 DOI: 10.1002/jpln.200800021 *J. Plant Nutr. Soil Sci.* 2009, *172,* 704–712

Impact of effective microorganisms and other biofertilizers on soil microbial characteristics, organic-matter decomposition, and plant growth

Mario Schenck zu Schweinsberg-Mickan[1]* and **Torsten Müller[1,2]**

[1] Department of Soil Biology and Plant Nutrition, Faculty of Organic Agricultural Sciences, University of Kassel, Nordbahnhofstraße 1a, 37213 Witzenhausen, Germany
[2] Present address: Institute of Plant Nutrition (330), University of Hohenheim, 70593 Stuttgart, Germany

Abstract

Incubation and pot experiments were conducted to investigate the impact of commercially distributed biofertilizers (effective microorganisms [EM], BIOSTIMULATOR, BACTOFIL-A, and BACTOFIL-B) on soil microbial-biomass content and activity, net N mineralization in soil, and growth of *Lolium perenne.* According to the manufacturers, the products tested are based on microbial inoculants or organic growth stimulants, and are supposed to influence soil microbial properties and improve soil conditions, organic-matter decomposition, and plant growth. In the incubation experiment (40 d, 20.6°C, 50% maximum water-holding capacity), EM was repeatedly applied to soil together with different organic amendments (nonamended, chopped straw, and lupine seed meal). Under the experimental conditions of this study, no or only marginal effects of EM on organic C, total N, and mineral N in soil could be observed. In soil treatments without any organic amendment, EM suspension slightly enhanced microbial activity measured as soil CO_2 evolution. In soil with easily degradable plant residues (lupine seed meal), EM suspension had a suppressive effect on microbial biomass. However, comparisons with sterilized EM and molasses as the main additive in EM suspension showed that any effect of EM could be explained as a pure substrate effect without the influence of added living organisms. In the pot experiment with *Lolium perenne* (air-conditioned greenhouse cabin, 87 d, 16.8°C, 130 klxh d^{-1} light quantity), the products EM, BIOSTIMULATOR, BACTOFIL-A, and BACTOFIL-B were tested in soil with growing plants. The products were repeatedly applied for a period of 42 d. Within this study, no effects of the different biofertilizers on mineral N in soil were detectable. There were clear suppressive effects of all tested biofertilizers on microbial-biomass content and activity. Comparisons with sterilized suspensions showed that the effects were not due to living microorganisms in the suspensions, but could be traced back to substrate-induced processes.

Key words: biofertilizers / effective microorganisms / EM / BIOSTIMULATOR / BACTOFIL / *Lolium perenne* / mineralization / soil organic matter

Accepted July 30, 2008

1 Introduction

Currently, many different microbial biofertilizers are available for agricultural use. The manufacturers claim that their products enhance plant growth and yield and improve soil conditions, due to the addition of beneficial microbial inoculants to soil and by stimulation of soil microorganisms. Biofertilizer suspensions are usually applied to soil in very low concentrations. Often the microbial composition of the biofertilizers is not specified in detail, making it difficult for the users to evaluate the product. The effectiveness of these products has yet to be proven scientifically.

One of the world's most commonly used biofertilizers in this context is the so-called "effective microorganisms" (EM). Effective microorganisms was developed in the 1970s by Teruo Higa, University of Ryukyu, Okinawa, Japan (*Higa*, 1991). The exact microbial composition of EM is kept secret. Beside other minor literature, Higa promoted EM in two books (*Higa*, 2002a, b). In Germany, EM is commercially available under the product name EM-1 (EMIKO GmbH, Euskirchen-Kirchheim).

According to the package declaration, EM-1 suspension contains "a selection of groups of microorganisms, in particular, lactic acid bacteria, yeast, photosynthetic bacteria", which are mainly used in food production. Among other things, EM-1 is claimed to improve the biological status of the soil, increase soil organic matter (SOM), improve germination and root development, increase photosynthesis, and increase plant growth and yield (*Emiko,* 2003).

To some extent, the proposed mechanisms behind the effect of EM on soil and other media are not consistent with today's scientific knowledge, *e.g.*, coexistence/symbiosis of aerobic and anaerobic soil organisms, anaerobic activity of photosyn-

* Correspondence: M. Schenck zu Schweinsberg-Mickan;
e-mail: mschenk@uni-kassel.de

thetic bacteria in soil under aerobic conditions (*Higa*, 2002a, b). Nevertheless, experiments with positive effects of EM on crop quality and growth as well as on soil properties and in particular on microbiological indicators of soil quality have been reported in literature not subject to any peer-review procedure (*e.g., Senenayaka* and *Sangakkara*, 1997, 1999; *Xu* et al., 2000; *Anonymous*, 2004). Due to a lack of control treatments with sterilized EM suspensions or suspension additives, the reported experiments cannot distinguish between effects of living microorganisms and pure-substrate effects. In a long-term field experiment with different crops, no effects on yield and soil microbiology of EM applications caused by living microorganisms could be detected. All observed effects could be related to pure-substrate addition of the EM application (*Mayer* et al., 2007).

The original EM-1 suspension can be used directly. But, EM-1 is usually reproduced by fermentation after addition of molasses and water (then called EM-A, see section 2). EM-A is used diluted at application rates equivalent to 5–10 L EM-1 ha^{-1} (*Daenecke* and *Zschocke*, 2002; *Emiko*, 2003).

Other biofertilizers similar to EM are BIOSTIMULATOR, BACTOFIL-A, and BACTOFIL-B (Agrinova GmbH). According to the package declaration, BIOSTIMULATOR is based on N-fixing and P-mobilizing bacteria, especially *Bacillus subtilis*, natural humates, and algae extract (*Ascophyllum nodosum*). Among other things, BIOSTIMULATOR is claimed to increase soil microbial activity, plant nutrient availability, and plant growth. BACTOFIL-A and BACTOFIL-B are supposed to contain different combinations of *Azospirillum brasilense, Azospirillum lipoferum, Azotobacter vinelandii, Bacillus megaterium, Bacillus polymyxa, Bacillus circulans, Bacillus subtilis, Pseudomonas fluorescens, Streptomyces albus*, and *Micrococcus roseus* and furthermore growth stimulators, phytohormones, and vitamins synthesized by bacteria. It is claimed that BACTOFIL-A and BACTOFIL-B mobilize plant nutrients, fix atmospheric N, increase soil microbial activity, increase productivity, and increase plant resistance against pathogens.

