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ORIGINAL ARTICLE

Impact of forest organic farming change on soil microbial C turnover using ^{13}C of phospholipid fatty acids

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Abstract Situated in the fast-developing country of Brazil, the Atlantic rainforest Mata Atlântica faces risks generated by population growth-derived problems. Conversion of forest to agriculture has led to a mosaic landscape with fragments of secondary forest in agricultural land. This disturbance to a naturally well-adapted ecosystem prompts rapid soil degradation. Therefore, here we compared soil C incorporation into soil microorganisms and their turnover in typical land-use systems such as primary forest, secondary forest, and agricultural land at the Atlantic Plateau of São Paulo, Brazil. In C_3 and C_4 plants having different $^{13}C/^{12}C$ compositions, a C_3–C_4 vegetation change was induced using maize, a C_4 plant. We measured the $\delta^{13}C$ composition of individual phospholipid fatty acids (PLFA) because PFLA are specific of typical microbes. Results show that statistical analysis of soil PLFA allow differentiation of four microbial units: (1) Gram-positive bacteria; (2) anaerobic Gram-positive bacteria; (3) fungi, vesicular–arbuscular mycorrhizal fungi, and Gram-positive bacteria; and (4) actinomycetes and Gram-positive bacteria. We also found that soil organic matter is cycled for longer time in primary forest ecosystems, of mean turnover time of 28 years, than in agricultural ecosystems with mean turnover time of 4 years for organic farming and 8 years for conventional farming. Calculation of maize-derived carbon of each microbial unit suggested that fungi and vesicular–arbuscular mycorrhizal fungi dominate microbial activity in primary forest whereas Gram-negative bacteria are prominent in the agricultural sites. To conclude, we found that PLFA profiles are sensitive to land-use conversion, and their compound-specific stable-isotope analysis can strongly discriminate between different managements.

Keywords Mata Atlântica · Disturbance · Carbon-13 · Link between soil microbial structure and its function · Phospholipid fatty acid · SOM turnover · C incorporation

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1 Introduction

World population growth and its political and ecological consequences will be one of the central issues in the twenty-first century. Especially developing countries in the Southern hemisphere will be affected by a greater demand for living space and the production of food. Soil degradation, caused by soil mismanagement, as well as severe droughts accelerates this phenomenon (Lal 2009a). Thus, the need for identifying processes, practices, and policies that govern sustainable management of soil resources is more critical now than ever before (Lal 2009b). Situated in

the fast-developing country of Brazil, the Atlantic rainforest Mata Atlântica faces the dangers generated by these problems. Since the sixteenth century, the time when the colonizers arrived, more than 95% of the native forest vegetation has been replaced by agro-ecosystems and living space, being one of the largest destruction of a natural forest ecosystem without reforestation (Diegues 1995). Land clearance and intense farming systems endanger land conservation, raising an urgent need to implement appropriate land management which has a large scale perspective but acts at the local level (Zuazo and Pleguezuelo 2008). Soil is one of the key driving factors of sustainability as it provides ecosystem services to food production activities (Lal 2008), especially in the tropics where soil fertility is typically low. Appropriate soil management techniques such as reduced or no tillage are inevitable for diversification and sustainability in food production, the two main millennium goals in agriculture (Zuazo et al. 2008; Roger-Estrade et al. 2009).

The key driving factor for mineralization of soil organic matter (SOM) and nutrient supply is composition and activity of the soil microbial community (Zech et al. 1997). The microbial biomass belongs to the labile SOM pool and is therefore highly relevant to soil fertility in tropical soils (Zech et al. 1997). In the past, mainly physicochemical analysis, for example pH, cation exchange capacity, total organic carbon, and plant available nutrients were used to describe the effect of stress to soil (Kaur et al. 2005). However, the soil microbial community reacts faster to disturbance and is therefore a more appropriate indicator of soil health (Kaur et al. 2005). To study the soil microbial community structure, phospholipid fatty acid (PLFA) profile analysis has obvious advantages compared to other conventional culture-based methods, as it accounts for a larger proportion of the soil microbial community (Tunlid and White 1992). Phospholipid fatty acids are useful biomarkers of the soil microbial community as the relative abundance of certain PLFA differs significantly between specific groups of microorganisms (Tunlid and White 1992; Zelles et al. 1994) allowing individual groups of microbes to be identified in soils (Gattinger 2001; Zelles 1999). Hence, the microbial community should be studied as such and in terms of microbial diversity for assessing the real picture of an ecosystem, which may reflect the immediate displacement of organisms by stress and long-term effects on (e.g., carbon) processes caused by successions in the microbial community (Kaur et al. 2005).

Studying carbon processes, different methods are used to characterize and quantify carbon turnover in soils. In arable lands, storage of soil organic carbon (SOC) is a function of carbon additions, e.g., organic amendments, crop residues, and carbon losses such as CO_2, dissolved organic carbon through organic matter decomposition. A well-known technique for calculating these carbon processes is the litter bag experiment as described by Berg et al. (1993). Rezende et al. (1999) showed that this method underestimates litter decomposition because the mesh of the nylon bag does not allow access by soil macro fauna. Experiments in the Mata Atlântica rainforest using "covered litter" have provided more accurate results (Rezende et al. 1999). Oven-dried litter samples were laid on a cleared area of the soil surface and covered with nylon mesh held down with mails. In this manner, the entry of soil fauna was permitted (Rezende et al. 1999).

For a quantitative analysis of SOC dynamics, it is necessary to trace the origin of the soil carbon compounds and the pathways of their transformation (Flessa et al. 2000). Studies at molecular level imply that the dynamics of natural substances are more complex than previously recognized having considerable geochemical and environmental implications (Lichtfouse 1997). Thus, using stable carbon isotope experiments represents a fruitful approach to study organic matter transformations in soils (Lichtfouse et al. 1995). For this reason in our study, the method of Rezende et al. (1999) was further improved using a ^{13}C natural abundance tracer technique. Maize straw (C_4 plant) was laid on the soil surface cleared from surrounding C_3 litter and covered with nylon mesh as described by Rezende et al. (1999). In this manner, the transformation pathway of the C_4-derived carbon could be determined. Such tracer experiments at natural ^{13}C abundance (C_3–C_4 transition) are a useful tool for field studies (Glaser 2005) because the contribution of isotopically distinct carbon sources within different chemical compounds can be evaluated (Lichtfouse 1995). With the advance in analytical techniques, especially with the development of gas chromatography–combustion–isotope ratio mass spectrometry (GC-C-IRMS), it is possible to analyze stable isotope ratios of a number of biomarkers with excellent sensitivity and with respect to both concentration and isotope ratio (Brenna et al. 1997; Hayes et al. 1990; Lichtfouse 2000; Glaser 2005). This approach allows the identification and quantification of PLFA with concurrent determination of ^{13}C incorporation, thus linking microbial structure and function.

