loess that has been cultivated as an arable soil for a long time. Long-term annual mean temperature is 9.0 °C and annual rainfall averages at 872 l m^{-2} (310 m above sea-level). A seven year crop rotation included potatoes, winter wheat 1, beet roots, winter wheat 2, and three years of grass-clover in all systems. Our investigation included the following agricultural systems, where the amount of organic fertilizer corresponded to 1.4 livestock units ha^{-1} which resulted in a mean annual amount of 2 Mg organic matter ha^{-1} in all organically fertilized treatments:

unfertilized: Unfertilized since 1978, but amended with bio-dynamic preparations.

bio-dynamic: Fertilized with aerobically composted farm-yard-manure (FYM) (C/N=8) and amended with mineral and herbaceous

preparations according to biodynamic farming practice (Kirchmann 1994).

organic: Fertilized with slightly aerobically rotted FYM (C/N=11) according to the organic farming practice in Switzerland.

conventional: Fertilized with stacked, anaerobically rotted FYM (C/N=12) and an additional mineral fertilization according to the official Swiss extension service, integrated pest management.

mineral NPK: Unfertilized from 1978 until 1985, but then fertilized with NPK according to the official extension service, integrated pest management.

We investigated soils from winter wheat plots. Wheat has been cultivated in two consecutive years in two parallel crop rotation subunits of the trial. Soils from 0-20 cm were sampled in March 1995 and March 1996 at tillering and two months after the harvest of winter wheat in October 1995 as bulked samples from each of the four field replicates. In 1995 wheat followed red beets and in 1996 potatoes in the crop rotation. In the laboratory, soils were kept at 4 °C until they were sieved (2 mm) and water adjusted to 50 % water holding capacity (ca. 24 % H_2O of dry matter).

Data on soil pH (0.1 M KCl, 1/10; weight/vol) organic C and N (CHN analyzer, LECO) are given in table 1.

Table 1. pH, organic carbon (C_{org}), total N (N_t) and C/N ratio in soils from the DOC field trial (n=4). Different letters indicate significant differences at p=0.05

	pH (0.1 M KCl)	C_{org}	N_t	C/N
unfertilized	5.1 ab	1.32 a	0.140 a	9.58 a
bio-dynamic	5.7 a	1.61 b	0.169 b	9.56 a
organic	5.4 a	1.34 ab	0.148 ab	9.11 a
conventional	5.2 ab	1.37 ab	0.132 ab	10.36 a
mineral NPK	4.9 b	1.41 ab	0.156 ab	9.18 a

2.2 Soil microbial biomass

Soil microbial biomass carbon (C_{mic}) was estimated by chloroform-fumigation-extraction (CFE) according to Vance *et al.* (1987). CFE was done on 25 g subsamples that were extracted with 100 ml of a 0.5 M K_2SO_4 solution. Total organic carbon (TOC) in soil extracts was determined by infrared spectrometry after combustion at 850 °C (DimaTOC, Dimatec, Essen). Soil microbial biomass was then calculated according to the formula:

$$C_{mic} = E_C / k_{EC}$$

E_C= *(TOC in fumigated samples - TOC in control samples)*

$k_{EC} = 0.45$ (Joergensen 1995; Martens 1995)

2.3 Carbon source utilisation assay

Soils were preincubated at 22 °C for 5 days. After comparing different extractants and dilutions we decided to use the extraction procedure that gives the highest values for average well colour development (AWCD) and the lowest variability among repeated measurements. 10 g (dry matter) of soil was suspended in 100 ml of extractant on a rotary shaker at 300 rev min^{-1} for 30 min. Soil suspensions, prepared with a 0.9 % NaCl-solution showed higher values and lower variation than with 0.1 % sodium hexametaphosphate, where an addition of $CaCl_2$ was necessary to precipitate dispersed clay minerals (Haack *et al.* 1995). NaCl extracts were allowed to settle for 10 min to clear the supernatant, which was diluted tenfold to obtain a final dilution of 10^{-2}.

