

Harsh summer conditions caused structural and specific functional changes of microbial communities in an arable soil

W. LEVY, V. RADL, B. RUTH, M. SCHMID, J. C. MUNCH & R. SCHROLL

Institute for Soil Ecology, GSF – National Research Centre for Environment and Health, Ingolstädter Landstraße 1, 85764 Neuherberg, Germany

Summary

The mineralization of the herbicide 3-(4-isopropylphenyl)-1,1-dimethylurea (isoproturon) was reduced after the dry and hot summer 2003 in a soil profile placed in a field lysimeter. A different isoproturon mineralization pattern remained in soil material taken at two different soil depths (0–5 cm and 15–20 cm), although soil material was re-equilibrated at adequate climatic conditions. Special soil microcosms were designed to determine if the changes in this special soil function ‘isoproturon mineralization’ were related to the climatic scenario of summer 2003. These microcosms were filled with lysimeter soil from the 15–20 cm depth and the temperature and dryness of summer 2003 were simulated. Afterwards, soil samples were taken from the microcosms and re-equilibrated under controlled conditions for 4 weeks. Subsequently, isoproturon mineralization was investigated. The soil microbial community reduced drastically its original capability of isoproturon mineralization in the course of the model experiments.

Analysis of 16S-rRNA by denaturing gel gradient electrophoresis (DGGE) revealed substantial differences in the band patterns of the bacterial communities from both depths of the field lysimeter soil and from the soil incubated in microcosms. The different soil microbial biomass determined by microcalorimetry reinforced these results. In conclusion, the factors higher temperature and smaller soil moisture content generated important and enduring changes in the microbial community structure and therefore in specific soil functions of the community, as shown here by the function of isoproturon degradation. Results are discussed in connection with environmental conditions and conservation tillage.

Introduction

According to ECCP (2003) and IPCC (2002), climate change will not only cause an increase of the average temperature of the globe, but also a change in the fluctuation and intensity of precipitation and temperature (Shearer, 2005). Drought and flood events are anticipated for several regions in the world. Global changes are also predicted to introduce changes in agricultural ecosystems (Chartzoulakis & Psarras, 2005). In current publications, for example, on the one hand effects of global warming on the diversity of the soil microbial community were reported (Sowerby *et al.*, 2005), and on the other hand a decrease in soil enzyme activity with drought increase was demonstrated (Sardans & Penuelas, 2005).

Summer 2003 was unusually hot and dry in the southern part of Germany. One of the first identifiable effects of this unusual summer was a drastic re-intensification of the process of forest

decline in Bavaria (Waldzustandsbericht, 2004). Therefore, this very specific climatic scenario of 2003 could also have affected other ecosystems, as given for agro-ecosystems in the form of a yield decline in that year. In particular, our system under study was a lysimeter facility that allows analysis of specific soil systems under outdoor conditions. Laboratory studies of the fate of the herbicide isoproturon in the ‘Feldkirchen’ soil were conducted in autumn 2003, with samples taken from 0–5 cm and 15–20 cm depths from the lysimeter. From experience with isoproturon in this ‘Feldkirchen’ soil in earlier experiments, we noticed in the 2003 experiments an unexpected and drastic change in isoproturon mineralization (shown by the length of the lag period, in the total mineralization capability, and in the mineralization pattern at both soil depths after summer 2003; see Figure 3a below). In earlier experiments, Schroll & Kühn (2004) determined a mineralization pattern characterized by consecutive lag, exponential and plateau phases, which represent degradation behaviour known as a ‘metabolic degradation dynamic’ (Torstensson, 1980). Even

Correspondence: R. Schroll. E-mail: schroll@gsf.de

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after 4 weeks of re-equilibration of soil from the upper 5 cm of the lysimeter profile under constant laboratory conditions (water potential -214 kPa, 20°C), this degradation dynamic was reduced dramatically to a nearly constant and weak daily isoproturon mineralization rate which resulted in reduced total herbicide mineralization after summer 2003. Changes in the isoproturon degradation behaviour with soil taken from the 15–20 cm depth were not as pronounced as in the surface soil.

The unusual weather conditions and the clearly detected and specific changes in isoproturon degradation after summer 2003 led us to investigate possible interrelationships between both events. The aim of this study was to determine if the changes in isoproturon mineralization were related strongly to soil temperature and water content, and therefore if the microbial community of the soil might have been influenced permanently by these environmental conditions.

Materials and methods

Chemicals

International Isotopes (Munich, Germany) provided ^{14}C -ring-labelled 3-(4-isopropylphenyl)-1,1-dimethylurea with a radiochemical purity $>98\%$. Non-radioactive isoproturon was provided from Agrevo (Höchst, Frankfurt, Germany). The specific ^{14}C -radioactivity of the applied isoproturon standard was 686 Bq μg^{-1} . Scintillation cocktails for determination of the radioactivity were from Packard (Groningen, the Netherlands).

