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Degradation and humification of maize straw in soil microcosms inoculated with simple and complex microbial communities

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Summary

Microbial communities are responsible for soil organic matter cycling and thus for maintaining soil fertility. A typical Orthic Luvisol was freed from organic carbon by thermal destruction at 600°C. Then the degradation and humification of ^{14}C-labelled maize straw by defined microbial communities was analysed. To study the role of microbial diversity on the humification of plant material, microcosms containing sterilized soil were inoculated with a natural microbial community or with microbial consortia consisting of bacterial and fungal soil isolates. Within 6 weeks, 41 ± 4% of applied ^{14}C-labelled maize straw was mineralized in the soil microcosms containing complex communities derived from a soil suspension, whilst the most efficient communities composed of soil isolates mineralized less than 35%. The humification products were analysed by solution state ^{13}C-NMR-spectroscopy and gel permeation chromatography (GPC). The analyses of humic acids extracts by solution state ^{13}C-NMR-spectroscopy revealed no difference in the development of typical chemical functional groups for humic substances during incubation. However, the increase in specific molecular size fractions of the extracted humic acids occurred only after inoculation with complex communities, but not with defined isolates. While it seems to be true that redundancy in soil microbial communities contributes to the resilience of soils, specific soil functions may no longer be performed if a microbial community is harshly affected in its diversity or growth conditions.

Introduction

Biological processes are of significance for the ecological functions of arable soils, and thus for maintaining soil quality. Of special importance in this respect is the organic matter content with its input and output dynamics: a loss of organic carbon in agricultural soils due to mineralization and leaching can be balanced by the incorporation of crop residues (Perucci *et al*., 1997). Crop residues are humified by the soil microbial communities, which make an important contribution to the naturally occurring carbon and nutrient cycles and to the maintenance of soil fertility. For this reason, soil microbial diversity has been considered as an indicator for soil quality. However, the importance of soil microbial diversity in soils in terms of its ability to degrade natural organic matter is as yet poorly understood (Nannipieri *et al*., 2003). Previous investigations of microbial processes were limited to culturable species. These studies were hampered by neglecting a large portion (up to 99%) of the native microbial community and by the possibility that those organisms do not represent the most dominant species *in situ*. These cultivable microbes are often characterized by fast growth rates under optimal conditions. However, these so called *r*-strategists have been recognized as key players during the early stage of plant residue degradation (Liebich *et al*., 2006).

Recently, the relationship between biodiversity and ecological functions has attracted attention (Degens, 1998; Griffiths *et al*., 2000; Griffiths *et al*., 2001; Setälä & McLean, 2004; Wohl *et al*., 2004) by focusing on theories for ecosystem functioning such as the redundant species hypothesis, the predictable change hypothesis and the idiosyncratic response hypothesis (Mikola & Setälä, 1998). According to the redundant species hypothesis changes in community composition may not necessarily be linked to functional changes in a certain environment,

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as other members of the community will fill any gap opened by the disappearance of species formerly performing a specific process. According to the predictable change hypothesis and the idiosyncratic response hypothesis, either predictable or unpredictable changes in soil functioning may result from structural changes in community composition. In agricultural soils, many typical soil functions, such as the degradation of organic matter, seem to follow the redundant species hypothesis, and a reduction of biodiversity has generally little effect on many soil processes that are taken over by other members of the community (Nannipieri *et al*., 2003).

In our study, we focus on the role of microbial diversity and redundancy for microbial degradation of crop residues as an important function in soil organic matter turnover. We established 10 different artificial consortia of extremely low diversity (consisting of soil isolates). In order to overcome variable behaviour of selected consortia, we set up consortia of comparable biodiversity rather than varying biodiversity. The degradation and humification of maize straw by the artificial consortia was compared with a complex microbial community of high diversity (inoculated by a soil suspension). The degradation of maize straw was followed as $^{14}CO_2$-release from the ^{14}C-labelled plant material and the degree of humification was analysed by solution state ^{13}C-NMR and size exclusion chromatography of humic acids extracts (cf. Figure 1).

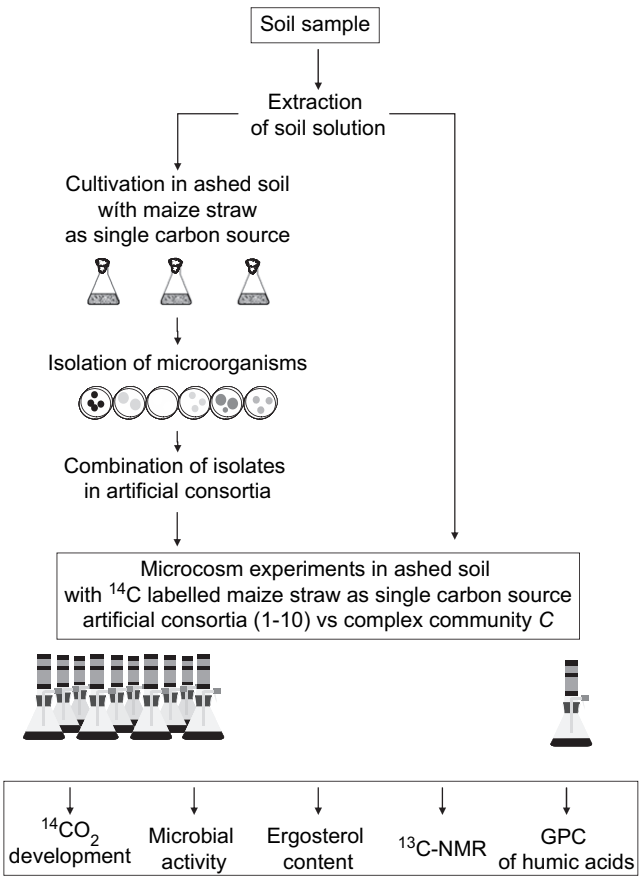

Figure 1 Flowchart of experimental approach and analyses.