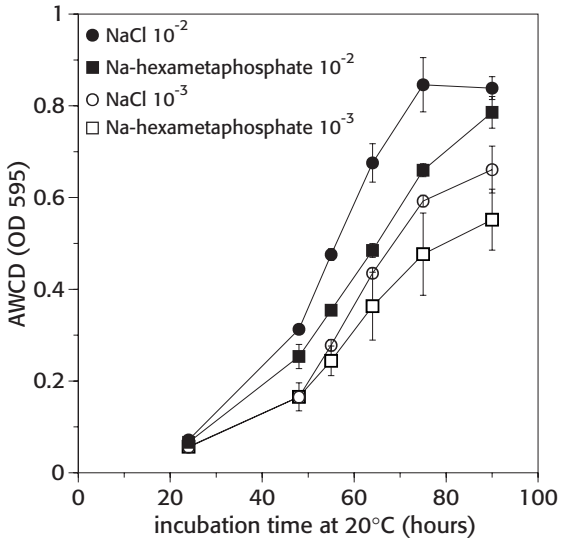

Fig. 1. Average well colour development in Biolog microplates as affected by the solution used for extraction of microorganisms and by the dilution of the soil suspension (n=3).

This suspension (125 µl per well) was directly inoculated to Biolog GN microplates (Biolog Inc. Hayward, Cal., USA) (Garland and Mills 1991). Microplates were kept at 22 °C until colouration became visible, then the plate absorbance was measured repeatedly (BioRad, Model 450). For data processing we used set points, where the average well colour development reached values between 0.6 and 0.7 absorption units. This was usually the case after 60 to 65 hours of incubation at 22 °C. Data were processed in the following ways:
Raw difference (RD): $X - X_0$ where X is the raw value of each well and X_0 the OD_{595} of the water blank, negative scores were set to zero.
Average well colour development (AWCD): $\sum RD/95$
Number of utilized substrates: Number of substrates with RD > AWCD

Diversity was calculated according to population ecology by using raw difference data: $-\sum p_i (lnp_i)$ where p_i is the ratio of activity on a particular substrate to the sum of activities on all substrates.

2.4 Statistics

Biolog results were analyzed with CANOCO software from Microcomputer Power, Inc. (Ithaca, N.Y.) (Jongman *et al.* 1995). For diversity calculations substrates were treated as individual species. AWCD transformed data were used to perform principal component analysis (PCA).

3. Results and Discussion

3.1 Soil microbial biomass and activity

The different agricultural systems, represented in the DOC field trial, mainly differing in fertilization and plant protection strategy, resulted in marked differences in soil microbial biomass (C_{mic}) and microbial activity (Mäder *et al.* 1995). After 18 years, C_{mic} in soils of the two biological plots was significantly higher than in conventional soils (Fig. 2). The organic manure treatment in the conventional plots did not cause a significant difference to the mineral fertilizer treatment and the unfertilized plot. Corresponding results were found in system comparison trials at Darmstadt (D) (Bachinger 1995; Raupp 1995) and Järna (S) (Pettersson *et al.* 1992), indicating that organic rather than conventional farming exerts positive effects on the size and activity of the soil microbial biomass (Mäder *et al.* in press).

Although microbial biomass and soil basal respiration were highest in the biodynamic treatment, we found the lowest qCO_2 in these plots (Fig. 3), indicating that the microbial populations of these plots utilize the organic substrates more efficiently. The key to understanding differences of microbial metabolic activity among the treatment plots is supposed to lie within the community structure of soil microorganisms.

The ratio of microbial biomass C to total soil organic C was also higher in the biological plots than in the conventional and especially the plots with mineral fertilizer exclusively (Fig. 4). Since this ratio is indicating organic matter quality and availability it suggests that long-term amendments with fertilizers of different quality will result in a change of organic matter pools and that fertilizers are favouring soil organisms to a different extent (Anderson and Domsch 1989).

3.2 Substrate utilization by soil microbial communities

In a first step we attempted to find site differences by the use of univariate measures of substrate utilization patterns. Microbial functional diversity as indicated by the Shannon index showed the same ranking over the whole

incubation period of the microplates, but at different levels. Differences due to the DOC-treatments were obtained in samples from March 1995 and March 1996, where the bio-dynamic plots showed highest values and the conventional treatment the lowest (Fig. 5). In samples from October 1995 under emerging grass-clover and two months after the winter wheat harvest differences between the systems became very small. Possibly a different microflora has developed after incorporation of straw and residues, resulting in a more diverse substrate utilization of the microbial communities of all treatments.