This investigation is based on the following hypotheses: (1) Living microorganisms added with the above-mentioned biofertilizers increase microbial biomass and microbial turnover activity in soil; (2) the above mentioned biofertilizers enhance plant growth. To test the hypotheses, an incubation experiment and a pot experiment with *Lolium perenne* were conducted. The aim of the incubation experiment was to investigate the impact of EM-1 after fermentation with molasses (EM-A) under conditions with different plant residues on SOM decomposition, soil N mineralization/immobilization, soil microbial biomass, and soil microbial respiratory activity. The aim of the pot experiment with *Lolium perenne* was to investigate the impact of EM-1 (unfermented as basic suspension EM-1), BIOSTIMULATOR, BACTOFIL-A, and BACTOFIL-B on soil microbial biomass, soil microbial respiratory activity, and plant growth.

For interpretation of the data, a comparison between suspensions containing living microorganisms on the one hand, and sterilized suspensions, pure-suspension additives (molasses), and simply demineralized water on the other, will be crucial for distinguishing between effects due to the addition of living microorganisms, pure substrate, and wetting effects.

2 Materials and methods

2.1 Incubation experiment

Portions of 1000 g soil on an oven-dry basis (clay silt, $pH_{(CaCl_2)}$ 7.3, total C [C_t] 1.16%, total N [N_t] 0.13%, sieved at 2 mm, 50% water-holding capacity) were mixed with either 0.5% chopped wheat straw (STR: *Triticum aestivum* L., 5 mm, C : N 103, C input 0.23%, N input 0.002%) or yellow lupine seed meal (LUP: *Lupinus luteus* L., 2 mm, C : N 6.8, C input 0.23%, N input 0.033%), or left unamended (NON). The soil was incubated in open preserving glasses (3 L) for 40 d in an air-conditioned greenhouse cabin at 20.6°C. The average light quantity during incubation was approx. 130 klxh d^{-1}. The soil water content in the pots was maintained at 50% maximum water-holding capacity by weighing every 5 d and adding demineralized water.

In accordance with the instruction manual, EM-A was produced by anaerobic fermentation of EM-1 (*Emiko*, 2003). A volume of 15 mL molasses was dissolved in 470 mL hot (70°C) demineralized water. After cooling down to 32°C, 15 mL basic suspension of EM-1 was added. The fermentation took place at 32°C for 7 d in plastic bottles. The pressure developing in the bottles was removed by briefly opening the bottles. Sterilized EM-A (EM-ster.) was prepared from the final EM-A suspension by sterilization at 95°C for 1.5 h. After sterilization, the stored suspension did not show any further gas production which would have been an indicator of ongoing fermentation as a result of insufficient sterilization. A separate molasses suspension (MOL) was prepared analogously to EM-A by dissolving 15 mL molasses in 485 mL hot (70°C) demineralized water. All suspensions were diluted 1:100. During soil incubation, EM, EM-ster., MOL, or demineralized water (H2O) were applied to the soil surface without further mixing at the beginning and subsequently every 10 d. The suspensions were newly prepared for each application. Application rates were 25 mL diluted suspension per pot. If upscaling from the soil surface in the pot to a hectare base, the application rates of EM were equivalent to 5 L EM basic suspension (EM-1) ha^{-1} or 166 L EM-A ha^{-1}, respectively. The added amounts of C and N in EM, EM-ster., and MOL suspension per application are shown in Tab. 1. Beside H2O, EM-ster. and MOL were included as additional control treatments to distinguish between effects of living organisms (EM) and pure-substrate or nutrient effects caused by dead microorganisms (EM-ster.) or additives (MOL). In total, the incubation experiment had 12 different treatments including all possible combinations of the three organic amendments (STR, LUP, NON) with the four different suspensions (EM, EM-ster., MOL, H2O).

The parameters organic C [C_{org}] and N_t were measured at the end of the incubation in all soil samples. Soil was ground with a ball mill (Retsch). The finely ground soil was dried at

Table 1: Added amounts of C and N in EM, EM-ster., and MOL suspension per application.

Suspension	C / μg application^{-1}	N / μg application^{-1}
EM	3275	61
EM-ster.	3519	68
MOL	3435	62

60°C. Soil was analyzed for C_t and N_t after oxidative combustion at 1000°C, reduction of N oxides, and gas-chromatic separation using an automated elemental analyzer (Vario EL, Elementar). The amount of C_{org} was calculated after measuring the inorganic bound carbonate in a Scheibler apparatus (*Schaller*, 2000).

For all other measurements, soil samples were taken after 0, 5, 15, 25, and 40 d. This also included a slight mixing of the soils. Soil microbial biomass C and N (C_{mic} and N_{mic}) were estimated by chloroform-fumigation extraction (CFE) in all soil samples (*Brookes* et al., 1985; *Vance* et al., 1987). Two portions equivalent to 50 g oven-dry soil were taken from each incubation glass. One portion was fumigated for 24 h at 25°C with ethanol-free $CHCl_3$. Following fumigant removal, the soil was extracted with 200 mL 0.5 M K_2SO_4 by 30 min horizontal shaking at 200 rev min^{-1} and filtered (Schleicher & Schuell 595 ½). The other nonfumigated portion was extracted similarly at the time fumigation commenced.

All extracts were stored in a freezer until analysis could be carried out. Organic C in the extracts was measured as CO_2 by infrared absorption after combustion at 850°C using a Dimatoc 100 automatic analyzer (Dimatec). Microbial biomass C was calculated as follows: $C_{mic} = EC / k_{EC}$, where EC = (organic C extracted from fumigated soils) – (organic C extracted from nonfumigated soils) and $k_{EC} = 0.45$ (*Wu* et al., 1990; *Jörgensen*, 1996). Total N bound in the extracts was measured as NO_2^* by chemo-luminescence detection after combustion at 850°C using a Dimatoc 100/Dima-N automatic analyzer. Microbial biomass N was calculated as follows: $N_{mic} = EN / k_{EN}$, where EN = (total N extracted from fumigated soils) – (total N extracted from nonfumigated soils) and $k_{EN} = 0.54$ (*Brookes* et al., 1985; *Jörgensen* and *Müller*, 1996).

Mineral N (N_{min}) was measured as NH_4^+ and NO_3^- in the 0.5 M K_2SO_4 extracts of the nonfumigated soils, using segmented continuous-flow analysis (Evolution II, Alliance Instruments) followed by spectrometric detection. Total mineral N is expressed in μg N_{min} (g soil)$^{-1}$.