Soil

The soil was taken from the agricultural location 'Feldkirchen' ($48^{\circ}9'\text{N}$, $11^{\circ}44'\text{E}$, elevation 521 m, Southern Germany) and transferred as a monolith into field lysimeters (1.12 m diameter \times 2 m depth) in 1996. This calcaric regosol had an Ap horizon (33% sand, 34% silt, 33% loam, pH (CaCl_2) 7.2 and 2.70% organic carbon) of depth 30 cm, followed by a C horizon. In September 2003, soil samples were taken from the lysimeter at 0–5 cm and 15–20 cm depths, with soil from these two depths referred to as the upper soil and the deeper soil, respectively.

Soil water retention curves were determined on disturbed and sieved soil material (≤ 2 mm) compacted to a density of 1.3 g cm^{-3} (Schlichting *et al.*, 1995) and extrapolated to tensions >1500 kPa (van Genuchten, 1980). Values for soil water potential in the various experiments were calculated from these soil water retention curves. Sieved soil material (≤ 2 mm) was re-equilibrated (bulk density 1.3 g cm^{-3} , water content 0.24 g g^{-1} , water potential -214 kPa, 20°C) for 4 weeks and subsequently used for isoproturon biodegradation experiments in the laboratory.

Microcosm design

In November 2003, deeper soil was taken from the lysimeter and placed in specially designed microcosms (22 cm \times 10 cm \times 15 cm; Figure 1) at a bulk density of 1.3 g cm^{-3} , adjusted to a water

content equal to -35 kPa and incubated for 10 weeks. The daily warm-up of the soil surface was done by adjusting the distance of lamps (100 J s^{-1}) from the microcosms with soil surface temperature as a control parameter. The warm-up period was 12 hours day^{-1} . The soil surface was kept at a maximum temperature of $40 \pm 1^{\circ}\text{C}$ (daytime) and a minimum temperature of $20 \pm 1^{\circ}\text{C}$ (night). The average soil temperature, and the periodicity and amount of water to be added to the microcosms, were based on measurements of soil temperature and precipitation at the GSF lysimeter station in June and July 2003. The calculated amount of water to be added to the microcosms was 40% of the total precipitation in June and July 2003 (110 mm). This decision was based on the absence of plant roots that take up approximately 60% of the precipitation that enters the system under agriculture. The microcosms were watered at three different times to simulate natural rainfall. The whole water addition per microcosm (980 ml in 8 weeks) was equivalent to 44 mm precipitation. A water potential of -35 kPa was defined as the maximum soil moisture content at the bottom of the microcosms (a depth of 12 cm) to avoid greater wetness than the normal conditions in the lysimeter soil system. Soil water content and soil temperature were measured with sensors (Ruth & Munch, 2005) placed at depths of 1, 6 and 12 cm from the soil surface (Figure 1a).

Parallel to the microcosm incubation, deeper lysimeter soil was kept at 20°C , at a bulk density of 1.3 g cm^{-3} and at a moisture potential of -35 kPa in the dark as a control.

At the end of the incubation period, the soil water potential in the upper layer (0–1 cm) of microcosms was around -600 MPa and in the deeper layer (12–13 cm) around -35 kPa. Subsequently, soil from the upper and deeper layers in the microcosms and the microcosm control were each pooled, homogenized separately, and finally re-equilibrated (bulk density 1.3 g cm^{-3} , water content 0.24 g g^{-1} , water potential -214 kPa, 20°C , 4 weeks) prior to isoproturon biodegradation experiments and to further microbial analysis.

Isoproturon biodegradation assay

In general, soil samples (50 g dry weight) from the lysimeter, microcosm experiments and control soil were supplied with isoproturon (125 kBq) in incubation flasks. The ^{14}C -labelled herbicide standard was applied and mixed in 3.5 g dry soil (dried for 24 hours at 105°C) and then distributed homogeneously in moist soil (equivalent to 46.5 g dry weight soil). After isoproturon application, the soil samples were brought to a water content of 0.30 g g^{-1} soil and a water potential of -35 kPa, and compacted again to a bulk density of 1.3 g cm^{-3} . Thus, all the biodegradation experiments in this study were conducted at environmental conditions which are close to the optimal conditions for pesticide mineralization in agricultural soils (Schroll *et al.*, 2006). The flasks were connected to a closed system with serial traps that allowed the separate determination of the volatilization and mineralization (formation of $^{14}\text{CO}_2$)