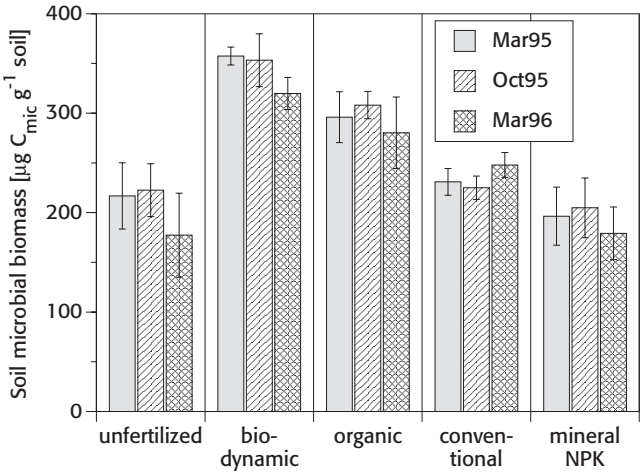

Fig. 2. Soil microbial biomass in field plots of the DOC-experiment at three sampling dates under winter wheat. The columns represent the mean of four field replicates (Standard error bars shown).

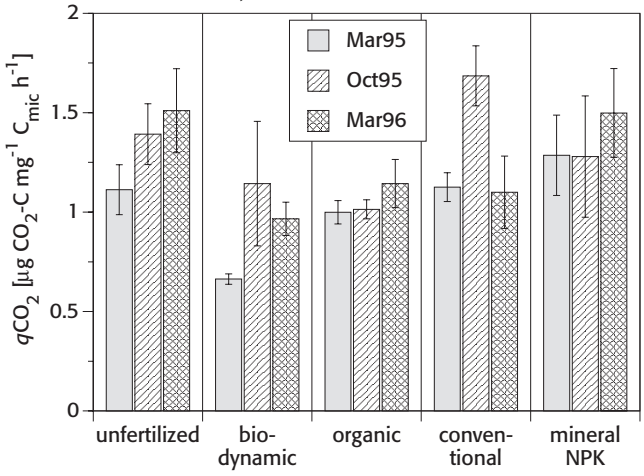

Fig. 3. Metabolic quotient for CO_2 (qCO_2) in plots of the DOC field trial at three sampling dates under winter wheat. Columns are the mean of four field replicates.

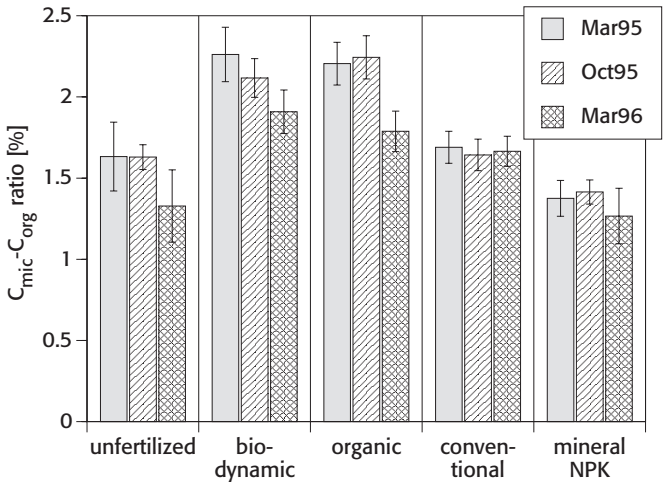

Fig. 4. C_{mic}-to-C_{org} ratio in plots of the DOC field trial in March 1995 and 1996. Columns are the mean of four field replicates (Standard error bars shown).

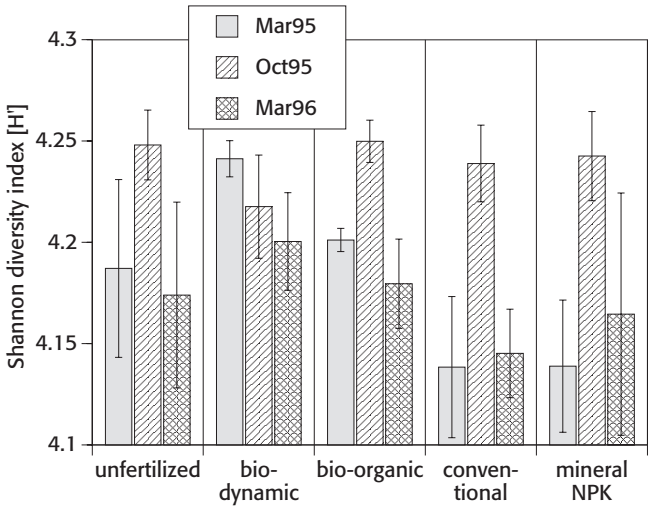

Fig. 5. Microbial functional diversity (Shannon Index) at three sampling dates under winter wheat. The columns are the mean of the four field replicates (Standard error bars shown).