Soil CO_2 evolution was measured every 5 d according to *Isermeyer* (1952). The CO_2 was trapped in NaOH. During CO_2 trapping, the preserving glasses were closed temporarily. The amount and concentration of NaOH were adapted to the expected soil CO_2 evolution in accordance with earlier experiments. The trapped CO_2 was calculated from the remaining alkalinity in the NaOH solutions titrated with HCl. The cumulative CO_2 evolution was simply calculated by linear interpolation between the measuring days. Soil CO_2 evolution is expressed as μg CO_2-C (g soil)$^{-1}$. All data are given on an oven-dry basis (24 h, 105°C). Each application took place after soil sampling.

2.2 Pot experiment with *Lolium perenne*

Portions of 2000 g field-moist soil (clay silt, $pH_{(CaCl_2)}$ 7.3, C_t 1.32, N_t 0.15, sieved at 2 mm) were filled into 2 L containers (Ø 16 cm). In each pot, 1.6 g seeds of *Lolium perenne* L. were sown and subsequently covered with 2–5 mm soil. The pots were placed in an air-conditioned greenhouse cabin (15.8°C) for 87 d. The average light quantity during the experiment was about 130 klxh d^{-1}. The soil water content in the pots was maintained by keeping a constant amount of water in saucers below the pots.

EM-1 (EM), sterilized EM-1 (EM-ster.), BIOSTIMULATOR (BIO), sterilized BIOSTIMULATOR (BIO-ster.), BACTOFIL-A (BAC-A), sterilized BACTOFIL-A (BAC-A-ster.), BACTOFIL-B (BAC-B), sterilized BACTOFIL-B (BAC-B-ster.), or demineralized water (H2O) were applied to the soil surface at the beginning and subsequently every 2 weeks during the first 6 weeks. The suspensions were newly prepared for each application. The sterilized suspensions were heated to 95°C for 1.5 h. Each suspension was diluted. All application rates were 25 mL diluted suspension per pot for each application. In contrast to the incubation experiment, EM-1 was used without additional fermentation. Related to the soil surface in the pots, the application rates of EM, BAC-A, and BAC-B were equivalent to 10 L ha^{-1} and of BIOSTIMULATOR 3 kg ha^{-1}.

Soil microbial biomass and mineral N were measured by CFE as described for the incubation experiment. Prior to CFE, intact roots and plant particles that would have interfered with the estimation of microbial biomass, were removed using a combined wet sieving-sedimentation method (*Müller* et al., 1992; modified by *Mayer*, 2003). Two aliquots of 50 g were taken from the soil samples 0–15 cm. Both samples were horizontally shaken for 30 min at 200 rev. min^{-1} with 100 mL 0.05 M K_2SO_4 in plastic vessels. The suspension was poured through a 2 mm sieve, retaining bigger roots, plant particles, and small stones. An additional 50 mL 0.05 M K_2SO_4 were used to rinse the vessels and to pass the soil quantitatively through the sieve. Stones were returned to the suspension. After approx. 30 min of sedimentation in plastic beakers, fine roots swimming on the surface of the suspension were removed by hand-picking. Finally, the suspensions were filtered (Schleicher & Schuell 595 ½). Again, an additional 50 mL 0.05 M K_2SO_4 were used to rinse the plastic beakers into the sieve. In total, 200 mL 0.05 M K_2SO_4 were used in this first extraction step. The CFE was continued with the two soil aliquots including the filters.

CO_2 evolution from soil was measured every week *in situ* using a portable differential CO_2/H_2O infrared gas analyzer (CIRAS-1, PP-Systems) with a soil-respiration chamber (SRC-1, PP-Systems). The chamber (area 78 cm^2, volume 1117 cm^3) was placed on the soil surface, and the rate of CO_2 increase inside the chamber was monitored for a maximum of 120 s. The CO_2-evolution rate was automatically calcu-

lated and expressed in mg CO_2-C m^{-2} h^{-1}. The cumulative CO_2 evolution was calculated by linear interpolation between the measuring days. Cumulative CO_2 evolution is expressed as g CO_2-C $(m^2 \text{ soil})^{-1}$.

The plant dry-matter production was measured three times during the experiment. The plants were cut-off 0.5 cm above the soil surface and dried at 105°C for 18 h. All data are given on an oven-dry basis (24 h, 105°C). Each application took place after soil samplings.

2.3 Experimental design and statistical analysis

The incubation experiment was set up in a completely randomized block design with four experimental parallels. The pot experiment was laid out with two different experimental sets. One set was used for destructive soil sampling and harvesting of plant material in a completely randomized block design with three experimental parallels, while the other set was used for *in situ* soil-CO_2 measurement in a completely randomized block design with four experimental parallels. Analyses of variance were calculated for each measuring date. Multiple differences of means were tested for significance using a Tukey HSD-test ($p \leq 0.05$). All statistical calculations were carried out using the statistical-analysis software SPSS 10.0 for windows (SPSS-Inc., 1989–1999).

3 Results

3.1 Incubation experiment

The contents of C_{org} in soil after the incubation differed significantly between the treatments with addition of wheat straw (STR, 1.26% C_{org}), lupine seed meal (LUP, 1.21% C_{org}), and without organic-matter (OM) amendment (NON, 1.14% C_{org}). LUP supply led to significantly higher N_t contents (0.17% N_t) compared to NON, whereas STR and NON were equal in N_t contents (0.14% N_t) at the end of the experiment (data not shown).

Application of EM-A suspension (EM), sterilized EM-A suspension (EM-ster.), and molasses (MOL) had no significant effects on C_{org} in soils without organic amendment (NON) when compared to the application of pure demineralized water (H2O, 1.13% C_{org}). Lowest C_{org} values were measured in NON with EM application (1.11% C_{org}), which were significantly lower than EM-ster. (1.15% C_{org}) and MOL (1.16% C_{org}). Within the organic amendments (LUP and STR), no significant differences of C_{org} could be found. In LUP with EM application, the N_t content was significantly higher than in LUP with H2O, but not significantly different to that with EM.-ster. and MOL. Within NON- and STR-amended soils, no significant differences of N_t could be found.