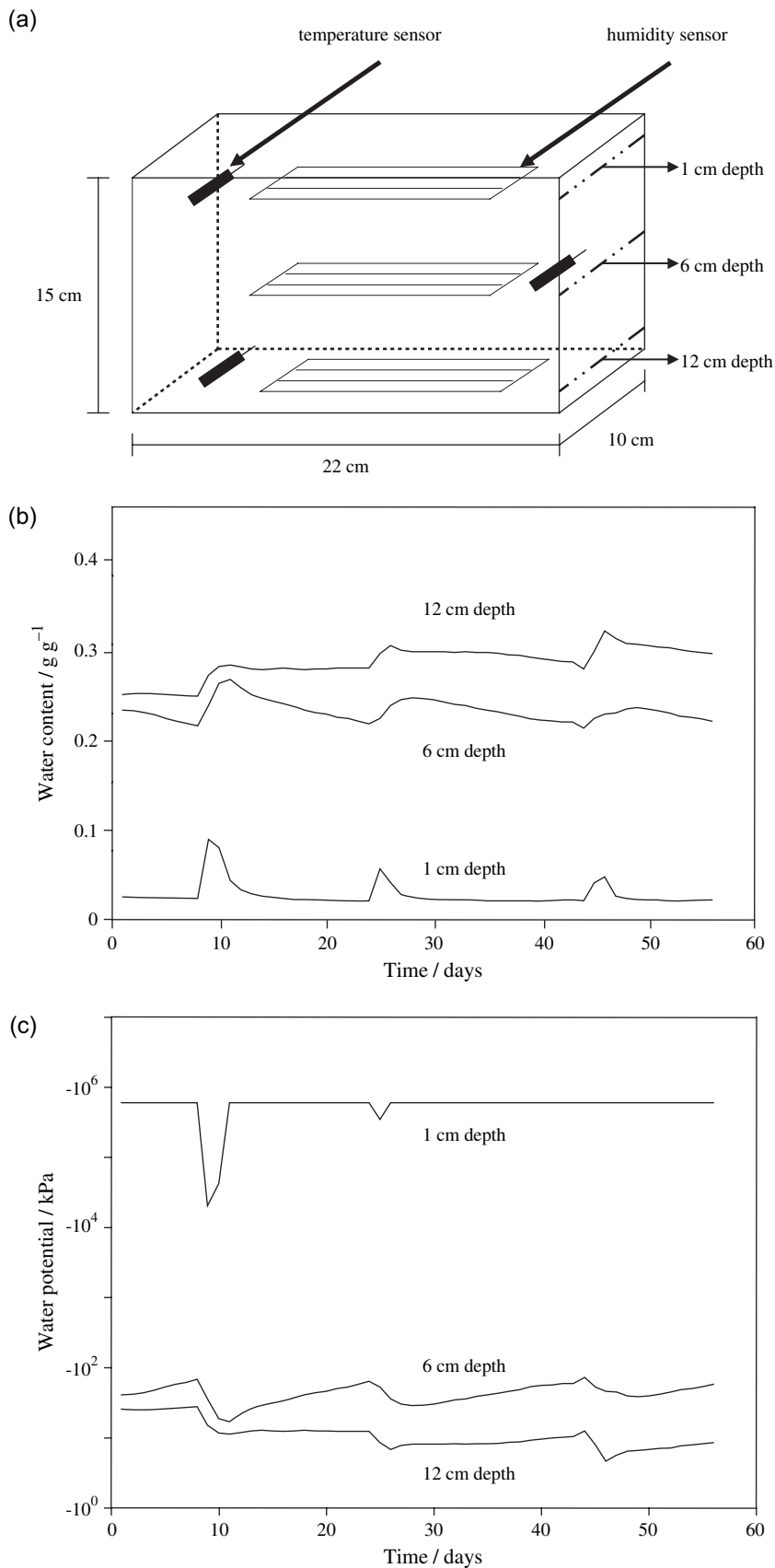


Figure 1 (a) Microcosm dimensions and location of soil temperature and soil humidity sensors; (b) soil water content and (c) soil water potential in the microcosms at 1, 6 and 12 cm depth during incubation.

of the applied pesticide. Three times a week, the system was aerated for 1 hour with an air flow rate of 1 l hour⁻¹ and then the solutions from the trapping system (Schroll *et al.*, 2004) were collected. The analyses of the ¹⁴C-substances were performed in a scintillation counter (Wallac Winspectral 1414, Turku, Finland). All biodegradation experiments were conducted in at least quadruplicate.

Quantitative analysis of the physiologically active bacterial fraction

In general, soil material for total cell counts and DNA extraction was stored at 4°C in the dark at the end of all experiments and equilibrated prior to analysis.

Cell fixation and immobilization on polycarbonate filters. Soil samples were prepared and fixed according to previously described protocols (Amann *et al.*, 1990; Stein *et al.*, 2005). For further fluorescence *in situ* hybridization analysis of the bacterial composition with fluorescent labelled oligonucleotide probes, the fixed samples were immobilized on polycarbonate filters by already described standard protocols (Glöckner *et al.*, 1996; Perntaler *et al.*, 2001).

Fluorescence in situ hybridization (FISH). This was done to investigate the physiologically active bacterial population in the soils with the protocols of Manz *et al.* (1992) and Amann *et al.* (1990). We used an equimolar mixture of the probe EUB-338 (Amann *et al.*, 1990) and two newly described derivatives (Daims *et al.*, 1999) to detect almost all known bacteria. All oligonucleotides (Table 1) were purchased from Thermo Hybaid, Division Interactiva (Ulm, Germany). Hybridization with carbo-cyanine Cy3-labelled probes was done with 35% deionized formamide in the hybridization buffer. The prepared filters were hybridized on standard glass slides for microscopy for at least 1.5 hours at 46°C, and mounted with 200 µl hybridization buffer and 20 µl fluorescently labelled probe (50 ng µl⁻¹). The filters were washed intensively with distilled water to remove salts, DAPI-stained (see next section), air-dried and mounted with Citifluor antibleaching agent AF1 (Citifluor Ltd, Canterbury, UK) for further microscopic investigation.