Garland and Mills (1991) and Zak *et al.* (1994) identified different groups of chemically related substrates, of which the carbohydrates, carboxylic acids and amino acids were most numerous. By adapting the approach of Vahjen *et al.* (1995) we counted the number of substrates that showed higher absorption than the average plate absorbance. Differences among the number of these intensely

utilized substrates were small, but in most cases due to differences of carbohydrate utilization (Fig. 6). Especially in the March samples substrate utilization was greatly affected by carbohydrates. Carbohydrate utilization in the bio-dynamic soils was 50 % higher than in the conventional, whereas the other substrates did not differ that much. In October the relative proportion of each of the substrate groups was almost the same, showing differences to the conventional treatment of less than 10 %, however mean utilization of carbohydrates over the treatments was markedly higher at this sampling time than in the samples of March.

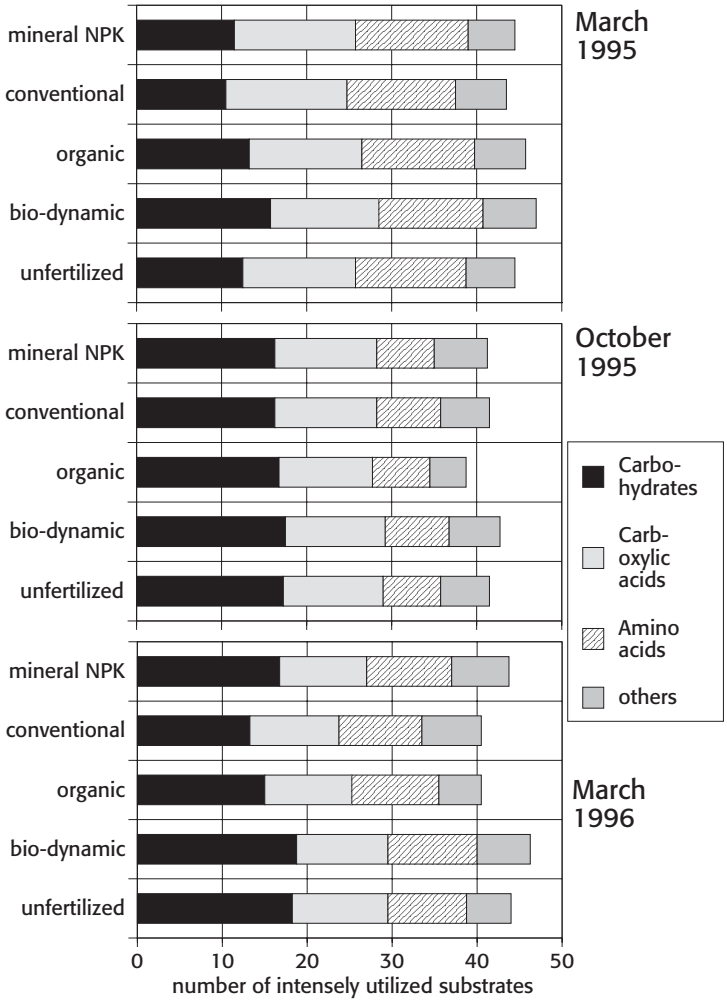

Fig. 6. Number of intensely utilized substrates (absorption > average plate absorbance) among chemically similar substrate classes. The columns represent the mean of four field and three microplate replicates.

Principal component analysis (PCA) applied to the absorption values transformed by the AWCD (Garland and Mills 1991) separated some of the DOC-treatments, by ordination of the first two principal components. The first two principal components accounted for more than 50 % of the total variance. However variation along with field replicates of the same treatments were often higher than variation between them (Fig. 7).

The substrate utilization patterns of microbial communities from the field trial soils showed distinct overlapping. However, in March 1995 the two organic systems showed a close ordination of the first two principal components as did the two conventional systems. In October 1995 the ordination points of the field replicates did not allow for a distinct grouping, but in March 1996 the three organically fertilized plots ordinated close by, whereas the unfertilized and minerally fertilized plots were far apart.

