Classification and monitoring of soil microbial biomass, N-mineralization and enzyme activities to indicate environmental changes

D. Tscherko and E. Kandeler

Klassifikation und Monitoring der mikrobiellen Biomasse, N-Mineralisation und Enzymaktivitäten zur Indikation von Umweltveränderungen

1. Introduction

Soils are natural bodies in the pedosphere and are thus a sensitive part of the overall ecosystem. Some of the hazards to soils include anthropogenic contamination, cultivation, urbanization and destruction. Certain soil components, however, will hardly change at all over the next century (e.g. mineral content), and monitoring thus should focus on those components expected to change more rapidly (e.g. organic and inorganic chemical components, soil animals and soil microorganisms). In this sense biomonitoring is the most integrated approach of soil screening as it monitors effects on organisms which are themselves part of the soil ecosystem (DESAULES and VON STEIGER, 1993) and which thereby impact in far reaching ways the quality and the multifunctionality of the soils. Microbial properties as indicators for human-induced soil changes need to be considered against the background of natural variation (BROOKES, 1993; VON STEIGER et al., 1996).This involves examining the effects of naturally occurring stresses, like water, temperature and substrate fluctuations, on soil microbial populations and their activities (DOMSCH, 1980; DOMSCH et al., 1983). Microbial properties also need to be generally scientically valid and accurately, precisely, easily and eco-

Zusammenfassung

Die Eignung bodenmikrobiologischer Parameter als Umweltindikatoren wurde auf fünf Bodendauerbeobachtungsflächen im Bundesland Salzburg untersucht. Die Analysen umfaßten die mikrobielle Biomasse und Enzyme des C-, N-, P-, und S-Kreislaufes (Xylanase, N-Mineralisation, Urease, Potentielle Denitrifikation, alkalische Phosphatase, Arylsulfatase). Die räumliche Variabilität der mikrobiologischen Parameter war im Vergleich zu den zeitlichen Schwankungen gering. Die Standortfaktoren Bodentyp und die Landnutzung, nicht jedoch die physikalisch-chemischen Bodenparameter, übten einen signifikanten Einfluß auf die mikrobiellle Biomasse und Enzymaktivitäten aus. Die untersuchten Flächen wurden nach den Perzentilgrenzen 30 und 70 der gemessenen Datensets als niedrig, mittel und hoch aktive Flächen klassifiziert.

Schlagworte: Monitoring, mikrobielle Biomasse, Enzymaktivitäten, Bodentyp, Klassifikation.

Summary

The potential of the microbial parameters as indicators for soil changes was assessed on five soil monitoring sites, differing in soil type and land use, in the province Salzburg, Austria. The spectrum of analyses comprised microbial biomass parameter and enzymatic activities of the C, N, P and S-cycle (xylanase, N-mineralization, urease, potential denitrification, alkaline phosphatase, arylsulfatase). While the spatial variability was low, the temporal amplitudes of the microbial biomass and activities during the year were substantial, limiting the potential to detect environmental changes. Overall, a strong influence of the soil type and land use on the microbial performance was detected. The percentiles 30 and 70 of the measured activity data were applied to delimit low, normal and high activity sites.

Key words: Monitoring, microbial biomass, soil enzymes, soil type, classification.

nomically measurable across a wide range of soil types and soil conditions. Finally, the property should be sensitive enough to indicate pollution but also sufficiently robust to avoid false alarms (BROOKES, 1993).

Many soil surveys have been conducted, and some of them have investigated biological properties of the soils (BROOKES, 1993; JURITSCH and WIENER, 1993; PHILIPP et al., 1994; ÖHLINGER, 1995; STRAALEN and KRIVOLUTSKY, 1996). Microbial screening in soil monitoring networks has received less consideration (MIRTL, 1996; VON STEIGER et al., 1996; HÖPER et al., 1997; JÖRGENSEN, 1997). A new concept by KANDELER et al. (1993) and JURITSCH (1995) attempts to fill this gap and involves not only monitoring soil physical and chemical parameters, but also microbial properties characterizing the functional diversity of the soil microorganisms.