DAPI staining. We determined the total cell numbers by counterstaining the hybridized filters with the DNA intercalating dye

DAPI (4'-6-diamidino-2-phenylindole). Therefore, filters were covered with 300 µl DAPI solution (1.75 µg ml⁻¹ double-distilled water) and incubated at room temperature for 10 minutes in the dark. After washing the filters with distilled water they were dried in the dark at room temperature.

Microscopic analyses. Fluorescence signals were detected by means of an epifluorescence microscope (Axioplan-2, Zeiss, Oberkochen, Germany) with filter sets for DAPI and the Cy3 label.

Determination of soil microbial biomass using microcalorimetry

We measured the development of the total microbial biomass with a thermal activity microcalorimeter (2277 Bio Activity Monitor, C3-Analysetechnik, Baldham, Germany). The soil samples were first equilibrated for 24 hours at 20°C and a water content equal to -214 kPa. Microbial biomass C was determined after substrate-induction (Sparling, 1983) with an additive of about 0.5% (related to dry weight) glucose (1 : 1 mixed with talcum powder) to the soil. Microbial biomass contents were measured before the onset in the second increase in heat production which results from microbial growth in the substrate-amended samples (Heilmann, 1993). Measurements were conducted in quadruplicate.

Analysis of bacterial community structure

DNA extraction. Total DNA from 500 mg soil was extracted using the Fast DNA[®] SPIN kit for soil following the manufacturer's instructions (Bio 101, Carlsbad, USA).

Polymerase Chain Reaction. The 16S-rRNA coding gene fragments were amplified with the bacterial universal primer pair f984GC/r1378 (Heuer *et al.*, 1997, Thermo Hybaid, Interactive Division Ulm, Germany; Table 2). The reaction mixture (total volume 100 µl) contained: 10 µl of 10 × reaction buffer (Invitrogen, Carlsbad, USA); 3 µl of 50 mmol MgCl₂; 5 µl of 2 mmol deoxynucleotide triphosphates each (dNTPs); 2 µl of 100 µmol forward and reverse primer; 2.5 units of Taq DNA polymerase (1 unit µl⁻¹, MBI Fermentas, St. Leon-Roth, Germany); 10 µl of 3% bovine serum albumin (BSA) (Sigma-Aldrich, Munich, Germany); 5 µl of dimethyl sulfoxide (Thermolectron Corporation, Ulm, Germany); and 58.5 µl nuclease-free, ultra-pure water. For each reaction, 40 ng of the

Table 1 Specificity and sequence of the oligonucleotides used

Probe	Specificity	Sequence 5' → 3'
EUB 338 I	Bacteria except <i>Planctomycetales</i> and <i>Verrucomicrobiales</i>	GCT-GCC-TCC-CGT-AGG-AGT
EUB 338 II	<i>Planctomycetales</i>	GCA-GCC-ACC-CGT-AGG-TGT
EUB 338 III	<i>Verrucomicrobiales</i>	GCT-GCC-ACC-CGT-AGG-TGT

Table 2 Characteristics of the bacterial universal primers used

Primer	Position	Sequence (5' → 3')
968FGC	968–984	AACGCGAAGAACCCTTAC GC clamp connected to the 5' end of 968F
1401R	1385–1401	CGGTGTACAAGACCC

isolated DNA was added. The PCRs were carried out in a programmable thermal cycler (Primus 96 MWG Biotech, Eberberg, Germany) with the following cycling conditions: (i) an initial denaturation step at 94°C for 7 minutes; (ii) 35 cycles of denaturation at 94°C for 1 minute; (iii) primer annealing at 54°C for 1 minute; (iv) elongation at 72°C for 1 minute; (v) cycling program completion by a final extension at 72°C for 10 minutes. After completion of the reaction, the thermal cycler was held at 4°C. The success of the PCR amplification was analysed with standard horizontal agarose gel electrophoresis after ethidiumbromide staining. All PCR experiments included negative and positive controls.

Denaturing gradient gel electrophoresis (DGGE). These analyses were done according to the protocol of Muyzer *et al.* (1996). The gel-running procedure was done as given in the Bio-Rad Manual (Bio-Rad Laboratories, Munich, Germany). Gel electrophoresis was performed at 100 V for 18 hours at 60°C. The silver staining of the gel was performed according to Heukeshoven & Dernick (1988).

Image analysis. Patterns of DGGE bands were analyzed with the program gel compar II 2.0 (Applied Maths, Kortrijk, Belgium) after digital image acquisition of the DGGE gel. The relation between band patterns was calculated by a similarity matrix by means of Dice's coefficient with band comparison. Position tolerance was set at 1.0%. A dendrogram describing the pattern similarities was generated using the unweighted pair-group method with mathematical averages (upgma).

Results

Soil water

The measured and calculated (van Genuchten, 1980) soil water retention curves of the sieved 'Feldkirchen' soil are shown in Figure 2.