The objective of this work was to investigate the microbial litter decomposition at typical land-use sites such as primary forest, big and small fragments of secondary forest, and agricultural land under organic and conventional farming in the Mata Atlântica rainforest region of Brazil using bulk and compound-specific ^{13}C stable isotope tracing of maize-derived carbon into PLFAs.

2 Material and methods

2.1 Investigation area

The investigation area was situated at the Ibíuna Plateau near Caucaia do Alto, about 40 km southwest of São Paulo,

Brazil (23°39′ S–23°47′ S, 46°55′ W–47°6′ W, 800–1,000 m above sea level). Temperature and precipitation in the southern part of the Atlantic rainforest ranged between 17°C and 24°C and 1,250–2,000 mm, respectively (Behling 1998). The climate in the region of São Paulo was characterized by mean winter temperatures of 15°C and mean annual rainfall of 1,700 mm (Ledru et al. 2005). Semi-deciduous forest and Cerrado are the major modern natural vegetation types (Hueck 1966). Cerrado in a strict sense is defined as tree and scrub woodland with 2–5 m tall trees and an open tree canopy (Behling 1998). Deeply weathered soils were classified as Ferralsol (FAO 1990). They are characterized by low pH values (3.4–5), extremely low effective cation exchange capacity (2–3 $cmol_c kg^{-1}$), a moderate to high base saturation (40–100%) probably due to fertilization, and a high SOM content between 2.6% and 7.6% including humic substances, high molecular weight organic substances such as polysaccharides and proteins, and simpler substances such as sugars and amino acids (Zech 2004).

2.2 Experimental design and sampling

The objective of this project was to study the effects of land management on the soil organic matter dynamics and microbial pool. The area is characterized by a fragmented landscape and a large lower mountainous Atlantic Forest area (Morro Grande Reserve). The fragmented area consists of secondary forest fragments in an agricultural landscape. The Morro Grande Reserve consists mainly of secondary forest as well, with only a minor part of mature forest. The study area was chosen because of its homogeneity in terms of type of forest, relief, the existence of a continuous area of secondary forest comparable to that of fragments, and the relative low amount of remaining native forest. A more detailed explanation of the study area can be found in Pardini et al. (2005). For this purpose, the following habitats were compared: Relics of primary forest (Morro Grande Reserve), large fragments of secondary forest (50–80 years old), small fragments of secondary forest (50–80 years old), and agricultural land under organic and conventional farming (20–30 years old). A random grid sampling design was used (BGZufGen program; Fig. 1). For avoiding edge effects, the outermost sub-plots were not taken into account. Each of these five habitats was represented by three independent plots (n=3). To investigate field heterogeneity, one of the three plots per habitat was intensively sampled, taking three sub-samples of this plot. These sub-samples were analyzed in the same way as the plot samples. Plot and sub-plot results were compared per habitat to investigate field heterogeneity.

In 2003 (September to October), the sampling sites were setup. One kilogram of dried maize (<4 cm pieces of leafs and main stems) was put onto 1-m^2 cleared areas of the soil surface and covered with nylon mesh (mesh size 2 mm) held down with nails. In this manner, the maize (C_4) was in direct contact with the soil surface and allowed entry of soil fauna. The mesh and regular removing of C_3 litter from the plots avoided input of surrounding C_3 vegetation from litterfall as well as leaching of soluble carbon from the litter. After 9 months, the maize was decomposed at the organic farming sites as no maize was visible at the soil surface. Therefore, new maize straw was put onto these plots again. To quantify the litter input, beside the plots, a further nylon mesh (1 m^2, nylon mesh 2 mm) was spanned in a height of 30 cm above the soil surface. Original litter beneath the nylon mesh was used to analyze the decomposition rate and compared with that of the maize.

Samples were taken in August 2004 after 11 months of incubation at the end of the dry season and in April 2005 after 18 months of incubation at the end of the rainy season. The litter samples taken in 2004 were dried and stored at 20°C in an air-conditioned laboratory and the soil samples taken in 2004 kept frozen until analysis in April 2005. Soil samples taken in 2005 were kept in refrigerator until analysis. For sampling, the 1-m^2 area was subdivided in 10×10 cm sub-plots. The soils from three random sub-plots (Fig. 1) were combined homogeneously to give one representative sample. Soil samples were taken with a cylindrical stainless steel drill (r=4 cm); divided into 0–2,

	A	B	C	D	E	F	G	H	I	J
1										
2				12	7		26	22	20	
3							16	3		
4		8	14		5			24		
5		17	2		18			15	21	
6			19		13			29	11	
7		4	30	1			28	9		
8		25	23	6						
9		10		27						
10										

Fig. 1 Plot design and sampling strategy for a long-term field incubation study with labeled plant material. *Individual numbers* indicate subsequent sampling positions within the grid. Please note that border cells are ignored due to exclusion of border effects. Therefore, plot design allows 64 destructive samplings. Sampling positions were determined randomly using the program BGZufGen

2–5, and 5–10 cm depth horizons; and stored in plastic bags. Maize and litter were stored in paper bags and dried. Reference soil samples (C_3) representing the original conditions were taken in April 2005 next to the maize incubation plots.

2.3 Sample preparation and instrumentation for bulk $\delta^{13}C$

The maize litter, original litter, and soil samples were oven-dried for 2 days at 50°C, manually cleaned with tweezers from residues such as insects or soil and ground, and weighed into tin capsules. Bulk $\delta^{13}C$ determinations were performed using a CHN-1110 Elemental-Analyzer (Carlo Ebra, Italy) coupled to a Thermo Finnigan IRMS under continuous flow of ultrapure Helium (6.0=99.9999%). Stable isotope measurements for carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) were done by comparison with known standard gases, which were calibrated against international standards (VPDB and atmospheric N_2, respectively). Laboratory standards (atropine for plant material and Leco soil standard for soil samples) were used as quality control (precision ~0.2‰ for C and 0.5‰ for N).

2.4 Soil microbial biomass analysis by fumigation-extraction method: quantification and $\delta^{13}C$ analysis

Soil microbial biomass C was determined by the fumigation-extraction method using a modified method of Gregorich et al. (2000). Soil samples from the maize incubation plots (0–2 cm) and the reference plots (0–10 cm) were analyzed. Fresh soil samples were sieved (<2 mm), and roots and plant residues were removed. The frozen samples taken in 2004 were thawed overnight in the refrigerator. Afterward, they were incubated overnight under conditions of room temperature, and 5 mL of distilled water was added. Koponen et al. (2006) showed that soil microbial biomass was not influenced by four repeated freeze–thaw cycles. Soluble C extract was obtained by extraction of 25 g of soil with 100 mL 0.5 M potassium sulfate (K_2SO_4).