Materials and methods

Soil characteristics and pre-treatment

Soils were taken from the plough layer of an arable Orthic Luvisol (FAO soil classification, 3.3% sand, 80.6% silt, 16.1% clay, 0.92% organic carbon, 0.081% total nitrogen, pH 7.0) from Merzenhausen, Germany (6°17′46″E, 50°55′47″N, 93 m altitude) and ashed at 600°C for 48 hours. This treatment resulted in a total depletion of organic substances in the soil and thus allowed the characterization of newly evolving humic substances in the microcosm experiments without interference from the native soil organic matter (Wais *et al*., 1996).

Isolation of soil microorganisms

To isolate typical bacteria and fungi involved in the turnover of maize straw, we inoculated 150 g ashed soil with 2 g fresh soil (Orthic Luvisol), in which we mixed, 9 g maize straw (air dried, pieces < 5 mm) as the sole carbon source. The soil moisture was kept at 40% of water holding capacity and initial liquid addition was as a soil solution equivalent medium according to Angle *et al*. (1991). The soils were incubated at 20°C in the dark.

After 1, 2, 3, 4, 7, 10, 13 and 26 weeks of incubation, 1 g of soil was taken from the soils using a sterilized spatula and vigorously shaken in 9 ml sterile extraction solution (0.1 g litre^{-1} NaCl, 0.02 g litre^{-1} $CaCl_2{\cdot}H_2O$, 0.2 g litre^{-1} $Mg_2SO_4{\cdot}7H_2O$, 5.0 g litre^{-1} Tween 80, pH 7.0) for 5 minutes. An aliquot of 0.5 ml of the supernatant was used to produce a dilution series with 10 times dilute sequential solutions down to a final dilution of 10^{-7}; 100 μl-portions of the dilutions 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} were plated on either Difco R2A (BD, Franklin Lakes, NJ, USA), soil extract agar (100 ml litre^{-1} soil extract, 1.0 g litre^{-1} glucose, 5.0 g litre^{-1} NaCl, 2.5 g litre^{-1} K_2HPO_4, 2% agar, pH 6.8, Führ, 1997), or malt extract agar (30.0 g litre^{-1} malt extract, 3.0 g litre^{-1} proteose peptone, 1.5% agar, pH 5.6).

Inoculated agar plates were incubated for 2 weeks at 20°C. Overall 200 bacterial and 50 fungal isolates were obtained. These cultured bacterial and fungal isolates were picked and purified by dilution plating. Isolates were then grouped by morphology. Representatives from the 25 most abundant groups were chosen for the artificial microbial consortia. To characterize the isolates in more detail, a genotypic approach (16S/18S rRNA phylogeny) was used. To obtain amplicons from 16S/18S rRNA genes, extracted DNA was amplified using the primer system F968 and R1401 according to Smalla *et al*. (1998) for bacteria and the primer system NS1 and NS2 according to White *et al*. (1990) for fungi. Amplified products were purified by the QIAquick PCR Purification Kit (Quiagen, Hilden, Germany). Sequencing reactions were run on an ABI

PRISM 377TM automated DNA sequencer (Applied Biosystems, Darmstadt Germany) and visualized using the software ABI Prism 377 XL collection (Applied Bioystems). Forward and reverse sequences of the same amplified DNA fragment were aligned and a consensus sequence was obtained for each sample using the ARB software package (Ludwig *et al*., 2004) or NCBI Blast search (Figure 2, Altschul *et al*., 1997).

Soil microcosms

Each microcosm consisted of a 250-ml Erlenmeyer flask with 100 g of ashed and <2-mm sieved soil. The flasks were equipped with a soda lime tube to trap $^{14}CO_2$ mineralized from the labelled maize straw (Anderson, 1975). As a source for new humus formation, 2 g maize straw was added (air dried, pieces < 5 mm, 1% of the mass ^{14}C-radiolabelled, with a total ^{14}C-activity of 5.6 kBq per microcosm (Wais, 1997)). Before the start of the experiment all microcosms were sterilized by autoclaving at 121°C and later reinoculated to ensure the same conditions in the microcosms with artificial microbial consortia or complex microbial communities (Figure 1).

Microcosms for the analysis of maize straw turnover by artificial consortia were inoculated as duplicates with one of 10 different mixtures of 13 soil isolates each (five isolates from R2A, five isolates from soil extract agar, and three isolates from malt extract agar), which were chosen at random (Figure 2). Prior to inoculation, the isolates were cultivated separately in nutrient broth (5 g litre^{-1} peptone, 3 g litre^{-1} meat extract). Ten ml of each culture was washed in 10 ml sterile 0.9% NaCl and the optical density at $\lambda = 436$ nm was adjusted to 1.0 (d = 1 cm) using sterile 0.9% NaCl, with fungal cultures being homogenized by gentle shaking if necessary. The consortia were combined from the same volumes of these individual culture solutions and 1 ml of each consortia solution was used for inoculation of the microcosms. The microcosms with complex microbial communities (denoted community *C*, three replicates) were inoculated with 1 ml of a soil suspension obtained by vigorous shaking for 5 minutes of 1 g fresh soil with 9 ml of Tween buffer (see above).

The culture mixtures and the soil solution, respectively, were pipetted together with water onto the soils. Subsequently, the soil moisture was adjusted to 40% water holding capacity. Additionally 4 ml H_2O were applied to compensate for the water uptake due to swelling of the maize straw. Furthermore, the microcosms were fertilized with calcium ammonium nitrate corresponding to 200 mg N kg^{-1} soil. The microcosms were incubated at 20°C in the dark for 6 weeks.

Mineralization of maize straw

The $^{14}CO_2$ produced in the microcosms from radio-labelled maize straw was collected in tubes containing 10 g soda lime by flushing the flasks with N_2. The fixed CO_2 was released with 50 ml 6 M HCl and collected in wash bottles containing 75 ml ethanolamine/methanol (3:7) solution. From each microcosm duplicate aliquots (total number of replicate measurements per treatment: $n_{14C} = 4$) of 1 ml were mixed separately with 10 ml of a scintillation cocktail (Instant Scint-Gel Plus, Canberra Packard, Meriden, CT, USA) and analysed by liquid scintillation counting (Tri-Carb 25600 TR, Canberra Packard).