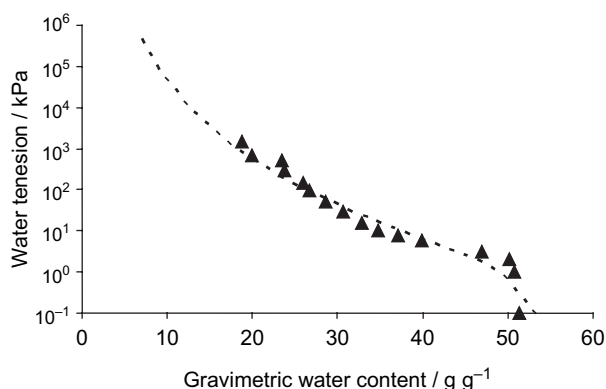


Figure 2 Measured (▲) and calculated (dotted line) (van Genuchten, 1980) soil water retention curves for sieved (≤ 2 mm) 'Feldkirchen' soil at a bulk density of 1.3 g cm^{-3} .

Mineralization of ^{14}C -isoproturon in soil from the field lysimeter: September 2003. The isoproturon mineralization in the upper soil (Figure 3a) had a 10-day lag phase, which was characterized by nearly constant and poor mineralization rates. This phase was followed by a more rapid degradation phase and the total amount of mineralized isoproturon after 28 days was $< 15\%$ of total ^{14}C applied. In contrast, the ^{14}C -isoproturon mineralization in the deeper soil (Figure 3a) was *c.* 35% of ^{14}C in the same period. The lag phase was around 7 days in this deeper soil.

Mineralization of ^{14}C -isoproturon in microcosm soil: climate scenario summer 2003. The development of soil water content and water tension in the microcosms in relation to the incubation time is given in Figure 1(b,c). The course of ^{14}C -isoproturon mineralization in the soil after the microcosm experiments and in the microcosm control soil is shown in Figure 3(b–d). Although the soil was re-equilibrated for 28 days prior to the biodegradation experiment, isoproturon mineralization was very different in the two microcosm layers. Soil in the upper layer (Figure 3b) had a poorer ^{14}C -isoproturon mineralization capability ($< 5\%$) after 66 days than soil material from the deeper layer ($< 30\%$, Figure 3d). There is no significant difference between the herbicide mineralization in the microcosm control (Figure 3c) and the soil from the deeper layer.

Microbial biomass

More microbial biomass was found in the microcosms in the deeper soil than in the upper layer (Table 3). The soil from the microcosm control had a microbial biomass comparable to the upper layer of the microcosms.

Cell counts determined by DAPI/FISH

Total cell numbers determined by DAPI and viable bacterial cell numbers determined by FISH were substantially greater in the deeper soil compared with the upper soil from the lysimeter (Table 3). In the microcosms, the cell counts from the deeper layer were around double those of the upper layer. The cell counts in the microcosm control were of the same order of magnitude as the cell counts in the deeper soil from the lysimeter.

Bacterial community structure

To characterize the bacterial community structure, we determined DGGE bands in soil samples from microcosms at both depths, the microcosm control and at both depths from the lysimeter soil (Figure 4). The microbial community was different in the various soil. Whereas the microcosm control and the deeper soil of the lysimeter had closed structures (95% similarity), two predominant bands (see arrows in Figure 4) – presented