At the beginning of the incubation experiment, N_{min} was between 7.3 and 17.4 μg N $(\text{g soil})^{-1}$, with highest values in LUP-amended soil. In treatments without organic amendments (NON), N_{min} increased steadily during the incubation. In the NON × H2O treatment, N_{min} increased up to a maximum of 23.2 μg N $(\text{g soil})^{-1}$ at the end of the incubation (Tab. 2). N_{min} was lowest in the NON × EM treatment, in which it was significantly lower than with NON × H2O, EM-ster., and MOL application at the end of the incubation. In the straw-amended soil (STR), N_{min} decreased rapidly to a very low level (<1.0 μg N [g soil]$^{-1}$, day 15). Absolute differences between the four STR treatments (STR × H2O, STR × EM, STR × EM-ster., and STR × MOL), although significant, are extremely small and not consistent during the incubation period. In the lupine-seed-meal treatments (LUP), N_{min} in-

Table 2: Mineral N (N_{min}) (μg N [g soil]$^{-1}$) in soil during 40 d of incubation. Different letters indicate significant differences (Tukey; $p \leq 0.05$) within NON, STR, and LUP treatments at the same sampling date. Standard deviation in parentheses.

Treatment	Days of incubation				
	0	5	15	25	40
	N_{min} / μg N $(\text{g soil})^{-1}$				
NON§ × H2O$	8.5 (± 0.7) ab	11.6 (± 0.8) ab	14.1 (± 0.5) a	16.7 (± 0.9) a	23.2 (± 1.2) b
NON × EM	7.3 (± 0.1) a	10.5 (± 0.9) a	13.4 (± 1.4) a	15.4 (± 2.1) a	19.2 (± 0.4) a
NON × EM-ster.	9.1 (± 1.5) ab	12.3 (± 1.1) ab	14.4 (± 1.0) a	16.4 (± 1.5) a	21.4 (± 1.7) b
NON × MOL	10.6 (± 1.4) b	13.0 (± 1.2) b	15.3 (± 1.4) a	17.5 (± 1.3) a	22.1 (± 1.6) b
STR × H2O	9.9 (± 0.8) a	0.9 (± 0.2) a	0.4 (± 0.1) a	0.5 (± 0.2) a	1.7 (± 0.2) b
STR × EM	11.7 (± 0.9) b	0.8 (± 0.2) a	0.8 (± 0.1) b	0.5 (± 0.3) a	1.1 (± 0.3) a
STR × EM-ster.	11.9 (± 0.6) b	1.6 (± 0.5) ab	0.4 (± 0.2) a	0.5 (± 0.4) a	1.2 (± 0.2) a
STR × MOL	12.4 (± 0.5) b	2.0 (± 0.9) b	0.7 (± 0.1) ab	0.6 (± 0.2) a	1.2 (± 0.3) a
LUP × H2O	13.4 (± 0.8) a	60.9 (± 9.5) a	224 (± 4) a	223 (± 13) a	248 (± 6) a
LUP × EM	14.6 (± 1.7) ab	64.0 (± 3.4) a	227 (± 9) a	229 (± 10) a	255 (± 12) a
LUP × EM-ster.	17.4 (± 1.1) b	59.5 (± 2.3) a	220 (± 10) a	224 (± 8) a	240 (± 13) a
LUP × MOL	15.5 (± 2.3) ab	54.3 (± 2.7) a	223 (± 9) a	216 (± 4) a	255 (± 15) a

§ Application of organic materials: NON = none, STR = wheat straw, LUP = yellow lupine seed meal.
$ Application of suspensions: H2O = water, EM = effective microorganisms, EM-ster. = sterilized effective microorganisms, MOL = molasses.

creased rapidly during the first days of incubation up to a level >200 μg N (g soil)$^{-1}$. Within the LUP treatments, no significant influence of H2O, EM, EM-ster., and MOL on N mineralization could be observed.

Microbial biomass C and N in the soil without OM amendment (NON) fluctuated around average values of 230 μg C_{mic} (g soil)$^{-1}$ and 41 μg N_{mic} (g soil)$^{-1}$ (Tab. 3). STR and LUP treatments showed an increase of C_{mic} and N_{mic} in soil compared to the relatively stable NON treatments, leading to significant differences between mean values of the three treatments (data not shown). Later on, C_{mic} and N_{mic} in STR and LUP treatments decreased again. At the end of the incubation, mean C_{mic} contents in STR and LUP treatments no longer differed from each other, but remained significantly larger than those in the NON treatments (day 40: NON < STR = LUP, Tukey: $p \leq 0.05$). Due to a high background of total soluble N in the nonfumigated soil extracts of LUP treatments, reliable calculations of N_{mic} where not possible at the last three sampling dates. Few significant differences could be detected between EM, EM-ster., MOL, and H2O treatments within NON, STR, or LUP. However, in none of these cases EM differ significantly from all of the other three treatments (H2O, EM-ster., MOL).

Cumulative CO_2-C evolution from soil as an indicator of total soil microbial activity increased with addition of the organic materials and was highest in the LUP treatment. During 40 d of incubation, averages of 348, 768, and 1646 μg CO_2-C (g soil)$^{-1}$ were released from soils in the NON, STR, and LUP treatments, respectively (Fig. 1). Within NON, STR, and LUP, the cumulative CO_2-C efflux at day 40 was lower in the H2O

Table 3: Microbial biomass C (C_{mic}) (μg C [g soil]$^{-1}$) and N (N_{mic}) (μg N [g soil]$^{-1}$) in soil during 40 d of incubation. Different letters indicate significant differences (Tukey; $p \leq 0.05$) within NON, STR, and LUP treatments at the same sampling date. Standard deviation in parentheses.