Measurements of the microbial biomass and various enzyme systems have been widely used to diagnose the soil state and to describe the effect of different influences (e.g. air pollutants, agricultural management, land use). So far, however, no valid classification system for microbiological variables has been available for assessing the specific measurements in relation to abiotic properties and land management. Thus, our specific objective is to develop and test such a system. Beyond that, the potential indicator value of the microbial parameters for environmental stress in general (e.g. heavy metals, pesticides, aromatic hydrocarbon-compounds) is investigated.

The current concept of soil monitoring is based on five monitoring sites, differing in soil type and land use, in the province Salzburg, Austria. The microbial properties were selected as recommended by KANDELER et al. (1993) and coincided with the criteria listed by BROOKES (1993) and EDWARDS et al. (1996). The parameters include the substrate-induced respiration (SIR) as a measure for the active microbial biomass, and dynamic soil processes including the N-mineralization and the enzymes of denitrification, urease, phosphatase, arylsulfatase and xylanase activity as indices of the nutrient cycles in the soil.

2. Material and methods

2.1 Site description

The sites consist of a circular central area (1000 m^2) surrounded by a protective border of 8–10 m width. Site 1–4 were established in 1994, Site 5 in 1995. Each site was homogeneous with respect to soil type, plant cover (grassland or arable land) and surface level. Calcaric *Fluvisol GL1*: site 1 (grassland, 2 cuttings a year) is located in an flat industrial area near Hallein at 450 m above sea level. Eutric *Cambisol GL2*: *Cambisol GL2* (grassland, 2 cuttings a year) is situated in an unpolluted landscape close to St. Koloman at 1005 m above sea level. *Cambisol GL2* represents a reference monitoring site for atmospheric pollutants. Eutric *Cambisol GL3*: site 3 (grassland, 2 cuttings a year) is located in a remote impact region near Saalfelden at 785 m above sea level. Calcaric *Fluvisol AL4*: site 4 (arable land, biological farming) has been established in the plain pastures of Salzburg city at 420 m above sealevel. Dystric *Cambisol GL5*: site 5 (grassland, 1 cutting a year) with a gentle slope is situated in a former mining area (diffuse Arsenic pollution of 60 ppm As) near Bischofshofen at 595 m above sea level. The soils are classified according to FAO-UNESCO (1994). The land use, soil type and physico-chemical properties of the sites are given according to JURITSCH (1995, personal communication) in Table 1.

Table 1: Soil chemical and physical properties of the monitoring sites (JURITSCH, pers. comm.)
Tabelle 1: Chemische und physikalische Eigenschaften der Bodendauerbeobachtungsflächen (JURITSCH, pers. Mitt.)

Site	Site name	Soil type (FAO)	Land use	Soil depth cm	pH ($CaCl_2$)	$CaCO_3$ %	C_{org} %	N_t %	Particle size distribution % <2 µm	2-60 µm	60-2000 µm
GL1	Hallein	Fluvisol	Grassland	0-5	6.80	1.05	3.5	0.43	7.0	57.2	35.8
				5-10	6.80	1.35	3.4	0.39	7.8	53.9	38.4
GL2	St. Koloman	Eutric Cambisol	Grassland	0-5	5.40	0.20	5.8	0.67	27.7	66.8	5.5
				5-10	5.50	0.20	5.8	0.66	28.7	66.3	5.1
GL3	Saalfelden	Eutric Cambisol	Grassland	0-5	5.34	0.17	3.5	0.42	13.3	57.0	29.8
				5-10	5.41	0.21	3.6	0.42	13.4	58.9	27.7
AL4	Salzburg City	Fluvisol	Arable	0-20	6.84	4.58	3.5	0.47	11.3	76.2	12.5
GL5	Bischofshofen	Dystric Cambisol	Grassland	0-5	6.15	0.12	4.3	0.45	10.9	69.1	20.0
				5-10	5.98	0.08	2.5	0.26	9.1	63.8	27.1

2.2 Soil sampling

Soil samples (4 replicates) were randomly taken from the protective area in spring and autumn 1995–1997. The sampling distance was approximately 5–10 m. *Cambisol GL5* has been sampled since 1996, *Cambisol GL2* was skipped in spring 1996 due to high snow cover. In the first monitoring year (1995) two sampling designs on the grassland sites were compared. Simultaneously, homogeneous sampling of the 0–10 cm layer was tested against stratified sampling of the 0–5 cm and 5–10 cm layers. The sampling depth of the arable land comprised the ploughed layer and was 0–20 cm. For each sample, 20–25 soil cores were taken using an auger, bulked and packed in a polyethylene bag. The fresh samples were stored at -20 °C until laboratory measurements commenced.