Soluble organic carbon was determined on a total organic carbon analyzer (Shimadzu 5000-A, Tokyo, Japan). Potassium hydrogen phthalate solution was used for calibration. The arithmetic mean was calculated from three replicates.

Soil microbial biomass was calculated as the difference of soluble organic carbon between fumigated and non-fumigated samples divided by 0.35 (Sparling et al. 1990). The solution samples were freeze-dried and weighed into tin capsules. $\delta^{13}C$ measurement was performed as described above for bulk $\delta^{13}C$ measurement for soil and litter. The $\delta^{13}C$ (per mil) value of the microbial biomass was calculated according to isotope mass balance of $\delta^{13}C$ of extracted carbon from fumigated and non-fumigated samples.

2.5 Sample preparation and instrumentation for compound-specific $\delta^{13}C$ analysis of phospholipid fatty acids

Soil samples (0–2 cm) from maize incubation sites (C_4) taken in 2005 were analyzed. Soil samples (0–2 cm) taken in 2003 next to the plots were used as a reference (C_3) to determine the possible influence of C_3–C_4 transition on microbial community structure. Extraction of phospholipids was performed using a modified method of Frostegard et al. (1991). Plastic centrifuge tubes were used for extraction because glass ones were not available. Therefore, one extraction was done without soil sample (control sample). Figure 2 shows a GC-C-IRMS chromatogram for PLFA from a standard (A), soil sample (B), and control sample (C) illustrating that no contamination by plasticizers or fatty acids from the plastic to the relevant PLFA can be observed.

Roughly 5 g of fresh and sieved (<2 mm) soil was weighed into a plastic centrifuge tube, and 100 μL of L-α-phosphatidylcholin-1,2-dinonadecanoyl (19:0, Larodan Fine Chemicals, Malmö, Sweden) was added as internal standard 1 (IS 1). Phospholipids were extracted by adding 18 mL of monophasic mixture of methanol–chloroform–citrate buffer (0.15 M citric acid, pH 4.0) in a 2:1:0.8 volume ratio, horizontally shaken, and subsequently centrifuged. The supernatant was decanted and extra volumes of monophasic mixture were added. Both extracts were combined, chloroform and citrate buffer were added, and the mixture separated overnight. Two times 3 mL from the lower organic phase (chloroform) were transferred into a new centrifuge tube, and the chloroform was subsequently evaporated. For compound-specific stable-isotope analysis, the residue was dissolved in a small amount of ethanol (absolute pure, >99.5%), quantitatively transferred into 5 mL glass vials, and kept at −18°C until lipid fractionation.

Total lipids were fractionated using silica column chromatography. The ethanol was evaporated under a gentle stream of nitrogen, and the residue was dissolved in 200 μL chloroform. Lipids extracted were loaded onto an activated (300°C over night), pre-conditioned (chloroform) silica column (0.063–0.200 mm), and fractionated into neutral lipids, glycolipids, and phospholipids with volumes of chloroform, acetone, and methanol, respectively. PLFA were hydrolyzed at 100°C for 10 min in alkaline solution and derivatized to fatty acid methyl esters (FAME) with BF_3 (boron trifluoride) in methanol according to Knapp (1979). In contrary to alkaline methanolysis proposed by Zelles (1999), this method is also suitable for pure fatty acid standards to quantify isotope fractionation during derivatization. One hundred ninety microliters of toluene and 10 μL of tridecanoic acid methyl ester (13:0 FAME; Sigma-Aldrich, Seelze, Germany; c=1 gL^{-1} in toluene)