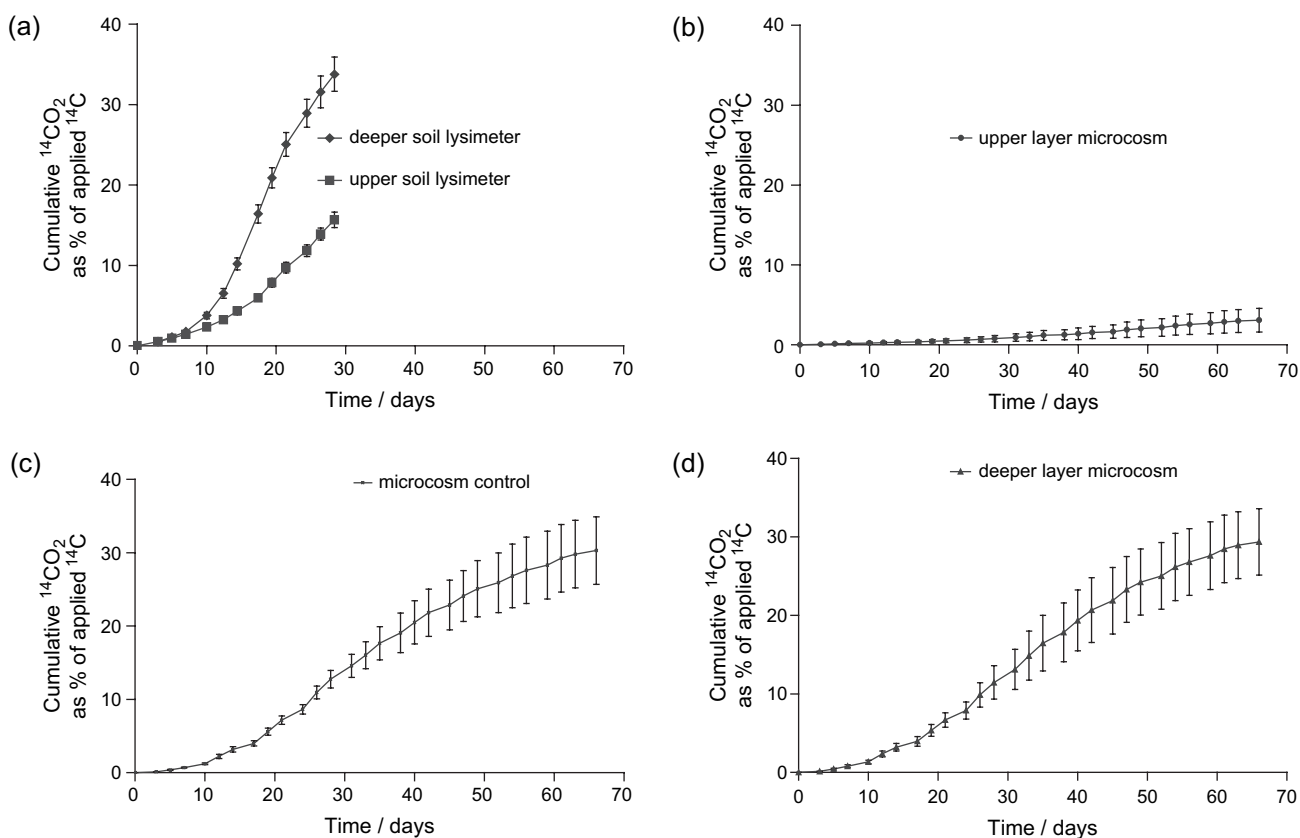


Figure 3 (a) ¹⁴C-isoproturon mineralization in soil from lysimeter (*n* = 4), (b) upper layer (*n* = 6), (c) microcosm controls (*n* = 4) and (d) deeper layer (*n* = 6). Bars are standard errors.

in lines from the deeper soil of the lysimeter – were not seen at the upper layer of the microcosm. The number of bands in this upper layer was in general less than in the other soil samples and had the least similarity (44%) to all the other soil samples. Further, the similarity between the upper and deeper soil from the lysimeter was very small at 60%.

Discussion

Isoproturon mineralization with soil from the field lysimeter

Although all soils were re-equilibrated prior to the biodegradation experiments, the upper soil from the lysimeter still had a tendency for a cometabolic degradation pattern, which implies the

Table 3 Isoproturon degradation and microbial parameters obtained in microcosms and lysimeter soil: accumulated ¹⁴CO₂ from applied ¹⁴C after 28 days incubation, cell counts and microbial biomass

Origin of the sample	Accumulated ¹⁴ CO ₂ ^a /% of ¹⁴ C applied after 4 weeks	Cell counts ^b /10 ⁷ cells g ⁻¹ dry wt soil		Microbial biomass carbon ^c /μg C × g ⁻¹ dry wt soil
		DAPI-stained cells	<i>In situ</i> hybridised cells (FISH)	
Microcosm upper layer	1.1 ± 0.4	4.3 ± 0.6	3.0 ± 0.4	362 ± 34
Microcosm deeper layer	12.9 ± 2.1	8.9 ± 0.7	6.8 ± 0.8	568 ± 7
Microcosm control	14.5 ± 0.8	52.8 ± 4.2	37.3 ± 3.3	329 ± 29
Lysimeter upper soil	15.7 ± 0.5	7.2 ± 1.4	5.2 ± 1.2	
Lysimeter deeper soil	33.8 ± 1.0	28.9 ± 1.8	23.9 ± 1.2	

^aStandard error (*n* = 6) for microcosms and for microcosm control (*n* = 4) and lysimeter soil (*n* = 4).

^bStandard error (*n* = 4).

^cStandard error (*n* = 4).

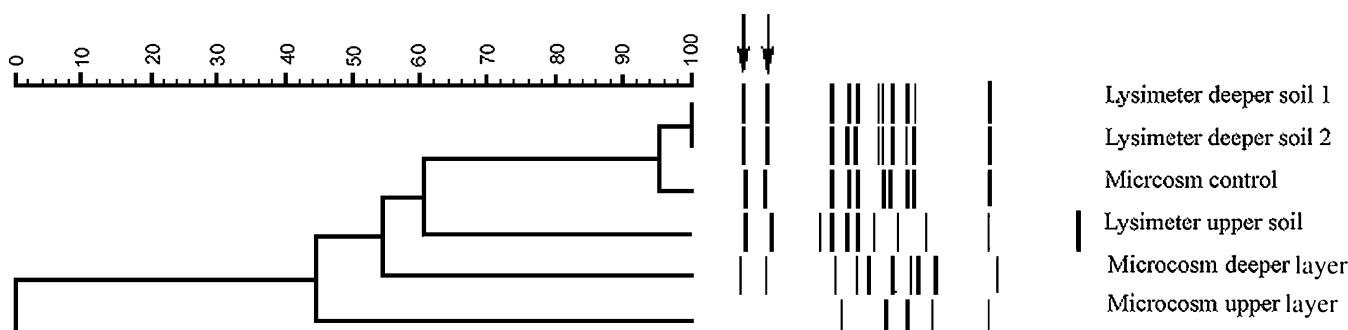


Figure 4 Cluster analysis of denaturing gel gradient electrophoresis (DGGE) gel of bacterial 16S rDNA PCR products from soil DNA extracts. Arrows show two predominant bands in soils except for microcosm upper layer. The deeper soil sample was repeated (1 and 2) as an internal quality control.