Soil ergosterol content

Soil ergosterol was extracted in triplicates from each microcosm (Eash *et al*., 1996; with a few minor changes described by Liebich *et al*., 2003). Briefly, 3 g of soil were homogenized in a mixture of 18 ml methanol + 6 ml potassium hydroxide-solution (40 g litre^{-1} KOH in 96% ethanol), followed by 30 minutes of incubation at 85°C. After cooling to room temperature, 5 ml distilled water were added. Subsequently these suspensions were vacuum-filtered and 5 ml water added to wash remaining ergosterol from the filter. The filtrates were extracted with n-hexane and concentrated under nitrogen. Ergosterol contents of 10 μl-aliquots were quantified by HPLC (column: LiChrospher Select-B 60 RP, Merck, Darmstadt, Germany) with a mobile phase of 80% acetonitrile in water (v/v) at 25°C and a flow rate of 0.8 ml minutes^{-1} (total number of replicate measurements per treatment: $n_{ergosterol} = 6$). Ergosterol-peaks were detected with a diode array detector (UV/Vis-Detektor UVD 340, Gynkotek, Germering, Germany) at 282 nm and identified by ergosterol standards. Ergosterol contents were not transformed into total fungal biomass, because this is still problematic because of variations between different fungal species and their metabolic state (Bermingham *et al*., 1995).

Characterization of humic acids

Soil samples of 20 g were fractionated with basic and acidic agents as described for the extraction of soil-bound residues (Khan & Ivarson, 1982; Liebich *et al*., 1999), without prior treatment with organic solvents. Prior to NMR analyses, the humic acids were washed three times with water to reduce the salt content and were subsequently dissolved in 3.5 ml 0.14 M deuterated NaOH (2–12 mg C ml^{-1}). ^{13}C-NMR spectroscopy was carried out in 10-mm tubes by inverse-gated decoupling as described by Wais *et al*. (1996) with an AMX 400 Bruker NMR spectrometer (100.6 MHz, Bruker, Rheinstetten, Germany). Acquisition time was 0.172 s, pulse width 45°, relaxation delay 1.0 s, and the decoupler off during relaxation. Line broadening was carried out at 50 Hz.

Gel permeation chromatography of humic acids was performed using an HPLC system (LB 506, Berthold, Wildbad, Germany; main column, MCX PSS; length, 300 mm; diameter, 8 mm; particle size, 5 μm; pore size, 1000 Å; PSS, Mainz, Germany) with K_2CO_3 (6 g litre^{-1}) as the mobile phase at a constant flow rate of 0.5 ml minutes^{-1}. Detection of the humic acids was performed by the aid of a diode array detector

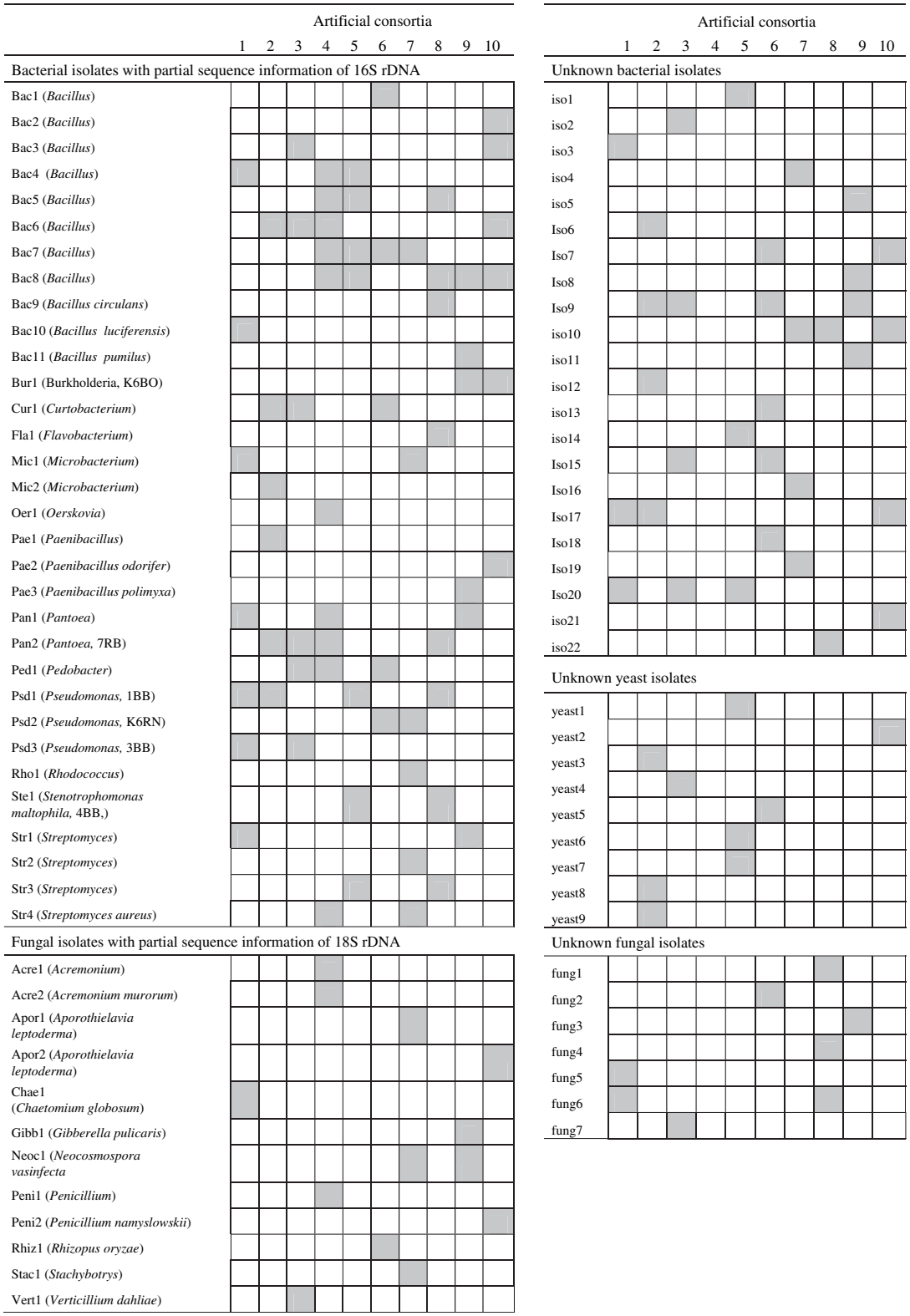

Figure 2 Overview of the artificial consortia and nearest neighbours in NCBI-Blast search (Altschul *et al*., 1997, only univocal species or genus with similarity of 97% or higher in 16S/18S rDNA partial sequences analysed). Acronyms in parentheses refer to Figure 5.