2.3 Chemical analyses

Chemical analyses were carried out on air-dried, sieved (2 mm) soil. The pH was determined by shaking 4 g soil material with 10 ml 0.01 M $CaCl_2$ solution. After 24 h the pH was measured potentiometrically. Total organic carbon content was determined by wet combustion of the soil with a mixture of 20 ml 0.33 M $K_2Cr_2O_7$ and 15 ml H_2SO_4. After 24 h Cr(III) in the filtrate was colorimetrically determined at 570 nm. Total nitrogen was determined by digestion of the organic N with H_2SO_4 conc. The NH_4^+-N in the digest was titrimetrically determined from the amount of NH_3 liberated by distillation with alkali. The carbonate content was determined by adding HCl (10 % vv^{-1}) to the soil and the volume of CO_2 that developed was measured in consideration of temperature and atmospheric pressure.

2.4 Microbiological analyses

Before microbiological analyses the frozen soil samples were allowed to thaw at 4 °C for 3 days and sieved (2 mm). Substrate-induced respiration (SIR) as a measure for the active microbial biomass was performed according to ANDERSON and DOMSCH (1978). The soil was amended with 4.0 mg glucose g^{-1} dry matter and incubated at 25 °C for 4 h. The CO_2 evolved was trapped in 0.05 M NaOH and titrated by 0.05 M HCl. N-mineralization was measured under waterlogged conditions according to KEENEY (1982) and KANDELER (1996). The samples were incubated at 40 °C for 7 d and the NH_4^+-N produced measured colorimetrically at 660 nm. Urease activity was assayed as described by KANDELER and GERBER (1988) using urea as a substrate. After incubation at 37 °C for 2 h, the NH_4^+-N formed was extracted with 1 M KCl and subsequently measured. Denitrification enzyme activity (DEA) was determined following the method of ZECHMEISTER-BOLTENSTERN (1996) using 1.07 mM KNO_3 / 0.7 mM chloramphenicol as a substrate and acetylene as an inhibitor of denitrification of N_2O (6 h at 25 °C). The N_2O-N produced was gas-chromatographically detected with an electron capture detector (Shimadzu GC 14). Alkaline phosphomonoesterase (APA) activity was assayed by using buffered disodium phenylphosphate (0.2 M borate buffer, pH 10, 20 mM phenylphosphate) as a substrate and incubating the samples at 37 °C for 3 h. The released phenol was estimated colorimetrically at 400 nm (HOFFMANN, 1968). Arylsulfatase activity (ASA) was measured according to TABATABAI and BREMNER (1970). After the addition of a p-nitrophenylsulfate solution, soil samples were incubated at 37 °C for 1 h. The p-nitrophenol released was colorimetrically quantified at 420 nm. Xylanase activity is described in detail by SCHINNER and VON MERSI (1990). Soil samples were incubated with 1.2 % xylan suspension and 2 M acetate buffer (pH 5.5) at 50 °C for 24 h. The produced reducing sugars were colorimetrically determined by the ferric-ferrocyanide reaction at 690 nm. The methods are described in detail in SCHINNER et al. (1996).

2.5 Statistical analyses

The results of the soil microbial activities were calculated on the basis of the oven-dry weight (105 °C) of soil and given as arithmetic means of 4 replicates and their standard errors (se). The data distribution passed the normality test (Kolmogorov-Smirnov test) and the homogeneity-of-variance test (Levene test). The two sampling designs were tested by comparing the measured mean of the homogeneous 0–10 cm samples with the calculated mean of the 0–5 cm and 5–10 cm layer samples (t-test). The percentiles of 30 and 70 were used to delimit the low, normal and high activity class. The Pearson correlation coefficient r was calculated between microbial and soil physico-chemical variables. The maximum critical range (LSD), calculated by the Student Newman Keuls test using time as the main factor, was applied to identify significant temporal changes and to estimate the potential of microbial parameters as bioindicators.