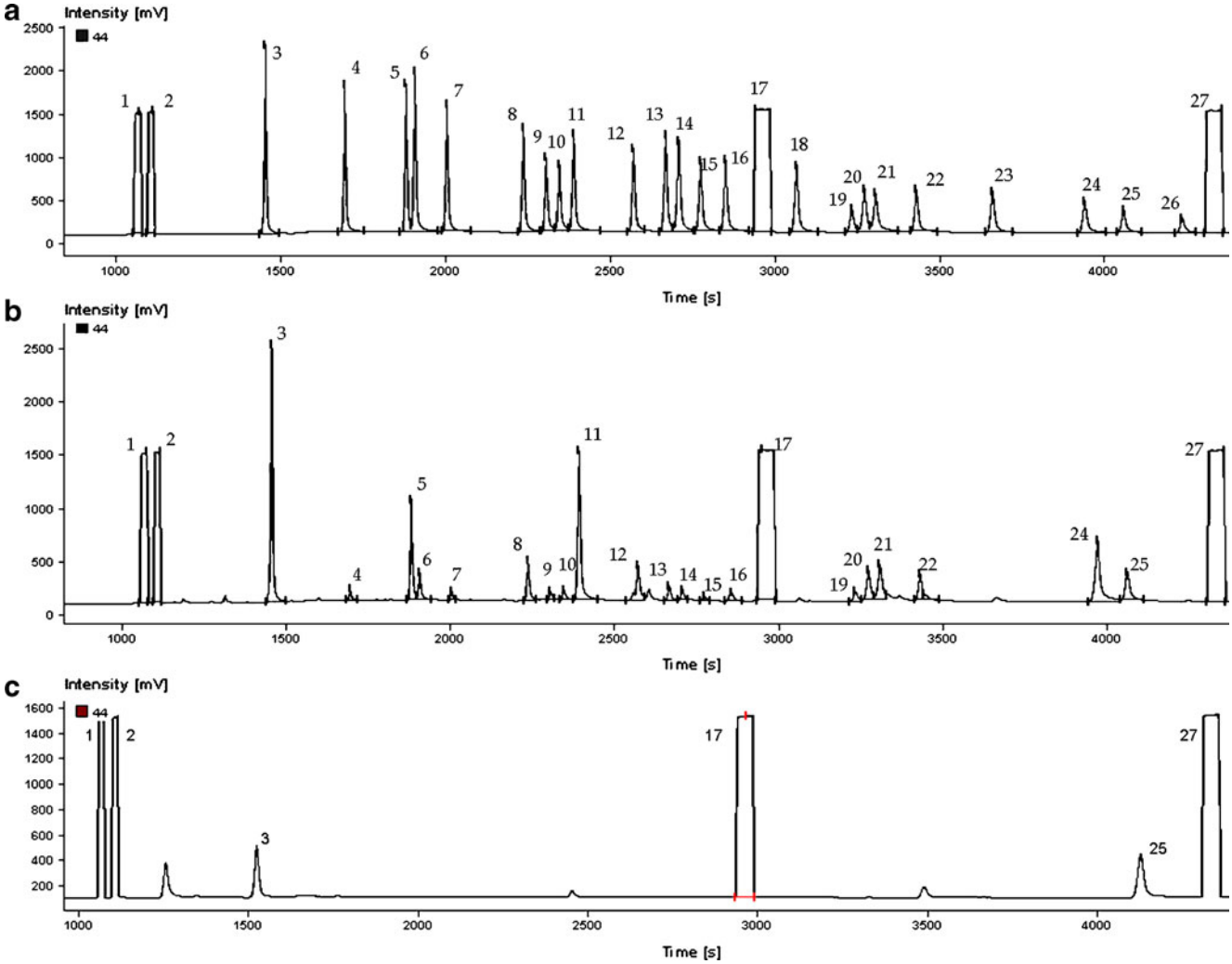

Fig. 2 Representative GC-C-IRMS chromatogram for phospholipid fatty acid analysis from standard (**a**), soil sample (**b**), and control sample without soil (**c**). CO_2 signal from mole weight 44 is shown. *1* reference gas, *2* reference gas, *3* 13:0 (IS 2), *4* 14:0, *5* i15:0, *6* a15:0, *7* 15:0, *8* i16:0, *9* 16:1ω7c, *10* 16:1ω5c, *11* 16:0, *12* 10Me16:0, *13* i17:0, *14* a17:0, *15* cy17:0, *16* 17:0, *17* reference gas, *18* 10Me17:0, *19* 18:2ω6.9, *20* 18:1ω9c, *21* 18:1ω7c, *22* 18:0, *23* 10Me18:0, *24* cy19:0, *25* 19:0 (IS1), *26* 20:4ω6, *27* reference gas

were added for sample dilution and as recovery standard, respectively. The samples were transferred into autosampler vials and kept at −18°C until measurement.

Stable carbon isotope ratios of pure fatty acids were used as standard compounds; internal standards, derivatization reagent (only methanol), and bulk $\delta^{13}C$ of samples were determined by continuous-flow elemental analysis–isotope ratio mass spectrometry (EA-IRMS) linking a Carlo Erba NC 2,500 (Thermo Finnigan MAT, Bremen, Germany) to a Delta^{plus} IRMS (Thermo Finnigan MAT, Bremen, Germany) via a Conflo II interface (Thermo Finnigan MAT; Bremen, Germany). During individual measurements, three pulses of CO_2 (99.7% purity; Riessner, Lichtenfels, Germany) were directly discharged into the IRMS for 20 s as reference gas for drift correction. Calibration of carbon isotope measurements was done by ^{13}C measurement of the certified standards sucrose (CH-6, IAEA, Vienna, Austria, $\delta^{13}C$=−10.47‰) and $CaCO_3$ (NBS 19, Gaithersburg, USA, $\delta^{13}C$=+1.95‰) under the same conditions.

Compound-specific stable isotope measurements of individual PLFA were performed using a GC-C-IRMS system consisting of a Trace GC 2,000 gas chromatograph (Thermo Finnigan MAT, Bremen, Germany) equipped with a split–splitless injector. For chromatographic separation, a BPX5 column (60 m×0.250 mm×0.25 μm film thickness of a cross-linked copolymer of 5% diphenyl- and 95% dimethyl-polysiloxane; SGE, Ringwood, Victoria, Australia) was used. The connection to the Combustion Interface III (Thermo Finnigan MAT, Bremen, Germany) for compound-specific $\delta^{13}C$ measurements and simultaneously for compound identification measurements via an ion trap organic mass spectrometer (Polaris GCQ, Thermo Finnigan MAT, Bremen, Germany) was accomplished with a VALCO crosspiece. A split ratio of about 9 was achieved by using

capillary diameters of 0.32 mm i.d. for the Helium flow into the IRMS and 0.05 mm i.d. into the ion trap MS. The temperature of the combustion and the reduction ovens were set to 940°C and 600°C for all measurements, respectively. Helium (99.996% purity; Riessner, Lichtenfels, Germany) was used as carrier gas. Isotope ratios were measured using a Delta^{plus} IRMS (Thermo Finnigan MAT, Bremen, Germany). Injection was done using an autosampler (AS 2000, Thermo Finnigan MAT, Bremen, Germany) with a 10-μL syringe with 70 mm needle length (IVA, Meerbusch, Germany). During all measurements, three pulses of CO_2 (99.7% purity; Riessner, Lichtenfels, Germany) were directly discharged into the IRMS for 20 s as reference gas for the calculation of relative $\delta^{13}C$ values and drift correction. Fatty acid notation was used as described by Crossman et al. (2004) and Zelles (1999).

2.6 Calculation of PLFA $\delta^{13}C$ values and correction for derivatization carbon and amount dependence

$\delta^{13}C$ values of individual PLFA of the soil samples were calculated from four replicate GC-C-IRMS measurements of the derivatives and four replicate EA-IRMS measurements of derivatization reagent according to Eq. 1,

$$\delta^{13}C_{PLFA} = \left(N_{Der}\delta^{13}C_{Der,\,corr} - F - N_{Me}\delta^{13}C_{Me}\right)/N_{PLFA} \quad (1)$$

where N is the number of C atoms of the PLFA derivative (N_{Der}), the original PLFA molecule (N_{PLFA}), and the carbon added via methylation (N_{Me}). F is a correction factor compensating for any offset between EA-IRMS and GC-C-IRMS measurements, discrimination during derivatization, amount dependence, etc. (Glaser and Amelung 2002; Gross and Glaser 2004; Schmitt et al. 2003). The correction factor F was determined individually for all PLFA using changes in $\delta^{13}C$ values of six derivatized standard solutions in the concentration range between 1.0 and 10 μg vial^{-1}. The $\delta^{13}C$ values of individual PLFA derivatives obtained by GC-C-IRMS analysis were corrected for reference gas drift ($\delta^{13}C_{Der,corr}$). In case of amount dependence, F in Eq. 1 is a function of IRMS signal intensity (Glaser and Amelung 2002; Schmitt et al. 2003). Due to the fact that there is no certified calibration standard available for GC-C-IRMS measurements, the recovery standard (13:0 FAME, IS 2) was calibrated against certified standards by means of EA-IRMS measurement. This value was used to calibrate GC-C-IRMS $\delta^{13}C$ measurements and to correct for systematic differences between EA- and GC-C-IRMS measurements as well as for discrimination during derivatization which is known to be significant but reproducible for methylation reactions (Docherty et al. 2001; Rieley 1994).