(UVD 160, Gynkothek, Germering) at a wavelength of 254 nm. All samples were washed with water and adjusted to a concentration of 2.5 g C litre^{-1} before the injection of 10 μl-aliquots. Calibration was done with polystyrol sulfonate standards (208 D, 840 D, 2290 D, 5280 D, 45 100 D, 86 500 D), which were detected at a wavelength of 200 nm, and evaluated by a specific fifth order polynomial calculated by the WinGPC 6.20 software (PSS, Mainz). Distribution of molecular masses was determined between 500 and 20 000 D with this polynomal.

Results

Mineralization of ^{14}C-labelled maize straw

After 1 week of incubation, all microcosms irrespective of the composition of the microbial inocula mineralized between 11% and 23% of the applied radiocarbon from the added maize straw (Figure 3). While artificial communities 4, 7 and 9 reached mineralization rates comparable with the complex microbial community, some of communities 1, 5 and 8 mineralized only about half of the radiocarbon compared with the complex microbial community. After 6 weeks, microcosms inoculated with the natural microbial community showed greater mineralization rates (41%) compared with the artificial consortia. Again, not all artificial consortia showed the same degradation rates and consortia 6 and 7 mineralized only slightly less than the complex microbial community (35% of applied radioactivity after 1 week of incubation). Interestingly, microcosms with the least turnover of maize straw, i.e. communities 1 and 2 with 14% and 17% mineralization of ^{14}C-labelled maize straw, evolved 75% of the total amount of $^{14}CO_2$ within the first week, while in all other treatments the portion of ^{14}C mineralized within the first week was less: between 53 and 69% in the mixed communities and 56% in community *C* with a complex microbial community.

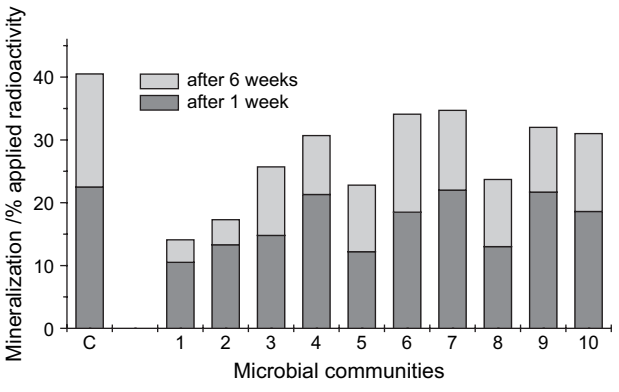

Figure 3 Mineralization of ^{14}C-labelled maize straw by complex communities (*C*) and consortia (1–10) consisting of soil isolates within 1 and 6 weeks of incubation (± standard deviation; applied radioactivity = 100%).

Microbial community characterization

After 6 weeks of incubation, the soil ergosterol content was measured as a fungal biomarker, and 8.8 μg ergosterol g^{-1} soil (dry weight) were found in those microcosms that had been inoculated with the natural community (Figure 4). Similar concentrations were also found in the microcosms that had been inoculated with the artificial consortia 4 and 8. Both communities were dominated by only one bacterial isolate (Pan2, Figure 5) as revealed by molecular analysis (data not shown). This isolate also dominated the bacterial communities in the two microcosms with the least ergosterol (communities 1 and 2). However, in these latter communities this isolate was not as dominant as in microcosms 4 and 8.

Comparison of humic acids extracts from maize straw and a native Orthic Luvisol

The solution state ^{13}C-NMR spectra shown in Figure 6 are representative of the starting and final products of the humification process. Spectrum A (recorded after 200 000 scans) shows a humic acids extract of maize straw, which had been added to an ashed soil, moistened and inoculated, but which had not been incubated (starting point). Thus, the signals are not influenced by the carbon matrix of the Orthic Luvisol used in our experiments. For comparison, spectrum B (recorded after 70 000 scans) shows a humic acids extract of this Orthic Luvisol from Merzenhausen (PB-MER). The maize straw extract spectrum is dominated by peaks with a chemical shift of 50–110 p.p.m. In contrast, this region is less developed for the humic acids extract from the Orthic Luvisol, whereas peaks with a chemical shift of 20–44 p.p.m. and 175 p.p.m. are dominant.

Separation of humic acids extracts by gel-permeation chromatography revealed molar masses of 2.2 kDa for the humic acids extract from maize straw and 3.2 kDa for the native humic acids from a typical orthic luvisol. The eluograms in Figure 7 show the molecular size distribution of a humic acids extract from a native soil (A) and a maize straw extract (B).

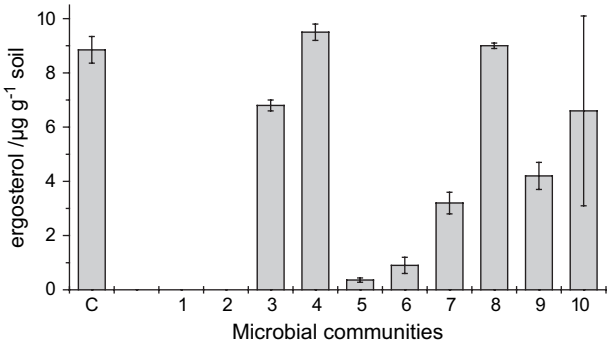

Figure 4 Soil ergosterol content in the microcosms 6 weeks after maize straw application (± standard deviation; no ergosterol was found in microcosms 1 and 2).