The relative coefficient of variance (CV_r) was used to assess the spatial variability within a site. Wilks' Lambda, the canonical correlation coefficient and the standardized canonical function coefficient of the discriminant functions (*DF*) were applied to evaluate the discriminatory importance of the microbiological properties and their sensitivity to soil type and soil conditions. Significance was accepted at the $P \leq 0.05$ level of probability.

3. Results and Discussion

3.1 Sampling approach

As a large part of the variation of the data can be attributed to the sampling design, two concepts (homogeneous against stratified sampling) were simultaneously applied on the grassland sites in the first year 1995. The comparison revealed that for both sampling events (spring and autumn) the calculated means of the measured activities in the 0–5 cm and 5–10 cm soil samples tended to be higher than the directly measured mean activities in the 0–10 cm homogeneous sample, although the differences were not significant (data shown for spring 1995). Coinciding with the findings reported by CAMPBELL and BIEDERBECK (1976), the temporal amplitudes of the microbial activities were significantly higher in the 0–5 cm than in the 5–10 cm layer, partly caused by the stronger temperature and water fluctuation in the surface area. In contrast, these amplitudes diminished in the homogeneous samples. In soil monitoring programs, stratified sampling is suggested as the surface layer is specifically affected by seasonal and anthropogenic impact.

3.2 Temporal Variability

Results on the temporal variability of microbial properties have widely been reported (GEHLEN, 1987; KOWALZCYK et al., 1987; BREMER and VAN KESSEL, 1992; BUCHANAN and KING, 1992; v. LÜTZOW, 1993; CAMPBELL et al., 1995; JÖRGENSEN, 1995; FRIEDEL et al., 1996; KANDELER and BÖHM, 1996). In this study sampling of different sites before and after the vegetation period enabled the variation of microbial population, caused by plant-specific influences, to be recorded (Figs. 1–5). Moreover, following the course of microbial biomass and activity of different independend sites, allowed the influence of soil type and climatic conditions to be assessed. While the *grassland Cambisols* Saalfelden (*GL3*) and Bischofshofen (*GL5*) showed low temporal variability, the fluctuations of microbial biomass and activity of *grassland Cambisol* St. Koloman (*GL2*) were substantial. The high altidute (1005 m) of St. Koloman, which results in stronger water and temperature conditions, might be responsible for the amplitudes. The highest temporal fluctuations on the *Cambisol sites* were observed for SIR, DEA, xylanase (Saalfelden, Bischofshofen), respectively for all microbial parameters (St. Koloman), except for ASA.

Within the sites of *Fluvisols* (grassland *GL1* and arable land *AL4*) the temporal variability was similar to that found for the Cambisols. Only APA showed higher fluctuations in the *Fluvisol sites* compared to those of the *Cambisol sites.*