2.7 Calculation of C_4 carbon content, mean turnover time, and fungal to bacterial ratio

2.7.1 Calculation of C_4 carbon content

Fraction of C_4 carbon incorporated into total SOC, soil microbial biomass (SMB), and PLFA were calculated according to Eq. 2,

$$x = \frac{\delta_{sample(SOM/SMB/PLFA)} - \delta_{C3(SOM/SMB/PLFA)}}{\delta_{C4(maize\ straw)} - \delta_{C3(vegetation)}} \quad (2)$$

where x is the fraction of incorporated C_4 carbon; δ_{C3} (denominator) is the $\delta^{13}C$ value of C_3 vegetation; δ_{C4} is the $\delta^{13}C$ value of maize straw; δ_{C3} (numerator) is the $\delta^{13}C$ value of soil organic matter (SOM), SMB, or PLFA from the reference sites without maize; and δ_{sample} is the $\delta^{13}C$ value of SOM, SMB, or PLFA from the maize incubation sites. Calculating the incorporated C_4 carbon of the PLFA, the arithmetic mean of the relevant fatty acids was used.

2.7.2 Mean turnover time

To evaluate how quickly the SOM in these systems shifts from C_3 to C_4 under different land-use systems and thus how fast soil microbial C is turned over, the output of a one-parameter model (Eq. 3) was compared to the bulk $\delta^{13}C$ data. As described by Burke et al. (2003), the model was based on the fact that the soil microbial community used one SOM pool. In the case of absence of substantial justification for dividing SOM into different compartments based on lability, the simplest models possible should be used (Bernoux et al. 1998). The mean turnover time ($1/k$) was estimated for SOM:

$$y = a \times (1 + \exp(-k \times t)) \quad (3)$$

where y is the bulk $\delta^{13}C$ value of SOM, a is the mean $\delta^{13}C$ value of maize straw, and t is the time since the beginning of the experiment.

2.7.3 Fungal to bacterial ratio

According to Kaur et al. (2005), the ratio of fungal to bacterial soil microbial biomass was calculated to study the state of soil microbial community using PLFA i15:0, a15:0, 15:0, i16:0, 16:1ω7c, 10Me16:0, i17:0, a17:0, 17:0, cy17:0, 10Me17:0, 18:1ω7c, 10Me18:0, and cy19:0 for bacteria and 18:2ω6.9 for fungi.

2.8 Statistical analysis

Statistical analyses were carried out using STATISTICA 6.0. Microbial community structure differences were

evaluated by principal component analysis (PCA). According to the Kaiser criteria, the number of factors (principal components) with an Eigenvalue greater than one was selected (Stoyan et al. 1997). Fatty acid methyl ester concentrations were converted to PLFA mole percentage. According to Zelles (1999), PLFA characteristic for a broad spectrum of bacteria (e.g., 14:0, 16:0, and 18:0) was excluded from analysis. Cluster analysis was performed with the factor values received from PCA to create similar microbial community groups. As distance unit, the Euclidian distance was selected. According to the Wards method, the fusion algorithm of hierarchic agglomerative process was used for creating clusters (Backhaus et al. 2003). Using the elbow criteria, the number of clusters was selected in that way that a further fusion would have led to rapid increase of the error square sum (Janssen and Laatz 1999).

To test the significant differences among different treatments and sampling dates, the Mann–Whitney *U* test was carried out. This test is the nonparametric alternative to the *t* test for independent samples (Stoyan et al. 1997). The interpretation of the Mann–Whitney *U* test is essentially identical to the interpretation of the result of a *t* test for independent samples (Stoyan et al. 1997).

3 Results and discussion

The aim of this work was to investigate soil microbial C turnover at typical land-use sites in the fragmented landscape of Mata Atlântica rainforest assessing bulk and compound-specific ^{13}C stable isotope tracing of maize-derived carbon into individual PLFA.

3.1 Litter input

Litter input at the primary forest sites was higher (27±3 $g\,m^{-2}\,month^{-1}$) compared to both secondary forest fragment sites (large 22±19 $g\,m^{-2}\,month^{-1}$; small 22±11 $g\,m^{-2}\,month^{-1}$; Table 1). Van Schaik and Mirmanto (1985) determined litterfall in undisturbed tropical rainforests ranging between 38 and 69 $g\,m^{-2}\,month^{-1}$. Li et al. (2005) reported litterfall in a tropical secondary forest in Puerto Rico of about 60 $g\,m^{-2}\,month^{-1}$. Therefore, litter input of primary and secondary forests at the Mata Atlântica site is in the same order of magnitude compared to other tropical regions and matches the observation from other studies (e.g., Cadisch et al. 1996; Camargo et al. 1999) that litterfall in secondary forest is lower than in primary forest.

3.2 Litter and maize decomposition

Litter decomposition was faster under secondary forest fragments (large 18±7 $g\,m^{-2}\,month^{-1}$; small 77 $g\,m^{-2}\,month^{-1}$) than under primary forest (1 $g\,m^{-2}\,month^{-1}$; Table 1), corresponding to the observation during sampling that organic matter in the primary forest accumulated up to a 10-cm-thick organic layer on the soil surface. In contrast to the original litter, maize decomposition on both secondary forest fragments (large 106±36 $g\,m^{-2}\,month^{-1}$; small 91±3 $g\,m^{-2}\,month^{-1}$) as well as on the primary forest sites (122±4 $g\,m^{-2}\,month^{-1}$) was much faster (Table 1). The highest value was observed on the organic farming site with 150±83 $g\,m^{-2}\,month^{-1}$ whereas on the conventional farming site decomposition was slower (79±14 $g\,m^{-2}\,month^{-1}$).

Table 1 Litter input and decomposition, fungal to bacterial ratio, and SOM mean turnover time

Land use	Litter input ($g\,m^{-2}\,month^{-1}$)	Litter decomposition ($g\,m^{-2}\,month^{-1}$)	Maize decomposition ($g\,m^{-2}\,month^{-1}$)	Index of fungal to bacterial ratio (0–2 cm) 18 months	SOM mean turnover time $y=a\times(1+\exp(-k\times t))$			
					a (‰)	k ($month^{-1}$)	R^2	$1/k$ (year)
Secondary forest large fragment	22±19[a]	18±7[b]	106±36[a]	0.10±0.03	−14.53	–	0.00	–
Secondary forest small fragment	22±11[a]	77[b]	91±3[a]	0.07±0.02	−13.85	0.009	0.87	9
Primary forest	27±3[a]	1[b]	122±4[a]	0.06	−13.59	0.003	0.93	28
Organic farming	–	–	150±83[a]	0.14±0.03	−12.01	0.02	0.96	4
Conventional farming	–	–	79±14[a]	0.14	−11.32	0.01	0.98	8

Except for calculation of mean turnover time, the arithmetic mean with ±1 standard deviation is presented

[a] October 2003 to April 2005

[b] August 2004 to April 2005

Different factors may have influenced decomposition of maize litter varying between the sites. Accelerated decomposition on the organic farming site could be attributed to the exposed location of the maize litter. Decomposition of litter and SOM are controlled by abiotic factors such as temperature, water availability, substrate quality, or UV light (Criquet et al. 2004). Additionally, in the tropics ants are known to accelerate litter decomposition (Murase et al. 2003; Zientz et al. 2005), and a high ant activity was observed on the organic farming site during sampling. The low value for the conventional farming plot may be due to the type of cultivation. The use of pesticides such as fungicide could lead to inhibition of microbial activity (Schmidt et al. 2000) in conventional farming. However, on the forest plots, the different biochemistry between forest leaves and maize might affect the different decomposition. A second crucial aspect might be the fact that the forest litter has already undergone a certain degree of decomposition compared with the maize.