Treatment	Days of incubation				
	0	5	15	25	40
	C_{mic} / μg C (g soil)$^{-1}$				
NON§ × H2O$	216 (± 10) a	223 (± 3) a	189 (± 35) a	218 (± 9) a	223 (± 13) a
NON × EM	226 (± 9) a	229 (± 10) a	232 (± 64) a	221 (± 10) a	240 (± 19) a
NON × EM-ster.	231 (± 3) ab	215 (± 6) a	267 (± 18) a	218 (± 18) a	238 (± 7) a
NON × MOL	247 (± 9) b	214 (± 8) a	221 (± 62) a	224 (± 14) a	231 (± 9) a
STR × H2O	290 (± 13) a	327 (± 4) a	284 (± 31) ab	291 (± 18) a	290 (± 12) a
STR × EM	284 (± 19) a	320 (± 22) a	374 (± 43) b	306 (± 5) a	308 (± 38) a
STR × EM-ster.	321 (± 16) a	332 (± 10) a	270 (± 63) a	310 (± 13) a	304 (± 14) a
STR × MOL	315 (± 23) a	305 (± 24) a	244 (± 51) a	302 (± 36) a	288 (± 38) a
LUP × H2O	639 (± 81) a	888 (± 76) b	526 (± 49) b	430 (± 15) b	320 (± 60) a
LUP × EM	749 (± 194) a	740 (± 55) a	391 (± 73) ab	380 (± 30) ab	317 (± 112) a
LUP × EM-ster.	697 (± 137) a	664 (± 40) a	459 (± 98) ab	354 (± 19) a	283 (± 16) a
LUP × MOL	781 (± 94) a	794 (± 69) ab	366 (± 63) a	351 (± 31) a	287 (± 22) a
	N_{mic} / μg N (g soil)$^{-1}$				
NON × H2O	45.5 (± 1.7) a	41.8 (± 1.6) ab	32.8 (± 7.3) a	39.0 (± 0.6) a	41.6 (± 3.7) a
NON × EM	47.4 (± 1.6) a	43.5 (± 1.7) b	41.2 (± 6.7) a	40.6 (± 2.3) ab	43.7 (± 2.3) ab
NON × EM-ster.	47.2 (± 2.0) a	39.8 (± 0.7) a	45.5 (± 3.6) a	41.8 (± 3.0) ab	40.8 (± 2.3) a
NON × MOL	45.8 (± 0.5) a	40.4 (± 1.6) ab	35.9 (± 5.9) a	44.3 (± 1.3) b	45.7 (± 2.7) b
STR × H2O	53.5 (± 2.7) a	53.8 (± 1.7) a	43.5 (± 4.9) a	53.5 (± 3.8) a	57.9 (± 2.3) a
STR × EM	53.1 (± 3.8) a	52.3 (± 2.9) a	56.2 (± 4.5) a	55.2 (± 0.7) ab	59.5 (± 4.3) a
STR × EM-ster.	52.7 (± 1.6) a	53.1 (± 1.5) a	44.1 (± 10.9) a	60.3 (± 2.1) b	54.8 (± 1.2) a
STR × MOL	51.3 (± 3.2) a	51.3 (± 1.9) a	45.1 (± 8.3) a	60.1 (± 5.4) b	58.7 (± 4.4) a
LUP × H2O	93.1 (± 7.2) a	232 (± 36) b	n.d.	n.d.	n.d.
LUP × EM	101.8 (± 20.3) a	165 (± 13) a	n.d.	n.d.	n.d.
LUP × EM-ster.	87.8 (± 12.1) a	209 (± 45) ab	n.d.	n.d.	n.d.
LUP × MOL	93.4 (± 7.8) a	190 (± 9) ab	n.d.	n.d.	n.d.

§ Application of organic materials: NON = none, STR = wheat straw, LUP = yellow lupine seed meal.
$ Application of suspensions: H2O = water, EM = effective microorganisms, EM-ster. = sterilized effective microorganisms, MOL = molasses, n.d. = not detected.

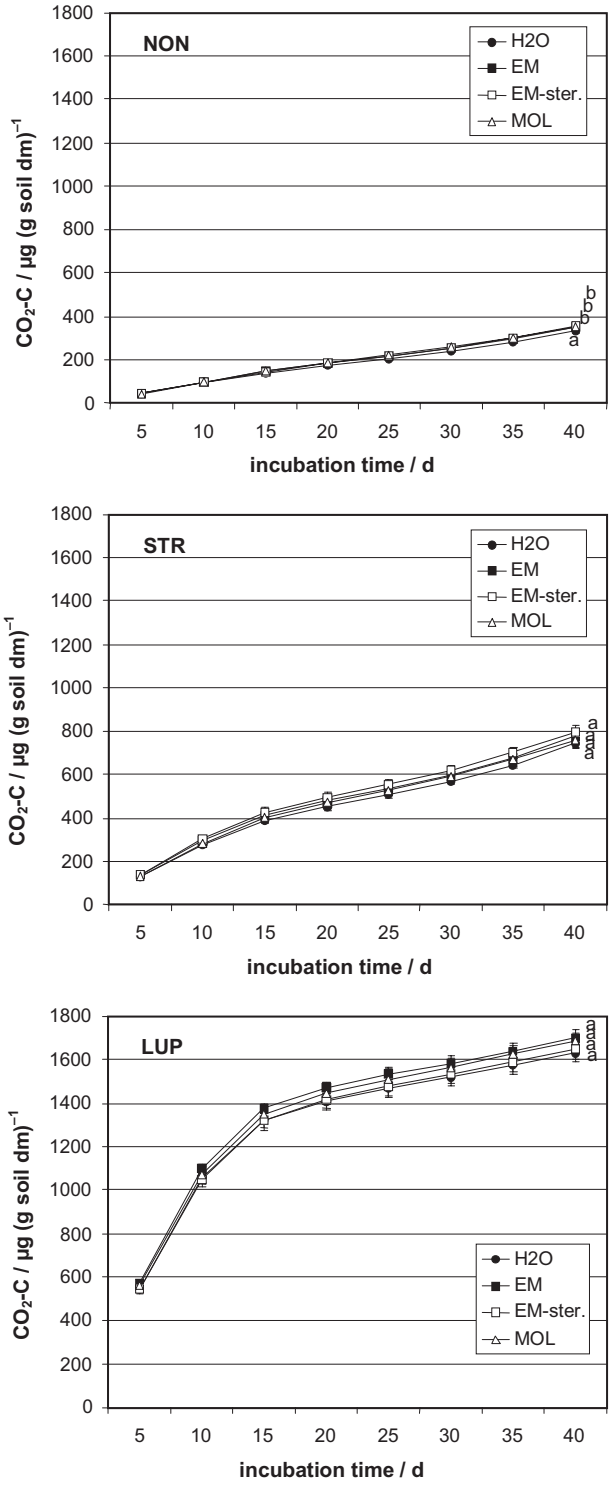

Figure 1: Cumulative CO_2-C evolution (µg CO_2-C [g soil]$^{-1}$) from soil during 40 d of incubation. Application of organic materials: NON = none, STR = wheat straw, LUP = yellow lupine seed meal. Application of suspensions: H2O = water, EM = effective microorganisms, EM-ster. = sterilized effective microorganisms, MOL = molasses. Different letters indicate significant differences (Tukey; $p \leq 0.05$) between applications after 40 d of incubation. Bars indicate standard deviation. Statistical indicators for earlier dates are not shown.

than in EM, EM-ster., and MOL treatments. But this effect was only significant within the NON treatments. Differences between EM, EM-ster., and MOL were very small and nonsignificant. No significant differences between H2O, EM, EM-ster., and MOL could be observed within the STR and LUP treatments. In the STR treatments, a nonsignificant tendency could be observed with highest CO_2 evolution in the EM-ster. and lowest in the H2O treatment.