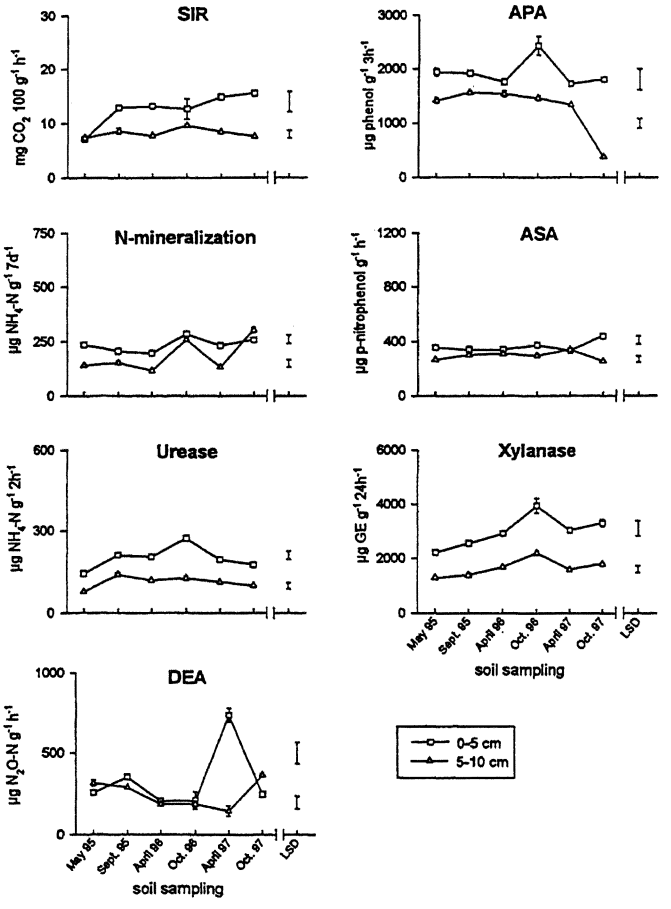

Figure 1: Temporal dynamics of the microbial biomass and enzyme activities of the grassland *Fluvisol GL1*. Different symbols are the means of the 0–5 and the 5–10 cm layer, in most cases, standard errors (whiskers) do not exceed size of the symbols.

Abbildung 1: Zeitlicher Verlauf der mikrobiellen Biomasse und Enzymaktivitäten auf der Grünlandfläche *Fluvisol GL1*. Unterschiedliche Symbole stellen den Mittelwert in 0–5 cm und 5–10 cm, Fehlerbalken den Standardfehler dar.

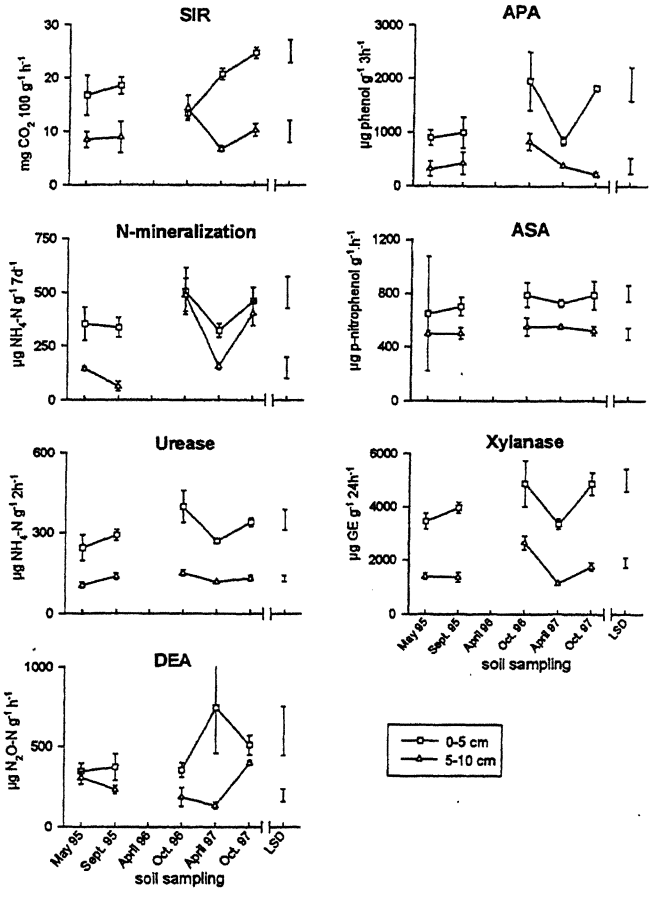

Figure 2: Temporal dynamics of the microbial biomass and enzyme activities of the grassland *Cambisol GL2*. Different symbols are the mean of the 0–5 and the 5–10 cm layer, in most cases, standard errors (whiskers) do not exceed size of the symbols.

Abbildung 2: Zeitlicher Verlauf der mikrobiellen Biomasse und Enzymaktivitäten auf der Grünlandfläche *Cambisol GL2*. Unterschiedliche Symbole stellen den Mittelwert in 0–5 cm und 5–10 cm, Fehlerbalken den Standardfehler dar.