concomitant oxidation of a non-growth substrate during the growth of a microorganism on an utilizable carbon and energy source (Torstenson, 1980). The deeper soil was intermediate between metabolic (growth-linked) and cometabolic degradation. Differences in the duration of the lag phases and in the total amount of mineralized ^{14}C -isoproturon were observed. This difference in the particular special soil function for isoproturon mineralization showed a significant change in the microbial degradation behaviour at the two soil depths. The cell counts in the upper and deeper soil differed by a factor of 4 (DAPI) to 5 (FISH), but the ability of the soils from the two depths to mineralize isoproturon differed by a factor of around 2 (Table 3). There were thus more microorganisms able to mineralize isoproturon in relation to the total bacterial community in the upper soil than in the deeper soil. Therefore, we assume that different communities with a different isoproturon degradation ability inhabit or are predominant in the two soil depths after summer 2003. This conclusion is reinforced by the results of the community structure analysis. The similarity between the upper and deeper soil was about 60%.

^{14}C -Isoproturon mineralization with soil from the microcosms

Despite re-equilibration, soil from the upper layer in the microcosms had lost the ability to mineralize isoproturon to such an extent that <5% ^{14}C was mineralized in 56 days of incubation (Figure 3b). The microorganism community of the upper layer had a very different mineralization ability compared with the deeper layer after 56 days of climate conditions that simulated summer 2003 and a subsequent re-equilibration of 28 days. The mineralization ability in the soil of the deeper layer of the microcosms did not decrease in comparison to the microcosm control soil. We think that the extreme changes in the water content of the upper layer affected the mineralization ability of the soil communities and, in the re-equilibration period, the microbial communities were not able to recover their initial isoproturon mineralization function. According to

Schimel *et al.* (1999) and Wilkinson *et al.*, (2002), the variation in soil moisture content and the nature of this fluctuation influence the sustainable composition of soil bacterial and fungal communities. Thus, soil moisture content change seems to be an important control of microbial community composition when large shifts in water content occur in short periods of time (Fierer *et al.*, 2003). In our soil, the climate conditions that happened in the particular summer of 2003 reduced the isoproturon mineralization function of the soil, and this function could not be reversed in the short term despite the re-wetting of the soil.

Strategies used by microorganisms for growth and survival under water stress reflect the nature and extent of water perturbations of their natural habitats (Harris, 1981). Depending on these capabilities, several microorganisms can withstand extreme plasmolytic and plasmoptotic water potential stress, but others cannot survive under extreme environmental conditions. We suppose therefore that the effect of summer 2003, as simulated in the microcosm study, placed an irreversible water stress on the isoproturon-degrading microorganisms in the upper layer.

Microbial soil parameters

The largest amount of bacterial cells was determined in the microcosm control, followed by the deeper layer of the microcosm. Comparing the two microcosm soil layers, the dryer the soil was, the smaller amount of cells detected. According to Pesaro (2004), cell lysis and spore formation can occur in the drying periods, thus decreasing the bacterial cell counts. The decreasing DAPI permeability made spores less detectable by cell fixation and staining procedures. The cell values determined in the deeper layer were less than those for the microcosm control. Given that the microcosms were filled with the same soil as the control, and given that the soil moisture contents were similar at this depth, such a decrease in the DAPI/FISH cell counts in the microcosms compared with the control soil was surprising.

The estimation of total biomass (microfauna, bacteria, fungi) showed smaller differences between microcosm control and both upper and deeper layers in comparison with results from the cell counts. The total microbial biomass from the upper layer decreased in comparison with the deeper layer. If a community change takes place, like in our experiment, this can affect some specific microorganisms more intensively than others. This effect is apparent in the different estimates of the bacterial (DAPI/FISH) and total microbial biomass (microcalorimetry) for the microcosm control on the one hand, and the upper and deeper layers on the other hand. This finding is reinforced by the DGGE patterns that show different predominant bands, i.e. from different predominant communities at the different soil depths. If we compare the pesticide mineralization achieved with the microbial parameters, with the control soil as reference, we observe that the control soil behaves differently in relation to the

microcosm layers (Figure 5). Although the control soil had greater cell numbers than the soil from both layers, pesticide mineralization was very similar to that in the deeper layer and much greater than in the upper layer. A large value was measured for total microbial biomass (microcalorimetry, which gives the sum of all microbiota, microfauna, bacteria, fungi) in the deeper layer, but similar and small values were measured in the control soil and the upper layer. In spite of the smaller biomass determined in the control soil, isoproturon mineralization was of the same order as in the deeper layer, which shows shifts in the development of microbiota.

We can conclude that the microbial biomass and the special isoproturon mineralization soil function, as well as the structural bacterial diversity in all the microcosms, were influenced by the simulated event of the unique conditions of summer 2003.