3.3 ^{13}C incorporation into soil organic matter and C turnover

^{13}C incorporation into soil by both fungi and bacteria is reflected by decreasing $\delta\ ^{13}C$ values in the order of initial litter, bulk soil, and PLFA samples (Table 2). In the upper 0–2 cm, the decomposition of maize litter (C_4) was reflected by a higher ^{13}C signature of SOM after 18 months (Fig. 3) while deeper layers (2–5 and 5–10 cm) showed no significant ^{13}C incorporation (data not shown). After 11 (data not shown) and 18 months (Fig. 3) of maize incubation, the maize-derived carbon (^{13}C content) of SOM (0–2 cm) in both large (10±3% and 12±3%, respectively) and small (9±3% and 8±4%, respectively) fragments of the secondary forest was significantly ($p<0.05$, for large fragment) higher than at the primary forest site (2±3% and 4%, respectively). After 18 months, the ^{13}C content of SOM was the highest at the organic farming site (17±4%) significantly ($p<0.05$) differing to both secondary forest fragments (Fig. 3).

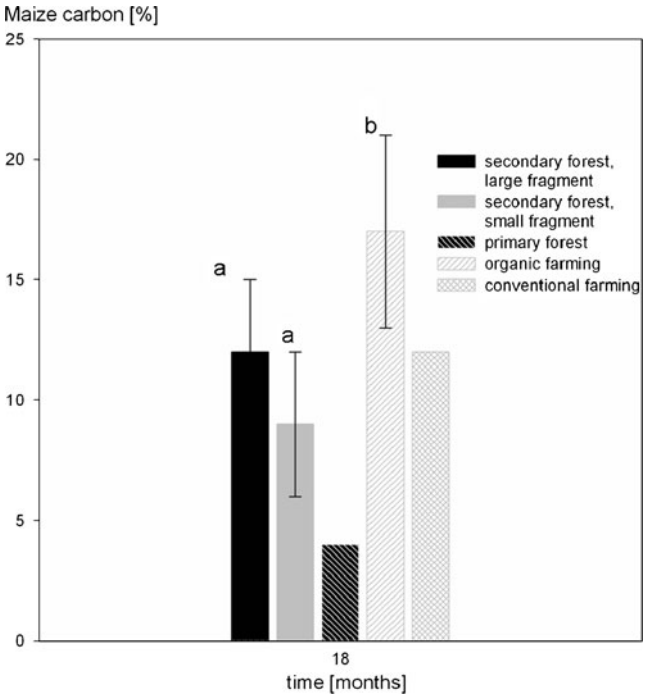

Fig. 3 C_4 maize carbon content of soil organic matter after 18 months of maize litter incubation for the upper 0–2 cm. *Error bars* indicate standard deviation and *b* represents significant ($p<0.05$) difference compared to *a*. Primary forest and conventional farming are represented by one plot because all others were destroyed

According to Santruckova et al. (2000), carbon turnover increases with increasing temperature explaining a comparable value of ^{13}C incorporation after 18 months under tropical conditions with the same value after 37 years under temperate conditions (Flessa et al. 2000). The higher C_4 content of SOM in both secondary fragments and agricultural plots compared to the primary forest does not correspond to the higher maize decomposition in the latter (Table 1). The sequence determined by ^{13}C tracer input (Table 2) is more logic than maize disappearance results (Table 1), which is a hint for the improvement of our method to the original approach of Rezende et al. (1999).

To evaluate how quickly SOM shifted from C_3 to C_4 under different land-use systems, the output of the one-

Table 2 Initial $\delta^{13}C$ values (per mil) of bulk litter, soil, fungal PLFA (phospholipid fatty acids), and bacterial PLFA

Land use	Litter	Soil	PLFA fungi	PLFA bacteria
Secondary forest large fragment	28.8±1.1	27.8±0.3	24.9±1.8	26.8±1.7
Secondary forest small fragment	28.2±0.5	27.5±0.4	24.4±2.0	26.8±2.0
Primary forest	28.8±0.7	27.5±0.3	24.1±3.0	26.6±3.3
Organic farming	–	23.6±0.6	18.9±1.8	20.1±1.0
Conventional farming	–	22.9	19.1±3.3	20.2±1.9

Shown are the arithmetic mean values with ±1 standard deviation. Concerning soil and PLFA only results from the upper layer (0–2 cm) are depicted

parameter model was compared to the bulk $\delta^{13}C$ data (Table 1). Mean turnover time for bulk SOM decreased in the order primary forest (28 years)>secondary forest (9 year)>agricultural sites (organic 4 years; conventional 8 years), indicating that SOM turnover on managed landscapes is much faster than on natural sites. This is consistent with other studies, where mean turnover time at a rainforest site was between 30 and 50 years depending on fractions size (Cadisch et al. 1996) and where estimated mean turnover time for agricultural site in the tropics was in the order of months and years (Post and Known 2000). Tiessen et al. (1994) concluded that under tropical forest to agriculture conversion, C loss was twice as rapid compared to natural systems.

Our results after 18 months of field incubation using ^{13}C labeling showed that managed landscapes such as organic and conventional farming had much faster carbon turnover compared to natural sites such as primary and secondary forest fragments resulting in higher ^{13}C content of SOM at the managed sites.

3.4 Soil microbial biomass

In our study, lowest soil microbial biomass C level was determined at the conventional farming site both at the end of the dry (245 μg Cg^{-1} dw^{-1}; data not shown) and wet (359 μg Cg^{-1} dw^{-1}; Fig. 4) season. On the organic farming site (822±143 μg Cg^{-1} dw^{-1}), microbial biomass was significantly ($p<0.05$) lower at the end of the wet season than on both forest fragment sites (large 1,681±548 μg C g^{-1} dw^{-1}; small 1,654±446 μg Cg^{-1} dw^{-1}; Fig. 4). However, the highest value was measured at the primary forest plot (2,619 μg Cg^{-1} dw^{-1}). Dinesh et al. (2004) suggested that deforestation and cultivation markedly reduces soil microbial activity as evidenced from the lower levels of soil microbial biomass C due to decreased nutrient availability.