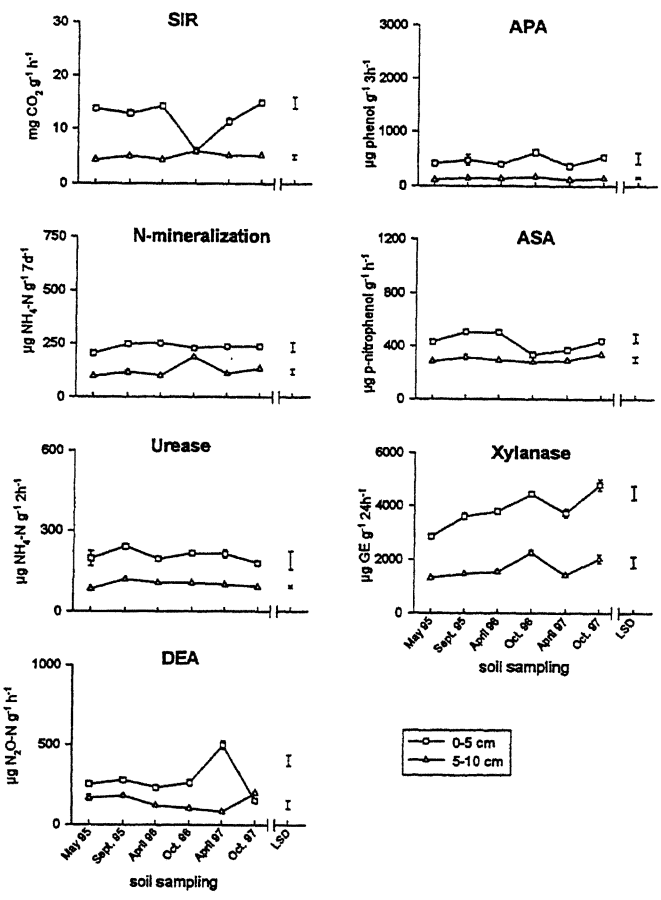

Figure 3: Temporal dynamics of the microbial biomass and enzyme activities of grassland *Cambisol GL3*. Different symbols are the mean of the 0–5 and the 5–10 cm layer, in most cases, standard errors (whiskers) do not exceed size of the symbols.

Abbildung 3: Zeitlicher Verlauf der mikrobiellen Biomasse und Enzymaktivitäten auf der Grünlandfläche *Cambisol GL2*. Unterschiedliche Symbole stellen den Mittelwert in 0–5 cm und 5–10 cm, Fehlerbalken den Standardfehler dar.

A striking feature of the investigations was the synchronous fluctuations of the microbial activities over time, regardless whether *Fluvisol, Cambisol,* arable or grassland soil. Especially, xylanase activity followed a periodical pattern, irrespective of the site. The observed temporal trend of xylanase and, to some degree, of N-mineralization (5–10 cm) is caused by the litter and plant residues at the end of the vegetation period. Remarkable are the steep increase in DEA of *Fluvisol GL1* (Hallein), *Cambisol GL2* (St. Koloman) and *Cambisol GL3* (Saalfelden) in spring 1997 (Fig. 1–3), likely produced by sufficient water saturation and soil N-mineralization during late winter and early spring, and the sharp decrease of APA within the *Fluvisols* (*GL1* and *AL4*) in autumn 1997 (Fig. 1 and 4).

Overall, the highest seasonal fluctuations were measured for APA, N-mineralization, DEA and xylanase activity, indicating the sensitive response of these enzymes to the site conditions (e.g. soil moisture, temperature, plant-specific influence). The smallest temporal amplitudes were recorded for urease activity and ASA. In order to eliminate the seasonal plant-specific effect sampling before the vegetation period is suggested (see next section).

The potential of microbial parameters to identify changes in successive samplings depends not only on the temporal variability, but also on the spatial variation within a site. The size of the critical range (LSD), accounting for the temporal and spatial variability, provides information on the potential of microbial properties to indicate environmental