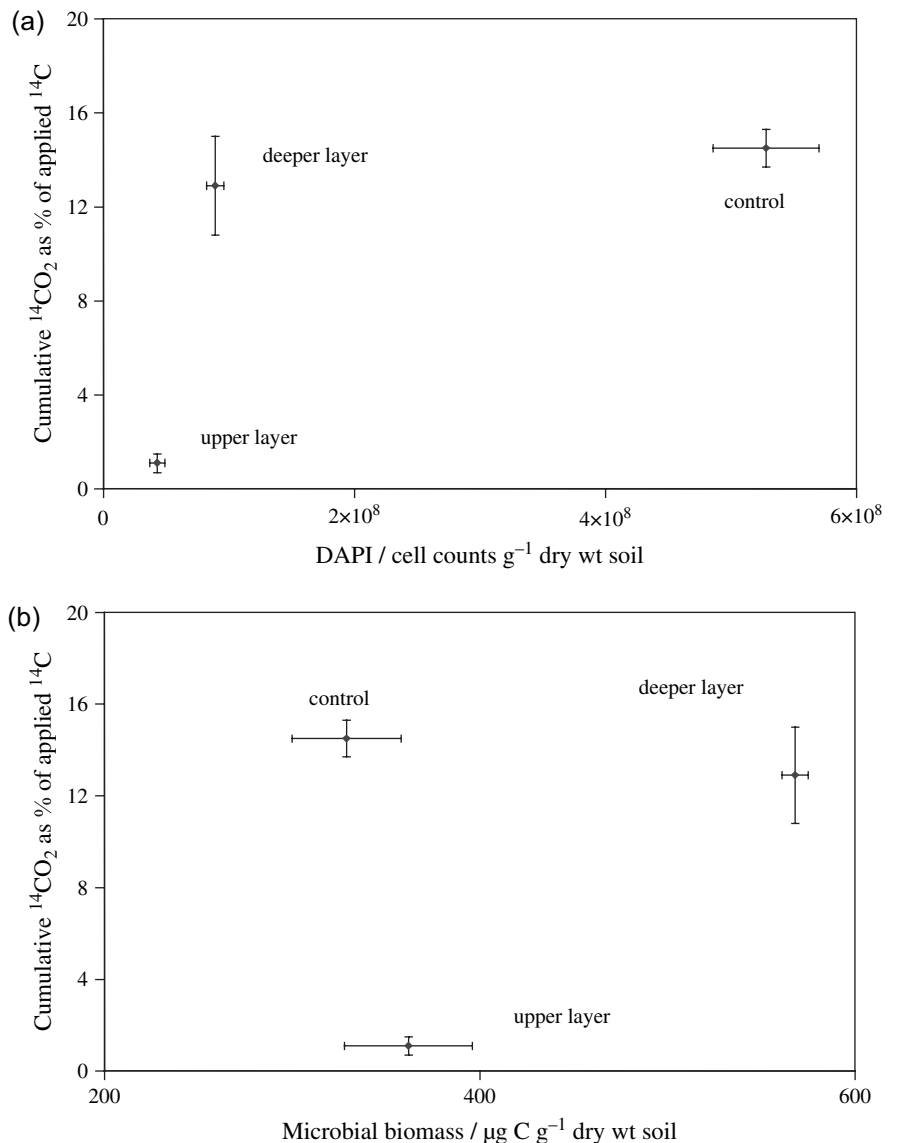


Figure 5 ^{14}C -Isoproturon mineralization in soil from microcosms and microcosm control and (a) the microbial parameters DAPI bacterial counts ($n = 4$) and (b) microbial biomass ($n = 4$) at the end of the incubation. Bars are standard errors.

Relevance for future agricultural management

As shown by these experiments, a drastic change of the environmental factors studied can deeply influence specific soil functions as well as structural microbial diversity in soils. Up to now, it is not known whether the observed change of soil quality is permanent or whether the soil will recover on its own in the long term.

We assume that the reduction of specific soil functions detected, for example in the upper soil layer of the 'Feldkirchen' soil, is a minor problem in relation to conventional soil tillage. By ploughing between 20 and 30 cm, soil will be inverted and microorganisms from deeper soil will populate the upper soil layer. However, in case of no-till, a farming method which is increasing in Europe (Holland, 2004), soil will not be inverted so often. Therefore, how will those microorganisms that survive extreme weather (for example, in the deeper soil) and are capable of mineralizing newly applied pesticides repopulate the upper layers? Short-range cell movements seem to be common in soil (Grundmann, 2004). Larger organisms such as earthworms (Singer *et al.*, 2001) can transfer microorganisms over large distances. But will those organisms move against a negative water gradient, i.e. from a wetter soil to a completely dry soil surface? Dighton *et al.* (1997) said that, beside the movement of soil biota acting as vectors for microorganisms, management practices play an important role in the dispersion of microorganisms in soils. Usually, isotruron is degraded more efficiently in soils when non-inversion tillage is conducted (Jordan, 2000), but the study of Jordan (2000) was carried out under temperate climatic conditions in the UK. However, what could happen under extreme climatic conditions? More information is needed to give answers on this complex problem.

Conclusion

A drastic change in functional and structural soil functions in a particular soil was caused by summer 2003. It needs to be shown whether this effect can be observed for other soils and in other regions. If the described climate could have a long-term effect on soil quality, strategies must be developed to minimize negative effects for the environment which could arise from this effect (e.g. ground water contamination by chemical residues). Ploughing of soils instead of no-till might be an easy method to solve this problem. Another possible strategy might be the application of specialized active microbial communities to damaged soils in order to improve the biological degradability of chemicals in soils as described by Schroll *et al.* (2004).

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