Seasonal variation of soil microbial biomass in 0–2 cm soil depth at the secondary forest sites was significantly ($p<0.05$) higher compared to variation among different land-use systems ($p>0.05$) with rainy season soil microbial biomass being significantly ($p<0.05$) higher than dry season soil microbial biomass (data not shown). The seasonal variation of soil microbial biomass at the farming sites was not significant ($p>0.05$) compared to the forest sites. Irrigation on the cultivated sites might be responsible for this phenomenon. These results follow the trend of greater wet season microbial biomass found by Li et al. (2005) in the humid tropics in Puerto Rico (dry season 460 μg Cg^{-1} dw^{-1}, wet season 804 μg Cg^{-1} dw^{-1}) who attribute this phenomenon to sensitivity of microbes toward soil moisture. Overall, soil microbial biomass at the forest sites underlies great seasonal fluctuations whereas the values on the farming sites are constant due to human cultivation.

3.5 Soil microbial community structure

Calculation of fungal to bacterial ratio for Mata Atlântica soils demonstrated a difference between both primary and secondary forest and agricultural sites. The index was lower at the primary (0.06) and secondary (large fragment 0.10± 0.03, small fragment 0.07±0.02) forest sites compared to the agricultural sites (organic 0.14±0.03, conventional 0.14) showing that the proportion of bacteria to fungi was higher on the forest sites. This result differs to Dinesh et al. (2004) who measured higher fungi abundance for the forest sites. Kaur et al. (2005) explained this by the fact that filamentous fungi are more sensitive to physical disturbances such as tillage than single-celled organisms like bacteria. However, Salamanca et al. (2002) showed that bacteria dominate the microbial biomass in tropical forest soils. Furthermore, Hackl et al. (2005) concluded that the pH value of the soil is the most prominent factor diverging microbial communities. pH values of the soil under study (data not shown) ranged from 3.4 to 5 (0–10 cm soil depth). According to Haider (1996), fungi dominate under acid conditions whereas bacteria dominate under less acid conditions. Thus, we conclude that in our investigation, differences in pH value of the agricultural and forest soils (if existing) is not the determining factor, as in this case the

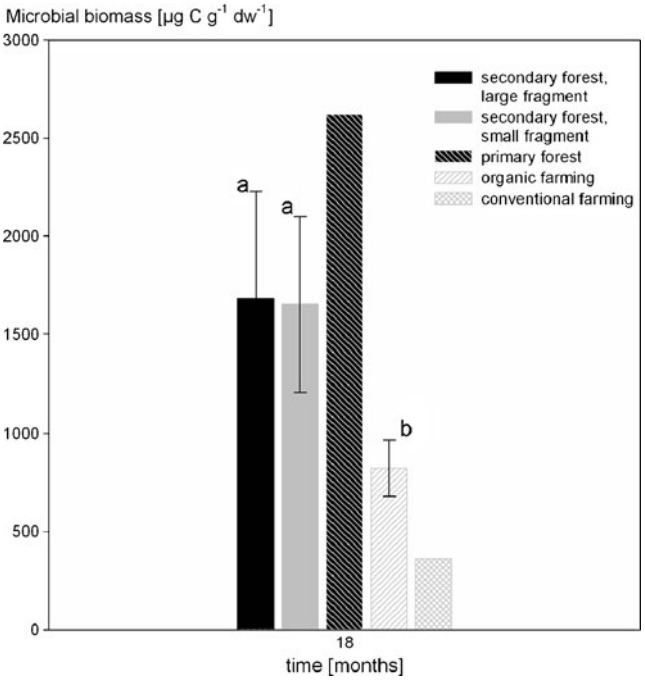

Fig. 4 Microbial biomass after 18 months of maize litter incubation for the upper 0–2 cm. *Error bars* indicate standard deviation and *b* represents significant ($p<0.05$) difference compared to *a*. Primary forest and conventional farming are represented by one plot because all others were destroyed

forest microbial community should be dominated by fungi due to lower pH values compared to agricultural sites which is not the case. Instead, the proportion of fungi is higher on the agricultural sites. As mentioned in Section 3.2, a high ant activity was observed on the latter. In the tropics, ants have an enormous abundance and live in symbiosis with fungi (Zientz et al. 2005). Thus, we rather assume that ants are the key driving factor for the high proportion of fungi at the organic farming site compared to the lower values for the forest plots.

Overall, we found that at the forest sites, the microbial community structure is quantitatively dominated by bacteria. Instead, the abundance of fungi is higher on the agricultural sites.

3.6 ^{13}C incorporation into different microbial groups

Analyzing ^{13}C incorporation into soil microbial biomass revealed no significant difference in terms of increasing or decreasing incorporation over time. However, using the combination of natural abundance ^{13}C labeling from maize litter with biomarker analysis (PLFA) offered the opportunity to identify four microbial groups using PCA of known indicator PLFA molar percentage (data not shown) and following cluster analysis with the factor values derived from the PCA (Table 3).

Calculation of ^{13}C incorporation revealed that individual microbial groups exhibited different substrate utilization depending on land-use type (Fig. 4). However, due to high standard deviation between field replicates, differences were statistically not significant. Therefore, only tendencies of different substrate utilization can be described. In contrast to the results of fungal to bacterial ratio (see Section 3.5), fungal ^{13}C incorporation at all forest plots tended to be higher than the microbial one whereas the situation on both farming sites is reversed (Fig. 5). Thus, at the forest sites, fungal abundance is relatively low compared to bacterial abundance (see Section 3.5), but fungal activity as expressed through ^{13}C incorporation is much higher than the microbial activity. However, discrepancies can be observed when focusing on the four different groups identified by PCA and cluster analysis (Table 3). As could be expected from turnover time of total SOM (Table 1), ^{13}C incorporation into individual microbial groups under primary forest tended to be the lowest. Furthermore, different microbial groups dominated ^{13}C incorporation at different land-use types. Under forest, ^{13}C incorporation decreased in the order of the group consisting of fungi, VAM fungi, and Gram-positive bacteria>actinomycetes and anaerobic Gram-positive bacteria>Gram-negative bacteria. Under agricultural soils, anaerobic Gram-positive and Gram-negative bacteria dominated C_4 substrate utilization while all other microbial groups were similar. Under organic farming, no difference was detected between the microbial groups. ^{13}C incorporation into Gram-negative bacteria was higher under agricultural soils than under forest soils.