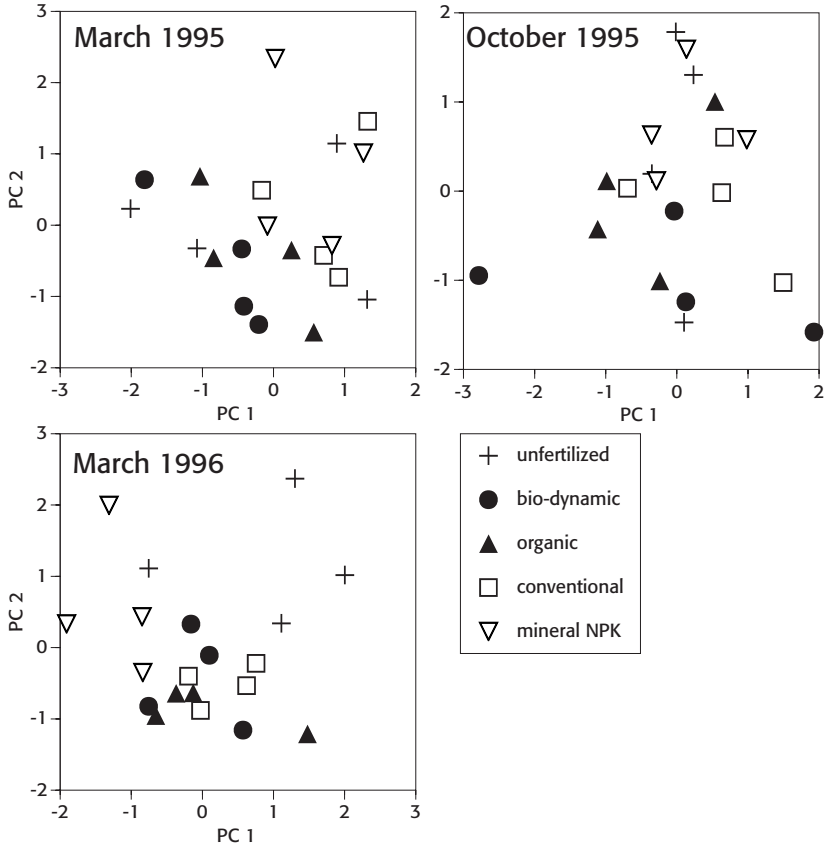

Fig. 7. Ordination of the first two principal components. Sample scores for each plot of the field trial represent the mean of three replicate microplates.

Variation within the field replicates can be explained by inhomogeneities, that were also observed with measurements of microbial biomass and activity.

Especially the subplots that were used in 1996 showed distinct variation among field replicates in the mineral and the unfertilized treatments. Ordination techniques that allow for the inclusion of covariables to account for block effects would be helpful.

Differences between the sampling dates in the same subplot (March and October 1995) are probably due to the soil disturbance after winter wheat cropping. Plant residues have been incorporated and farmyard manure and mineral fertilizers have been applied to the field plots two months prior to sampling. During this period a microflora might have developed that was not specifically adapted to the site, but to the nutrient and organic matter input. Therefore the substrate utilization patterns of the October sample did not differ among treatments.

Soils from different agricultural systems have been shown to differ in soil microbial biomass and activity. Holistic methods for their determination were little affected by sampling date. Substrate utilization reacted more sensitively to short-term management effects like tillage or fertilization rather than the long-term system effects.

The metabolic quotient for CO_2 in the two organic systems was markedly lower than in the two conventional treatments, indicating that the soil microbial population needs less energy for maintenance. A significantly higher diversity in organic soils was only found for the spring sampling in 1995. Hence, the hypothesis that differences due to community energetics are explainable by the functional richness and diversity of the soil microbial community needs further examination.

4. References

Anderson TH, Domsch KH (1989) Ratios of microbial biomass carbon to total organic carbon in arable soils. Soil Biol Biochem 21: 471-479

Anderson TH, Domsch KH (1990) Application of eco-physiological quotients (qCO_2 and qD) on microbial biomasses from soils of different cropping histories. Soil Biol Biochem 22: 251-255

Bachinger J (1995) Effects of organic and mineral fertilizer on chemical and microbial parameters of C- and N-dynamics and root parameters. Mäder P, Raupp J (eds) Effects of low and high external input agriculture on soil microbial biomass and activities in view of sustainable agriculture, 2nd meeting of the EU-concerted action (AIR3-CT94-1940), Oberwil, p 52-58