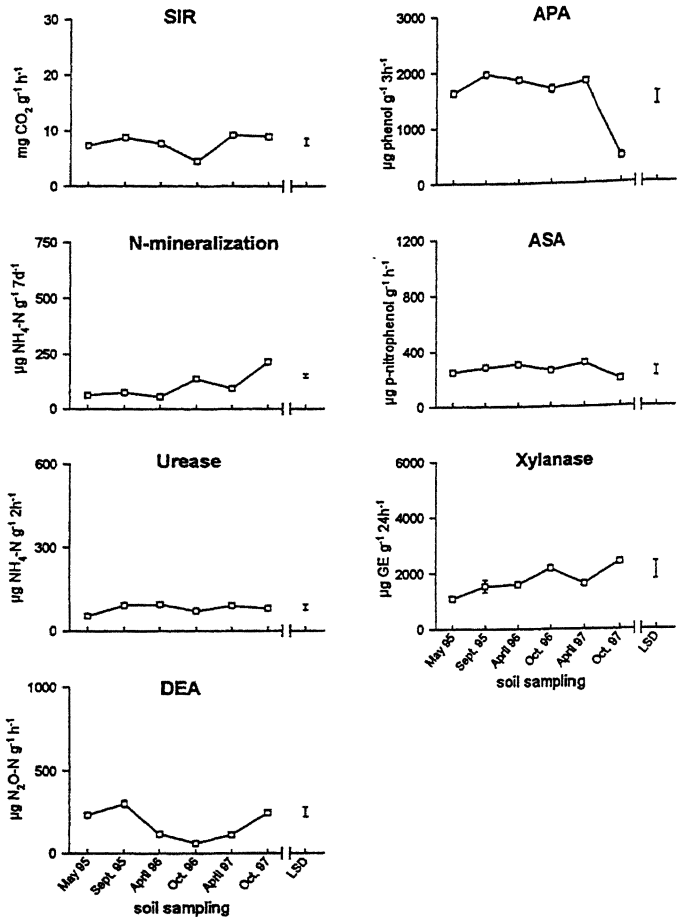

Figure 4: Temporal dynamics of the microbial biomass and enzyme activities of the arable land *Fluvisol AL4*. Given are the means of 4 replicates of the 0–20 cm layer, in most cases, standard errors (whiskers) do not exceed size of the symbols.

Abbildung 4: Zeitlicher Verlauf der mikrobiellen Biomasse und Enzymaktivitäten auf der Ackerfläche *Fluvisol AL4*. Datenpunkte stellen den Mittelwert (4 Wiederholungen) in 0–20 cm, Fehlerbalken den Standardfehler dar.

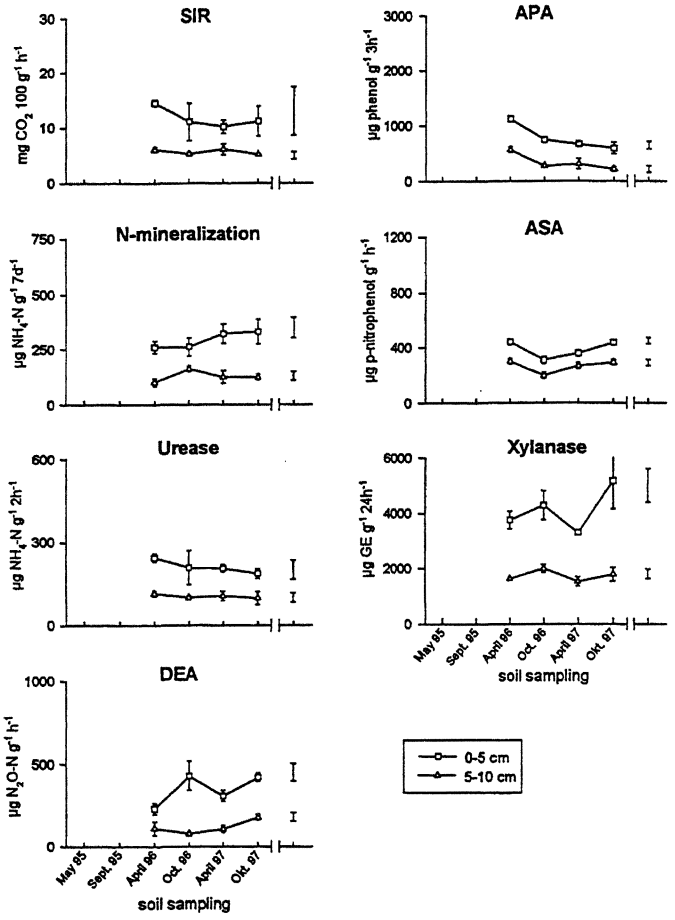

Figure 5: Temporal dynamics of the microbial biomass and enzyme activities of grassland *Cambisol GL5*. Different symbols are the mean of the 0–5 and the 5–10 cm layer, in most cases, standard errors (whiskers) do not exceed size of the symbols.