3.2 Pot experiment with *Lolium perenne*

Mineral N was very low between 0.9 and 1.4 µg N (g soil)$^{-1}$ at both sampling dates. No significant differences between biofertilizer and H_2O applications could be detected (data not shown). There was a general tendency, sometimes also significant, for C_{mic} and N_{mic} to be larger in the H2O treatment than in the other treatments at the end of the experiment (Tab. 4). However, no significant differences in C_{mic} or N_{mic} could be found between the corresponding nonsterilized and sterilized biofertilizer treatments.

Compared to the H2O treatment, both nonsterilized and sterilized biofertilizers already reduced the CO_2-C evolution from soil after the first application at the beginning of the experiment (Fig. 2). These differences increased with the duration of the experiment. At the end, the differences were significant for the BACTOFIL-A and BACTOFIL-B treatments. Nevertheless, no significant differences in soil CO_2 evolution were observed between nonsterilized and sterilized biofertilizers.

Plant dry matter was 1.8–1.9, 2.1–2.2, and 2.9–3.1 g pot^{-1} at days 35, 49, and 87, respectively. No significant differences could be observed between the different treatments within one sampling date. No impact of the added biofertilizers on dry-matter production of *Lolium perenne* could be detected.

4 Discussion

In the first part of this study, the impact of EM-1 after fermentation with molasses (EM-A) on SOM decomposition, soil N mineralization/immobilization, soil microbial biomass, and soil microbial respiratory activity was investigated after addition of different organic materials. As expected, the organic materials added to soil led to differences in C_{org}, N_t, and N_{min} contents in soil and reflected differences in the materials' quality. Contents of C_{org} and N_t at the end of the incubation experiment in soil with the organic amendments indicated a more distinctive decomposition of the added lupine seed meal than of the added wheat straw. This can simply be explained by the different C : N quotients and available C and N pools of the added organic materials lupine seed meal (C : N 6.8) and wheat straw (C : N 103) (*Knapp* et al., 1982; *Reinertsen* et al., 1983). Whereas N_{min} decreased in the straw-added soil, N_{min} increased rapidly during the first days of incubation after lupine addition to soil, indicating a nearly complete net immobilization during decomposition of the added straw and a fast net mineralization of added organic N after lupine addition to soil. In the soil without organic amendments, steady N mineralization could be observed. By simply subtracting mean N_{min} in the soil without organic addition from mean N_{min} in the soil

Table 4: Microbial biomass C (C_{mic}) (μg C [g soil]$^{-1}$) and N (N_{mic}) (μg N [g soil]$^{-1}$) in soil after 35 and 87 d in the pot experiment with *Lolium perenne.* Different letters indicate significant differences (Tukey; $p \leq 0.05$) within the same sampling date. Standard deviation in parentheses.

Treatment	Days			
	35	87	35	87
	C_{mic} / μg C (g soil)$^{-1}$		N_{mic} / μg N (g soil)$^{-1}$	
H2O§	202 (± 6) a	231 (± 6) b	37.6 (± 3.3) a	36.7 (± 1.9) a
EM	201 (± 6) a	212 (± 17) ab	35.5 (± 1.0) a	35.0 (± 1.6) a
EM-ster.	196 (± 5) a	199 (± 10) ab	33.5 (± 4.6) a	33.8 (± 0.7) a
BIO	196 (± 13) a	185 (± 12) a	35.2 (± 2.0) a	33.5 (± 1.7) a
BIO-ster.	207 (± 31) a	178 (± 5) a	36.9 (± 3.8) a	33.2 (± 1.4) a
BAC-A	199 (± 14) a	177 (± 9) a	35.7 (± 0.5) a	31.6 (± 1.6) a
BAC-A-ster.	219 (± 23) a	193 (± 34) ab	38.3 (± 5.1) a	33.4 (± 3.0) a
BAC-B	190 (± 25) a	193 (± 11) ab	35.4 (± 2.1) a	32.8 (± 1.1) a
BAC-B-ster.	200 (± 23) a	199 (± 4) ab	36.4 (± 2.6) a	33.2 (± 1.0) a

§ Applications: H2O = water, EM = effective microorganisms, EM-ster. = sterilized effective microorganisms, BIO = Biostimulator, BIO-ster. = sterilized Biostimulator, BAC-A = BACTOFIL-A, BAC-A-ster. = sterilized BACTOFIL-A, BAC-B = BACTOFIL-B, BAC-B-ster. = sterilized BACTOFIL-B.

with lupine addition at day 40, it can be estimated that 69% of the added lupine-seed N was net-mineralized during the whole incubation period. This is about double the 38% and 35% net N release from added lupine seed meal found by *Müller* and *von Fragstein und Niemsdorff* (2006) and by *Schmitz* and *Fischer* (2003), respectively, during incubation at only 15°C. However, net N mineralization and immobilization observed in the lupine- and straw-amended soils is in accordance with the common understanding of turnover of organic materials characterized by high and low C : N ratios (*e.g., Seneviratne*, 2000; *Palm* et al., 2001). Under the conditions of this study, a clear impact of the EM application on C and N turnover in soil with or without addition of different organic materials as claimed by the producers could not be observed. In particular, when comparing EM with sterilized EM, no effect related to the addition of added living microorganisms (EM) could be detected.

The increase of C_{mic} and N_{mic} in soil with straw and lupine addition compared to soil without OM addition indicates a considerable incorporation of OM into soil microbial biomass, especially after addition of lupine seed meal, which was also observed by *Müller* and *von Fragstein und Niemsdorff* (2006) during 64 d incubation at 5°C and 15°C. Very high initial values (day 0) in the lupine-amended soil indicate either microbial biomass added with the OM or a considerable amount of chloroform-labile C and N in the added lupine seed meal. The few significant differences detected between EM, sterilized EM, molasses, and water application within nonamended, straw-amended, and lupine-amended soil could not be related to one of the applications. An influence of EM application on the microbial-biomass status in soil as claimed by the EM producers could not be observed. Consequently, any effect can simply be explained by pure-substrate addition or by soil wetting. There is no evidence of effects caused by