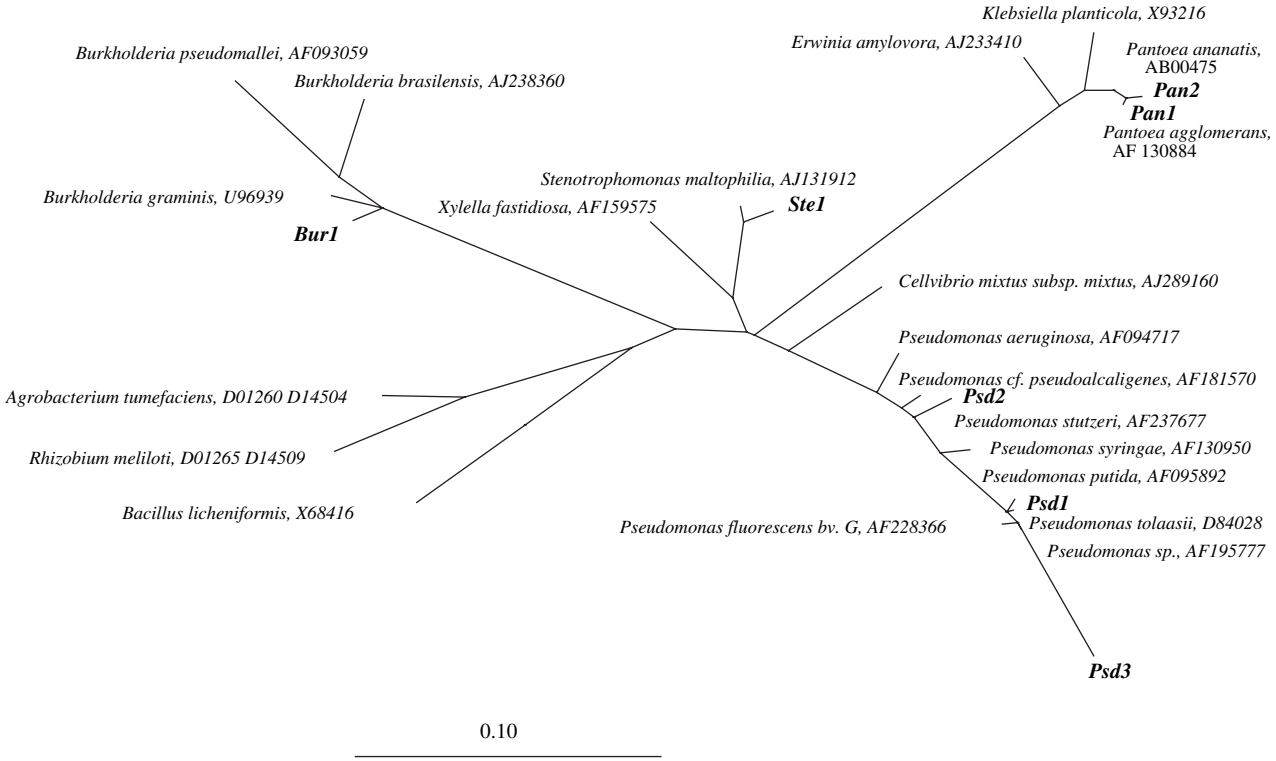

Figure 5 Phylogenetic classification of 16S rDNA sequence information for soil isolates (the scale bar indicates the number of changes per nucleotide).

Humic acids extracts after 6 weeks of incubation of maize straw with soil isolates

In order to analyse the humification of the straw material used, humic acids were extracted from selected microcosms (*C*, 1, 2 and 6) after 6 weeks of incubation, and measured by solution state ^{13}C-NMR spectrometry (200 000 scans). All spectra displayed characteristics typical of native humic acids after just 6 weeks of incubation with no substantial differences between the different communities (Figure 7), namely: high signal intensities at a chemical shift of 25–45 p.p.m. (lipids, waxes and aliphatic hydrocarbons, Hopkins *et al*., 2000) and at a chemical shift of 160–180 p.p.m. (carboxyl and carbonyl functional groups), while signals at a chemical shift of 60–110 p.p.m. (O-alkyl-C and di-O-alkyl-C functional groups typical of carbohydrates) were reduced compared with the NMR spectrum of the maize straw extract.

Whereas the ^{13}C-NMR spectra hardly differed as a function of the inoculum, separation of the humic acids extracts by gel-permeation chromatography led to different results. The elution volume of the measured extracts ranged from 5 to 11.5 ml, thus pointing to a wide range of molecular sizes, summarized in Figure 8 and Table 1. The humic acids extract from the 6-week-old microcosms containing the complex microbial community was characterized by molecular masses with a peak maximum of 2900 D, which means a substantial shift in the peak maximum from the straw material (2.2 kDa, Figure 8B) towards the native soil extract (3.2 kDa, Figure 8A). By contrast, the peak maxima of the humic acids extracts from the microcosms after inoculation with soil isolates (communities 1, 2, 6 and 7) ranged between 2.2 kDa and 2.4 kDa after 6 weeks of incubation, independent from the total amount of ^{14}C-maize straw mineralized.

Discussion

Within 6 weeks none of the 10 microcosms with soil isolates mineralized maize straw at the same rate as those with a complex microbial community. However, three different communities with soil isolates (communities 4, 7 and 9, Figure 3) were able to mineralize 21–23% of the applied radio-carbon after incubation for 1 week at a rate similar to the complex microbial community. Therefore, initial degradation steps are not necessarily linked to great microbial diversity. This is especially true for consortia 4 and 8, which were both dominated by only one bacterial species presumed to be phylogenetically related to *Pantoea* species (Figure 5).

In the artificial consortia with low microbial diversity, the species composition seems to be of crucial significance as only three communities out of 10 produce the same amount of $^{14}CO_2$ as the complex microbial community *C* within 1 week.