Besson J-M, Niggli U (1991) DOK-Versuch: Vergleichende Langzeit-untersuchungen in den drei Anbausystemen biologisch-Dynamisch, Organisch-biologisch und Konventionell. I. Konzeption des DOK-Versuchs: 1. und 2. Fruchtfolgeperiode. Schweiz Landw Fo 31: 79-109

Bossio DA, Scow KM (1995) Impact of carbon and flooding on the metabolic diversity of microbial communities in soils. Appl Environ Microbiol 61: 4043-4050

Garland JL, Mills AL (1991) Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level-sole-carbon-source utilization. Appl Environ Microbiol 57: 2351-2359

Haack SK, Garchow H, Klug MJ, Forney LJ (1995) Analysis of factors affecting the accuracy, reproducibility, and interpretation of microbial community carbon source utilization patterns. Appl Environ Microbiol 61: 1458-1468

Insam H, Amor K, Renner M, Crepaz C (1996) Changes in functional abilities of the microbial community during composting of manure. Microbial Ecol 31: 77-87

Insam H, Domsch KH (1988) Relationship between soil organic carbon and microbial biomass on chronosequences of reclamation sites. Microb Ecol 15: 177-188

Jörgensen RG (1995) Die quantitative Bestimmung der mikrobiellen Biomasse in Böden mit der Chloroform-Fumigations-Extraktions-Methode. Göttinger Bodenkundliche Berichte, Universität Göttingen, Göttingen,

Jongman RHG, Ter Braak CJF, Van Tongeren OFR (1995) Data Analysis in Community and Landscape ecology. 2/Ed. Cambridge University Press, Cambridge

Kirchmann H (1994) Biological dynamic farming - an occult form of alternative agriculture? J Agric Environ Ethics 7: 173-187

Mäder P, Fließbach A, Wiemken A, Niggli U (1995) Assessment of soil microbial status under long-term low input (biological) and high input (conventional) agriculture. Mäder P, Raupp J (eds), Effects of low and high external input agriculture on soil microbial biomass and activities in view of sustainable agriculture, 2nd meeting of the EU-concerted action (AIR3-CT94-1940), Oberwil (CH), p 24-38

Mäder P, Pfiffner L, Fließbach A, von Lützow M, Munch JC (in press) Soil ecology - The impact of organic and conventional agriculture on soil biota and its significance for soil fertility. Oestergaard TV (ed), Fundamentals of Organic Agriculture, Proc. of the 11th IFOAM International Scientific Conference, Copenhagen, DK

Martens R (1995) Current methods for measuring microbial biomass C in soil: Potentials and limitations. Biol Fertil Soils 19: 87-99

Odum EP (1969) The strategy of ecosystem development. Science 164: 262-270

Pettersson BD, Reents HJ, von Wistinghausen E (1992) Düngung und Bodeneigenschaften. Ergebnisse eines 32-jährigen Feldversuches in Järna, Schweden. Institut für biologisch-dynamische Forschung, Darmstadt

Raupp J (1995) The long-term trial in Darmstadt: Mineral fertilizer, composted manure and composted manure plus all bio-dynamic preparations. Raupp J (ed), Main effects of various organic and mineral fertilization on soil organic matter turnover and plant growth, 1st meeting of the EU-concerted action (AIR3-CT94-1940), Darmstadt, p 28-36

Reganold JP (1995) Soil quality and profitability of biodynamic and conventional farming systems: A review. Amer J Alternative Agric 10: 36-45

Tilman D, Wedin D, Knops J (1996) Productivity and sustainability influenced by biodiversity in grassland ecosystems. Nature 379: 718-720

Vahjen W, Munch JC, Tebbe CC (1995) Carbon source utilization of soil extracted microorganisms as a tool to detect the effects of soil supplemented with genetically engineered and non-engineered *Corynebacterium glutamicum* and a recombinant peptide at the community level. FEMS Microbiol Ecol 18: 317-328

Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass C. Soil Biol Biochem 19: 703-707

Winding A (1993) Fingerprinting bacterial soil communities using Biolog microtiter plates. In: Ritz K, Dighton J, Giller KE (eds) Beyond the Biomass: compositional and functional analysis of soil microbial communities, Vol. , John Wiley and Sons Ltd., Chichester, UK, pp 85-94

Zak JC, Willig MR, Moorhead DL, Wildman HG (1994) Functional diversity of microbial communities: a quantitative approach. Soil Biol Biochem 26: 1101-1108