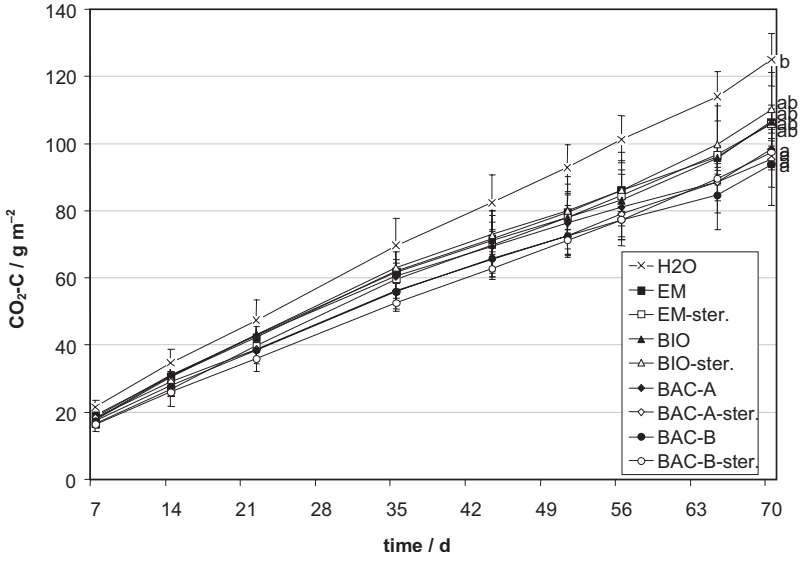

Figure 2: Cumulative CO_2-C evolution (g CO_2-C m^{-2}) from soil in the pot experiment with *Lolium perenne* during 70 d. Application of suspensions: H2O = water, EM = effective microorganisms, EM-ster. = sterilized effective microorganisms, BIO = Biostimulator, BIO-ster. = sterilized Biostimulator, BAC-A = BACTOFIL-A, BAC-A-ster. = sterilized BACTOFIL-A, BAC-B = BACTOFIL-B, BAC-B-ster. = sterilized BACTOFIL-B. Different letters indicate significant differences (Tukey; $p \leq 0.05$) between the applications after 70 d. Bars indicate standard deviation.

added living microorganisms (EM). There was a general tendency for C_{mic} in the lupine-amended soil with water application to be larger than in the other treatments (EM, EM-ster., and MOL). This might indicate a suppressive substrate-induced effect on the zymogene microflora developing on the easily available N-rich organic substrate.

Cumulative CO_2-C evolution from soil as an indicator of total soil microbial activity increased with addition of the organic materials and was highest in soil with lupine-seed-meal addition. Nearly 3% of the native soil organic C was metabolized in the nonamended soil. By simply subtracting cumulative soil CO_2-C evolution from soil without OM addition from those treatments with straw or lupine-seed-meal addition, it can be estimated that 18% and 58% of the C added with straw and lupine seed meal, respectively, was evolved during incubation. In all treatments with water application, the microbial activity was lower than in treatments with EM, sterilized-EM, and molasses application. This effect was significant only in soil without addition of OM. This indicates that C added with the suspensions, mainly molasses C, was highly available for microbial breakdown. However, the strong effects of the added straw and lupine seed meal may have overlapped small effects induced by the addition of the suspensions. Due to the absence of clear differences in CO_2-C release between EM, sterilized-EM, and molasses application within the three different OM treatments, no effects of added living microorganisms could be found. *Filho* et al. (1993) reported an enhanced CO_2-C evolution from soil after EM application, due to an improved decomposition of OM in soils. However, this conclusion was based on comparisons between treatments with and without EM only, and pure-substrate effects, which could have been identified by an additional comparison with sterilized EM, can therefore not be ruled out. *Van Vliet* et al. (2006) used DNA-fingerprinting technique (PCR-DGGE) to investigate if bacteria present in different EM-1 solutions after fermentation with molasses (EM-A) were able to maintain or reach significant relative abundance after addition to slurry manure. They found large variation in bacterial-community structure within different EM-1 solutions and within the fermented EM-A solutions. The EM-A addition to slurry manure had no effect on the bacterial diversity and decomposition of the OM. In the same manner, N mineralization, N uptake by plants and plant growth did not show any effect of EM.

In the pot experiment with *Lolium perenne*, the different biofertilizer applications had no effect on N mineralization and plant growth. But, in the soil with biofertilizer application, clear suppressive effects of the biofertilizer solutions on soil microbial biomass could be observed. However, no differences in soil microbial biomass between application of nonsterilized and sterilized biofertilizer suspensions could be detected. As in the incubation experiment, this might indicate suppressive substrate-induced effects of the biofertilizer suspensions on the zymogene microflora developing on easily available organic substrates (here rhizodeposits). This effect was also clearly visible by reduced CO_2-C evolution from soil with biofertilizer application in comparison to soil with water application right from the beginning of the experiment. However, these differences increased with the duration of the experiment and were significant for the BACTOFIL-A and BACTOFIL-B treatments at the end of the experiment. This indicated an inhibiting effect of the biofertilizers on microbial activity which was in contrast to the incubation experiment, where the addition of the suspensions to soils without organic amendment significantly increased soil CO_2 evolution. This contradiction can be simply explained by the addition of easily decomposable molasses to the different EM solutions in the incubation experiment. Also in the pot experiment, no significant differences in soil CO_2 evolution were observed between nonsterilized and sterilized biofertilizers and an effect of added living microorganisms could not therefore be detected.

4 Conclusions

Under the experimental conditions used here, the hypothesis that the investigated biofertilizers increase microbial biomass and microbial turnover activity in soil was not supported. In addition, the expectation that EM would increase plant growth was not confirmed here. Some minor positive effects of EM on net N mineralization and soil CO_2 evolution could be identified as pure-substrate effects. Possible suppressive effects of biofertilizers on zymogene soil microbial biomass and activity contradict the effects claimed by the producers. For generalization of these findings, further investigations must be conducted under different conditions. This should also include the investigation of potential negative effects on soil fertility and plant growth.

According to European Union Council Regulation (EEC) No 2092/91 (Annex I, 2.4), preparations of microorganisms (biofertilizers) may be used in organic farming to improve the overall condition of the soil or the availability of nutrients in the soil or in the crops, where the need for such use has been recognized by the inspection body or inspection authority. However, this regulation is based neither on a detailed listing of the biofertilizer ingredients nor on clear information about the effects of such products. Our investigation indicates that both are necessary to establish transparency on the biofertilizer market.

References

Anonymous (2004): Tomaten: Effektive Mikroorganismen – Durch Einsatz von Effektiven Mikroorganismen 9,5% Mehrertrag in Tomaten im Folienhaus. Research report, Bayerische Landesanstalt für Weinbau und Gartenbau, Veitshöchheim, Germany.