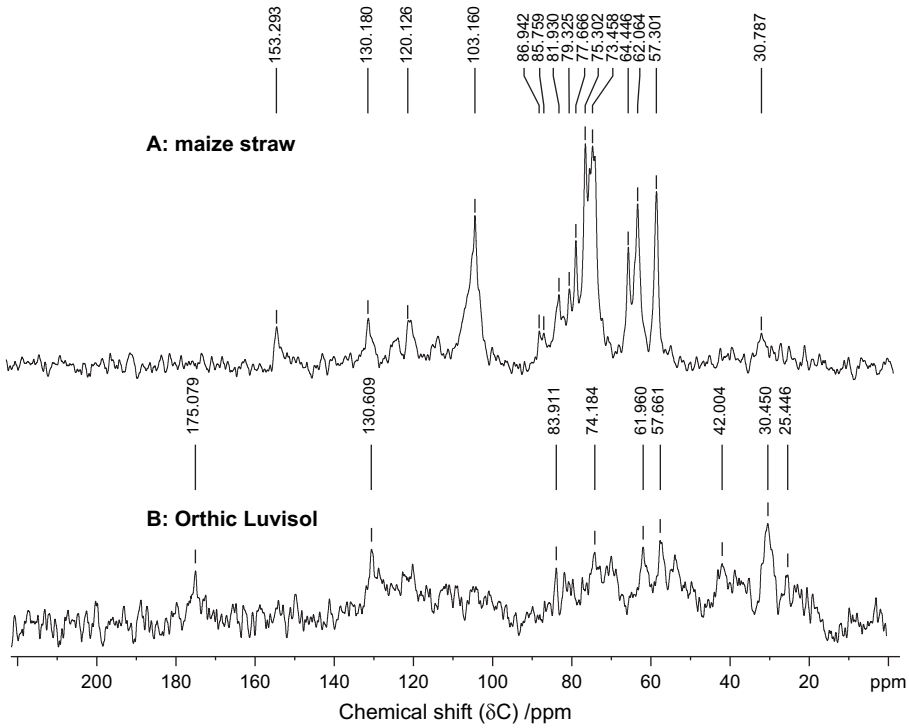

Figure 6 ^{13}C-NMR spectra of humic acids extracts from maize straw (a) and from the Orthic Luvisol (b).

In communities 1, 2, 5 and 8 only 60% of this amount was mineralized. After 6 weeks of incubation the mineralization for all artificial communities was less than that for the complex microbial community. Nevertheless, great differences were observed between the treatments. Whereas two microbial communities (6 and 7) achieved more than 80% of the $^{14}CO_2$ production of the complex microbial community, two other communities (1 and 2) mineralized less than 50% of this amount.

These findings are consistent with the redundant species hypothesis, which says that only a minimum number of species within an ecosystem is necessary for specific functions (Walker, 1992). In this respect, high diversity within a microbial community is accompanied by a specific degree of redundancy as a result of the ability of several species to perform a specific function, e.g. a particular degradation step. The removal of species is then redundant and does not lead to changes for this function, e.g. a particular degradation rate. According to this hypothesis, reduced biodiversity does not necessarily result in changes in soil microbial processes (Griffiths *et al*., 2001). However, Wohl *et al*. (2004) found a decreased rate of cellulose degradation if only one or two different species of cellulose degraders were present in a liquid culture, compared with 4 or 8 different species. Their results led to the conclusion that great diversity is still important in terms of quantitative changes. Setälä & McLean (2004) found similar results for the decomposition of coniferous forest humus and some model compounds by isolated soil fungi. Overall, it should be noted that in our experiments, which support the redundant species hypothesis, only a few soil functions were considered, i.e. the formation of $^{14}CO_2$ and of humic acids, and only these functions can be considered to be redundant or not. In contrast to the mineralization of maize straw as studied in our experiments, cellulose degradation is a single function of specific soil microorganisms, which thus may react in a more sensitive way to changes in biodiversity.

This interpretation of the mineralization rates is supported by the ^{13}C-NMR spectra of the extracted humic acids (after 6 weeks of incubation). The formation of functional groups typical of humic substances is obvious in the NMR spectra of the humic acids extracts of all treatments, in particular aliphatic compounds, aromatic compounds and carbonyl groups (Nardi *et al*., 2000). Differences between the spectra can be identified primarily for a chemical shift around 175 p.p.m., indicative of carboxyl, amide and ester groups (Hopkins *et al*., 2000). Corresponding signals occurred in the spectra of community 1 (with soil isolates) and community *C* (complex, Figure 7), and were also present in the spectrum of the Orthic Luvisol (Figure 5B), but not in that of maize straw (Figure 6A).

The degradation of readily available substances present in maize straw proceeds very rapidly, at least in those microcosms with mineralization rates of more than 20% of the applied radiocarbon within 1 week (complex microbial community *C* and