Table 3 Identification of microbial units by means of principal component and cluster analysis

Microbial group	Fatty acid	Factor load 1 77.4% explained variance	Factor load 2 16.5% explained variance	Microbial unit	Reference
Gram-negative bacteria group 1	18:1ω9c	−2.81	−0.58	Gram-negative	Crossman et al. (2005)
	18:1ω7c	−4.56	−1.14	Gram-negative	Crossman et al. (2005)
	i15:0	−2.82	−1.70	Gram-negative	Haack et al. (1994)
Anaerobic Gram-positive bacteria group 2	cy19:0	−6.03	3.70	Anaerobic Gram-positive	Zelles (1999)
Fungi, vesicular–arbuscular mycorrhizal fungi and Gram-positive bacteria group 3	a15:0	0.81	−0.40	Gram-positive	Zelles (1999)
	i16:0	0.60	0.003	Gram-positive	Zelles (1999)
	16:1ω7c	0.89	−0.95	Gram-negative	Treonis et al. (2004)
	16:1ω5c	1.10	−0.43	VA mycorrhizal fungi	Olsson et al. (1995)
	10Me16:0	−0.30	−0.13	Gram-positive	Waldrop et al. (2000)
	18:2ω6.9	−0.10	−1.61	Fungi	Olsson et al. (1998)
Actinomycetes and Gram-positive bacteria group 4	10Me18:0	2.87	0.54	Actinomycetes	Zelles (1999)
	10Me17:0	4.09	1.57	Actinomycetes	Zelles (1999)
	i17:0	2.28	0.47	Gram-positive	Zelles (1999)
	a17:0	2.64	0.55	Gram-positive	Zelles (1999)
	cy17:0	2.25	0.10	Gram-positive	Zelles (1999)

VA vesicular–arbuscular

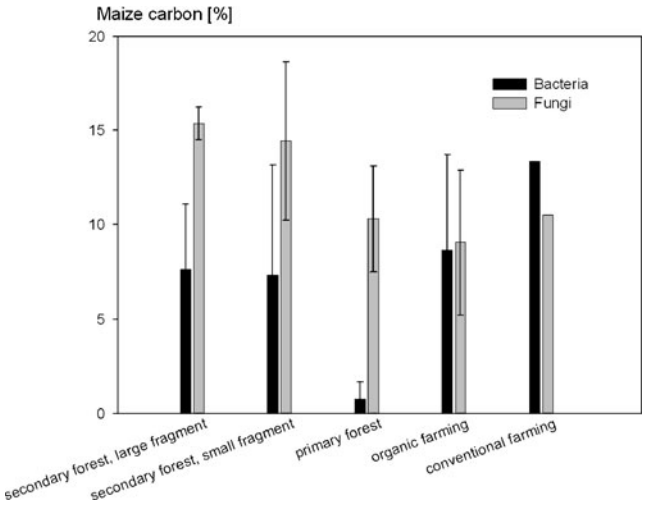

Fig. 5 C_4 carbon fraction of microbial community groups bacteria (group 1, 2, 4) and fungi (group 3). Shown are the results from PLFA at 0–2 cm depth after 18 months of maize incubation. *Error bars* represent±one standard deviation of three field repetition. Conventional farming is represented by one plot because all others were destroyed

According to Haider (1996), fungi and actinomycetes are better able to metabolize complex substrates such as cellulose and lignin than Gram-positive bacteria. This is related to the fungi's and actinomycetes' production of extracellular enzymes whereas Gram-positive bacteria are also able to degrade complex substrate but have slow growth rates. Gram-positive bacteria appear to be more important members of the microbial community in forest habitats than Gram-negative bacteria (Burke et al. 2003). In many studies, the relative concentration of cy19:0 has been observed to increase under stress conditions such as low oxygen level, starvation (Burke et al. 2003), or tillage practices (Kaur et al. 2005). Cyclopropyl fatty acids which are more stable and are not easily metabolized by the bacteria are synthesized by transmethylation of *cis*-monosaturated fatty acids (Kaur et al. 2005). This modification helps maintaining a functional living membrane by minimizing the membrane lipid losses or changes in membrane fluidity causes of cellular degradation during stress conditions (Guckert et al. 1986). The high ^{13}C uptake into fatty acid cy19:0 at the secondary forest sites reflects the stress conditions such as low oxygen level, starvation, or land use. The relation of increasing concentration of cy19:0 under such stress conditions has been observed by various investigators (Grogan and Cronan 1997; Guckert et al. 1986; Petersen and Klug 1994). This indicator fatty acid was the highest at the agricultural sites with 11±5% for the organic farming soil and 20% for the conventional farming site (data not shown).

Gram-negative bacteria are better able to metabolize easily available substrate and show therefore faster growth rate than Gram-positive bacteria (Burke et al. 2003). This result agrees with our conclusion that the labile pool of maize carbon at the forest plots was already metabolized after 18 months and that it was still available at the agricultural sites. One possible explanation for this surprising observation might be the fact that within the agricultural sites C (^{13}C) is turned over within the soil microbial biomass may be due to substrate limitation.

Overall, we identified different microbial groups upon their ^{13}C incorporation depending on land-use type. At the primary forest sites, fungi show high activity dominating ^{13}C incorporation and thus C turnover even though quantitatively bacteria are more abundant. At both secondary forest sites, fungi are dominating as well but Gram-positive bacteria also play an important role. At the farming sites, human soil cultivation caused a distinct activity of microbial groups. At the latter, Gram-negative bacteria are dominating and the stress indicator for land use such as cy19:0 fatty acid was detected.

4 Conclusion

Overall, soil management practices such as tillage were identified to affect both microbial community structure and function. PLFA profiles were found to be sensitive to land-use conversion, and their compound-specific stable-isotope analysis can strongly discriminate between different land management techniques expressing microbial abundance and its activity thus linking soil microbial structure and function.

Our results indicate that carbon turnover is much faster on disturbed sites, such as agricultural land (mean turnover time—4 years for organic farming and 8 years for conventional farming) or secondary forest sites than in the natural system of the primary forest (mean turnover time—28 years). Detecting different litter decompositions of original litter and maize on all sites, we recommend future investigations to analyze the probably different relevance of biochemical composition in contrast to abiotic factors of the environment. An important aspect in introduction of arable agriculture is that under cultivation, different microbial groups dominated the microbial community than under natural conditions leading to different carbon incorporation and respiration pathways. Calculation of maize-derived carbon (C_4 content) of each microbial unit suggested that fungi and vesicular–arbuscular mycorrhizal fungi dominate carbon turnover at the primary forest site even though quantitatively bacteria are more abundant. For the secondary forest sites, stress indicator fatty acid cy19:0 could be detected, and it was observed at higher levels at the agricultural sites. Furthermore, Gram-negative bacteria

appeared to be more important members of the microbial community in the agricultural sites than in the forest habitats.

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