Brookes, P. C., Landman, A., Pruden, G., Jenkinson, D. S. (1985): Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial nitrogen in soil. *Soil Biol. Biochem.* 17, 837–842.

Daenecke, A., Zschocke, A. K. (2002): Nach der Ernte: EM in den Boden. *EM Journal – Organ des EM e.V. Gesellschaft zur Förderung regenerativer Mikroorganismen* 1, 10–11.

Emiko (2003): EM – Zur Anwendung in Landwirtschaft, Garten, Wasser und Umwelt: Produktanleitung. EMIKO GmbH, Euskirchen-Kirchheim, Germany.

Filho, S. Z., Medeiros, P. R., Kinjo, S. (1993): Influence of EM on Organic Matter Decomposition in Soil under Controlled Conditions, in Parr, J. F., Hornick S. B., Simpson, M. E (eds.).: Third International Conference on Kyusei Nature Farming: Proceedings of the

Conference on Nature Farming for a Sustainable Agriculture. October 5–7, 1993, Santa Barbara, USA, pp. 242–243.

Higa, T. (1991): Effective microorganisms: A biotechnology for mankind, in Parr, J. F., Hornick, S. B, Whitman, C. E. (eds.): First International Conference on Kyusei Nature Farming: Proceedings of the Conference on Kyusei Nature Farming. October 17–21, 1989, Washington, D.C., USA, pp. 8–14.

Higa, T. (2002a): Eine Revolution zur Rettung der Erde: Mit Effektiven Mikroorganismen (EM) die Probleme unserer Welt lösen. 4th edn., Organischer Landbauverlag, Xanten.

Higa, T. (2002b): Die wiedergewonnene Zukunft: Effektive Mikroorganismen (EM) geben neue Hoffnung für unser Leben und unsere Welt. Organischer Landbauverlag, Xanten.

Isermeyer, H. (1952): Eine einfache Methode zur Bestimmung der Bodenatmung und der Karbonate im Boden. *Z. Pflanzenernähr. Bodenkd.* 56, 26–38.

Jörgensen, R. G. (1996): The fumigation-extraction method to estimate soil microbial biomass: Calibration of the k_{EC} value. *Soil Biol. Biochem.* 28, 25–31.

Jörgensen, R. G., Müller, T. (1996): The fumigation-extraction method to estimate soil microbial biomass: Calibration of k_{EN} value. *Soil Biol. Biochem.* 28, 33–37.

Knapp, E. B., Elliott, L. F., Campbell, G. S. (1982): Carbon, nitrogen and microbial biomass interrelationships during the decomposition of wheat straw: A mechanistic simulation model. *Soil Biol. Biochem.* 15, 455–461.

Mayer, J. (2003): Root effects on the turnover of grain legume residues in soil. PhD thesis, Fachbereich Ökologische Agrarwissenschaften, Universität Kassel, Germany.

Mayer, J., Scheid, S., Widmer, F., Fließbach, A., Oberholzer, H.-R. (2007): Wirkungen von Effektiven Mikroorganismen EM auf pflanzliche und bodenmikrobiologischen Parameter im Feldversuch, in Zikeli, S., Claupein, W., Dabbert, S., Kaufmann, B., Müller, T., Valle Zárate, A. (eds.): Beiträge zur 9. Wissenschaftstagung Ökologischer Landbau: Zwischen Tradition und Globalisierung. Universität Hohenheim, Stuttgart, Stiftung Ökologie und Landbau, Bad Dürheim, Hohenheim, Germany, pp. 41–44.

Müller, T., von Fragstein und Niemsdorff, P. (2006): Organic fertilizers derived from plant materials. Part I: Turnover in soil at low and moderate temperatures. *J. Plant Nutr. Soil Sci.* 169, 255–264.

Müller, T., Jörgensen, R. G., Meyer, B. (1992): Estimation of soil microbial biomass C in the presence of living roots by fumigation-extraction. *Soil Biol. Biochem.* 24, 179–181.

Palm, C. A., Gachego, C., Delve, R., Cadisch, G., Giller, K. E. (2001): Organic inputs for soil fertility management in tropical agroecosystems: Application of an organic resource database. *Agric. Ecos. Environ.* 83, 27–42.

Reinertsen, S. A., Elliott, L. F., Cochran, V. L. Campbell, G. S. (1983): Role of available carbon and nitrogen in determining the rate of wheat straw composition. *Soil Biol. Biochem.* 16, 459–464.

Schaller, K. (2000): Praktikum zur Bodenkunde und Pflanzenernährung, 7th edn., Geisenheimer Berichte, Band 2, Gesellschaft zur Förderung der Forschungsanstalt Geisenheim, Geisenheim, Germany.

Schmitz, H.-J., Fischer, P. (2003): Vegetabile Dünger in Substraten für den ökologischen Gemüsebau. *Gemüse* 2, 18–20.

Senenayaka, Y. D. A., Sangakkara, U. R. (eds.) (1997): Fifth International Conference on Kyusei Nature Farming: Proceedings of the Conference on Nature Farming and Effective Microorganisms for Agricultural and Environmental Sustainability. October 23–25, 1997 Faculty of Agriculture, University of Peradniya, Peradniya, Sri Lanka. Publisher: APNAN, Bangkok, Thailand.

Senenayaka, Y. D. A., Sangakkara, U. R. (eds.) (1999): Sixth International Conference on Kyusei Nature Farming: Proceedings of the Conference on Greater Productivity and a Cleaner Environment through Kyusei Nature Farming. October 28–31, 1999; University of Pretoria, Pretoria, South Africa. Publisher: APNAN, Bangkok, Thailand.

Seneviratne, G. (2000): Litter quality and nitrogen release in tropical agriculture: a synthesis. *Biol. Fertil. Soils* 31, 60–64.

Vance, E. D., Brookes, P. C., Jenkinson, D. S. (1987): An extraction method for measuring soil microbial biomass C. *Soil Biol. Biochem.* 19, 703–707.

van Vliet, P. C. J., Bloem, J., de Goede, R. G. M. (2006): Microbial diversity, nitrogen loss and grass production after addition of Effective Micro-organisms (EM) to slurry manure. *Appl. Soil. Ecol.* 32, 188–198.

Wu, J., Jörgensen, R. G., Pommerening, B., Chaussod, R., Brookes, P. C. (1990): Measurement of soil microbial biomass C by fumigation-extraction – an automated procedure. *Soil Biol. Biochem.* 22, 1167–1169.

Xu, H., Parr, J. K., Umemura, H. (2000): Nature Farming and Microbial Applications. Food Product Press, Binghamton, N. Y.