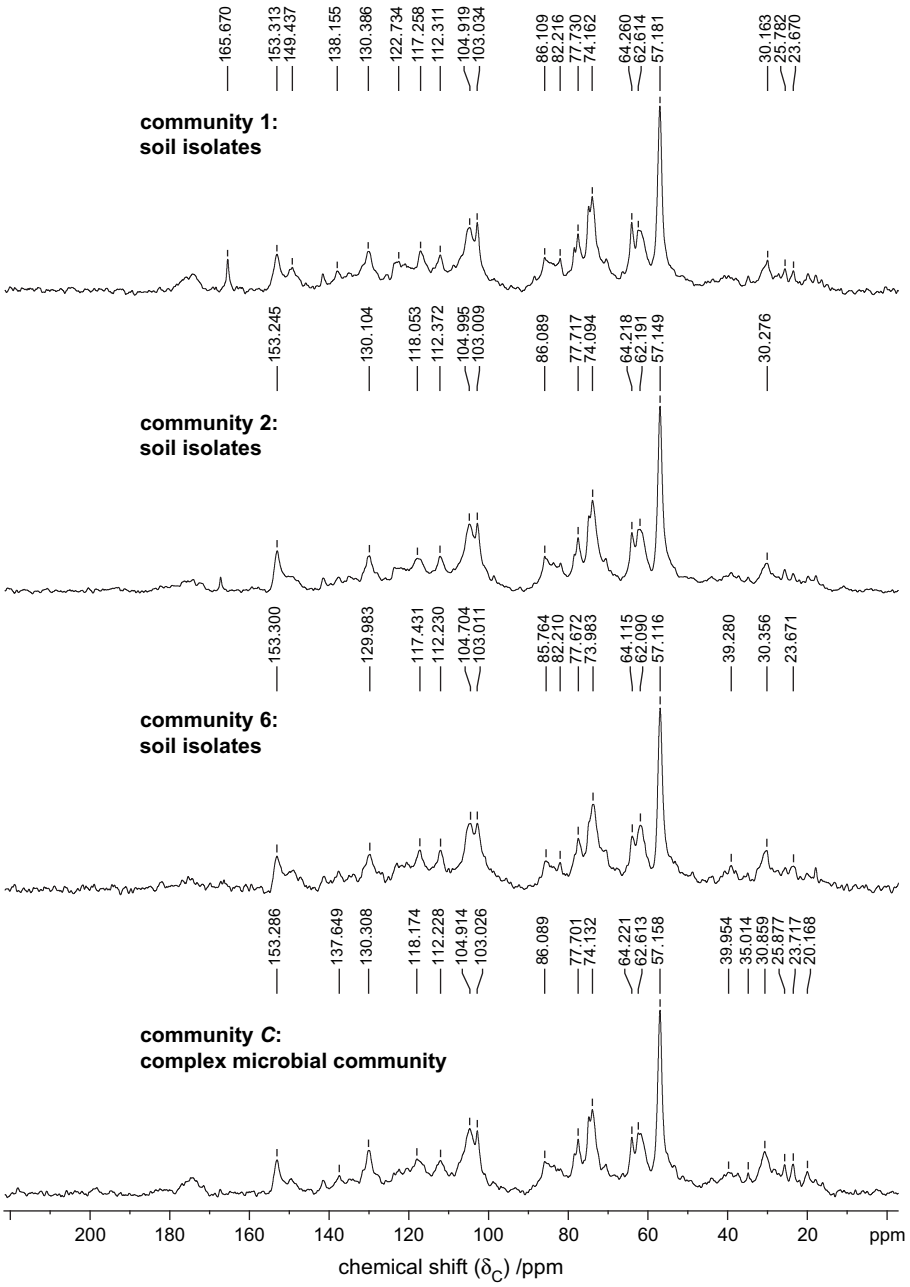

Figure 7 ^{13}C-NMR spectra of humic acids extracts after 6 weeks of incubation of maize straw in ashed soil inoculated with soil isolates (communities 1, 2, 6) or a soil suspension (community *C*).

communities 4, 7 and 9 with soil isolates, Figure 3). Similar results were also obtained by Webster *et al.* (2000) for the degradation of leaves of perennial ryegrass (*Lolium perenne*), the greatest mineralization rate being established within the first week after addition of the plant material. As proposed by Webster *et al.* (2000), it is possible that the microbial degradation of carbohydrates leads to proportional accumulation of more recalcitrant components, which are not mineralized as fast and which are thus responsible for the changes observed in the ^{13}C-NMR-spectra. This in turn means that the alkyl-C to O-alkyl-C ratio proposed by Baldock *et al.* (1997) as an indicator of the biodegradability of soil organic carbon is indeed suitable for characterizing the degree of decomposition of plant material. However, this indicator cannot be used to describe the formation of humic substances by microbial activity, because it merely describes the loss of carbohydrate-like structures in relation to aliphatic structures. It is thus possible to correlate the mineralization rates of maize straw with the

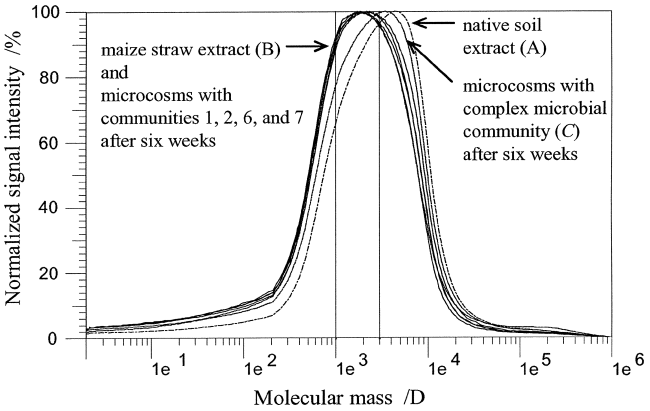

Figure 8 Molecular mass distribution of humic acids extracts from the Orthic Luvisol (A), from maize straw (B), and from the microcosms containing soil isolates (communities 1, 2, 6 and 7) and the complex microbial community (*C*); vertical lines indicate size limits in Table 1.

emergence of NMR signals in the range of alkyl, aromatic and carbonyl functions. The signals, which disappear first from the ^{13}C-NMR spectra during incubation (Figure 7), represent readily degradable structures, above all carbohydrates. Their ready mineralization is expected even in microcosms with a low microbial diversity, as the ability to degrade such compounds is generally widespread. However, because the quantities of ^{14}C-maize straw mineralized in the various microcosms differed with various combinations of organisms, it may be assumed that in addition to the utilization of readily available substrates, more complex substances can also be used by individual members of the microbial communities.

Microbial activity of the complex microbial community (which is considered as a community with great diversity) as well as of the artificial consortia consisting of soil isolates (representative communities with little microbial diversity) resulted in structural changes of the fresh organic material. In contrast, investigations of the humic acids by GPC did not show any perceptible change in retention times after 6 weeks of incubation in the microcosms with the soil isolates, in comparison with the starting material, whereas the humic acids from the microcosms with the complex microbial community increased in molecular size (Table 1 and Figure 8). It is therefore obvious that the microbial diversity indeed had an influence on the size distribution of the turnover products of crop residues but not on the formation of functional groups as analysed by solution state ^{13}C-NMR of the humic acids extracts. This means, while redundancy may still maintain some soil functions, others may be affected by reduced diversity. However, under suboptimal conditions, like moisture stress or anthropogenic perturbations, even rather resilient microbial processes may be significantly influenced, if the biodiversity (and thus the redundancy) is already reduced (Degens, 1998; Griffiths *et al*., 2000).

Salonius (1981) attempted to establish a quantitative relationship between the diversity and the degradation performance of microorganism communities in soil. He inoculated dry soil samples, sterilized by gamma radiation, with soil suspensions with different levels of biodiversity, but containing approximately the same biomass, and incubated these batches in closed vessels. The more species present in the inoculum, the greater was the measured respiratory activity. A similar argument was put forward by Setälä *et al*. (1998), who attributed changed carbon degradation in heterotrophic systems with reduced biodiversity to changes in the interactions between the various trophic levels. However, in our experiments, contrary to the expectation that changed diversity would influence the mineralization rate of organic material in soil the rate in some of the microcosms with soil isolates was similar to that of the complex microbial community (Figure 3).

Culturable bacteria are generally considered as *r*-strategists (Andrews, 1984) and their growth should be favoured after the addition of maize straw even in microcosms inoculated with the complex microbial community *C*. This may be the reason for comparable mineralization rates of the maize straw by soil isolates and the complex microbial community in the first week (Figure 3). After 6 weeks, more than 40% of the ^{14}C-carbon was mineralized. The readily available constituents of the maize straw had therefore been consumed and the selective conditions that initially encouraged the fast-growing species changed in favour of the slow-growing *K*-strategists. This reversal corresponds to the theoretical analyses by MacArthur & Wilson (1967), which proposed *r*-selection of the primary colonizers in a weakly colonized or uncolonized environmental compartment with sufficient nutrient supply. When such

Table 1 Calculated molecular masses from GPC peak maxima (Mp, see Figure 8) and molecular size distributions of the humic acids extracts (± standard deviation)

	Native soil extract	Maize straw extract	Extracts from the microcosms after 6 weeks of incubation Communities consisting of soil isolates				Complex community (C)
			1	2	6	7	
Mp (D)	3170 ± 53	2215 ± 37	2351 ± 0	2242 ± 79	2217 ± 112	2298 ± 156	2883 ± 48
< 1kD (%)	20.4 ± 0.9	31.4 ± 0.6	31.6 ± 0.9	33.3 ± 0.1	33.6 ± 0.4	31.9 ± 0.1	27.1 ± 0.4
1–3 kD (%)	30.9 ± 0.7	33.4 ± 0.3	31.7 ± 0.6	32.4 ± 0.0	33.6 ± 0.2	32.6 ± 0.1	30.9 ± 0.4
> 3kD (%)	48.8 ± 0.2	35.2 ± 0.3	36.7 ± 0.3	34.2 ± 0.1	32.8 ± 0.2	35.5 ± 0.3	42.1 ± 0.0

organisms are established, the selection pressure changes to the *K*-strategy. A comparable sequence in the occurrence of physiologically different groups of microorganisms has also been described by Hu *et al.* (1999).

No correlation was obtained by comparing $^{14}CO_2$ production with fungal biomass (ergosterol content) after 6 weeks of incubation. Hence it is highly interesting that some of the fungal isolates were not able to establish in some of the microcosms. This is most obvious for the microcosms with the greatest $^{14}CO_2$-production, which contained rather small amounts of ergosterol in the soils (communites 6 and 7 with 0.9 $\mu g\ g^{-1}$ and 3.2 $\mu g\ g^{-1}$, respectively), whereas community 4, which also showed a mineralization rate of more than 30% within 6 weeks, contained the greatest amount of soil ergosterol (9.5 $\mu g\ g^{-1}$). Generally, the fungal biomass does not seem to influence the formation of these structures typical of humic substances: the ergosterol content in the microcosms with soil isolates was <1 μg ergosterol g^{-1} soil in communities 1, 2, 5 and 6, and *c.* 9 μg ergosterol g^{-1} soil in the microcosm containing the complex microbial community *C* and some of the communities containing soil isolates (especially communities 4 and 8).

The molecular-size distributions shown in Figure 8 and Table 1 support the propositions of Wershaw (1993), Piccolo *et al.* (1996) and Wanner *et al.* (2000) that humic substances do not necessarily consist of large molecules, because more than 50% of the humic acids had molecular masses of less than 3.0 kDa. Because there is also a large portion of $> 30\%$ in all humic acids extracts >3.0 kDa, no further conclusions can be drawn regarding the humic acids of higher molecular weight representing either true macromolecules or rather agglomerations of small molecules.

Conclusions

The application of soil isolates was intended to elucidate the role of culturable organisms and the significance of high microbial diversity for the degradation and humification of dead plant material. Our experiments have shown that:

1 Within 6 weeks of incubation with different mixtures of soil isolates, mineralization of added ^{14}C maize straw and development of functional groups typical of humic substances was in some cases comparable with those that had been inoculated with a soil suspension. We attribute this to the selective conditions in the microcosms that favour fast-growing microorganisms at the start of incubation. Because culturable microorganisms are frequently *r*-strategists, which are characterized by high growth rates with an initially good supply of readily available carbon and energy sources, the defined communities have optimum conditions for rapid growth and carbon turnover.

2 The amount of fungal biomass does not correlate with the degradation of maize straw or the formation of humic substances.

3 Although NMR signals typical for humic substances increased in the course of incubation with soil isolates, no corresponding increase in the mean molecular size of the humic acids occurred as with complex microbial communities. This means that not all the mechanisms important for humification can be provided by communities with reduced diversity.

4 While it seems to be true that redundancy in soil microbial communities contributes to the resilience of soils, specific soil functions may no longer be performed, if a microbial community is harshly affected in its diversity or growth conditions.

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