Abbildung 5: Zeitlicher Verlauf der mikrobiellen Biomasse und Enzymaktivitäten auf der Günlandfläche *Cambisol GL5*. Unterschiedliche Symbole stellen den Mittelwert in 0–5 cm und 5–10 cm, Fehlerbalken den Standardfehler dar.

changes (Table 2). In practical, we suggest to apply soil microbial parameters with low temporal and spatial variability (parameters not sensitive to plant-specific influences and climate, e.g. urease, ASA, and to hot spots, e.g. SIR, N-mineralization, urease, ASA and xylanase), but with high sensibility to environmental stress (parameters sensitive to organic and inorganic pollutants, e.g. SIR, N-mineralization, urease, ASA and APA).

3.3 Spatial variability within the site

The spatial variability of the microbial variables was indicated by the relative coefficient of variation CV_r of the mean (Table 3). The CV_r ranged from 4.0–16.4 % in the grassland and from 0.5–14.1 % in the arable soil. The highest variation was calculated for APA (grassland, arable land) and DEA (arable land). Similar figures for grassland were observed by SPEIR et al. (1984) and HÖPER et al. (1997). For spatially related samples BONMATI et al. (1991) reported highest CV_r-values for urease and phosphatase activity within a 5 m interval in grassland (CV_r = 59.7 and 36.6 %, respectively) and ÖHLINGER et al. (1993) observed CV-values of 21 % for phosphatase within a range of approximately 40 meters in arable soils. VON STEIGER et al. (1996) reported that urease activity was spatially related with each other to a distance of 1 m (grassland) or 20 m (arable land) and SMITH et al. (1994) reported distances of 0.5–1.0 m for

Table 2: Comparison of the measured values of the 0–10 cm layer (0–10 cm) with the calculated means of the measurements in the 0–5 cm and 5–10 cm layer (mean). No significant differences (2–sample t-test) between the two sampling designs are detected. Data are shown for spring 1995.

Tabelle 2: Vergleich der gemessenen Aktivitäten aus der Tiefenstufe 0–10 cm mit den errechneten Mittelwerten aus den Tiefenstufen 0–5 cm und 5–10 cm (mean). Probenahmetermin Mai 1995.

	SIR		Urease		DEA		N-mineralization		APA		ASA		Xylanase	
	$mgCO_2\ 100g^{-1}\ h^{-1}$		$\mu gNH_4\text{-}N\ g^{-1}\ 2h^{-1}$		$ngN_2O\text{-}N\ g^{-1}\ h^{-1}$		$\mu g\ NH_4\text{-}N\ g^{-1}\ 7d^{-1}$		μg phenol $g^{-1}\ 3h^{-1}$		μg nitrophenol $g^{-1}\ h^{-1}$		μg GE $g^{-1}\ d^{-1}$	
	0-10cm	mean	0-10cm	mean	0-10cm	mean	0-10cm	mean	0-10cm	mean	0-10cm	mean	0-10cm	mean
Fluvisol														
GL1	6.1	7.3	104.2	111.2	254.6	316.9	154.6	196.7	1619	1646	340	315	1533	1950
GL1	6.9	7.0	122.0	115.1	234.5	261.8	154.6	175.0	1523	1695	334	306	1643	1716
GL1	7.3	7.3	127.0	115.4	239.2	266.7	155.8	187.1	1638	1655	340	300	1848	1715
GL1	7.6	7.4	124.5	98.7	224.7	305.0	183.7	189.8	1683	1711	349	320	2190	1636
Cambisol														
GL2	12.4	9.8	161.8	157.5	317.1	279.5	203.5	208.1	440	488	693	672	1936	2251
GL2	10.9	13.3	150.6	160.2	238.8	311.0	199.0	223.7	402	698	650	674	2231	2611
GL2	11.3	12.2	156.0	215.5	250.7	366.0	197.0	262.0	497	528	652	684	1997	2530
GL2	9.9	15.0	144.6	162.4	239.6	353.9	185.3	303.0	588	708	686	682	2030	2299
Cambisol														
GL3	8.3	8.8	116.0	121.2	208.7	236.6	139.2	151.4	248	273	317	354	2095	2109
GL3	9.0	9.1	76.2	144.8	202.0	207.5	126.8	145.6	297	263	335	368	1821	1976
GL3	6.5	9.7	141.0	118.0	154.1	215.5	113.0	156.8	214	285	320	371	1633	2165
GL3	8.5	8.9	154.8	175.2	179.4	173.6	140.5	149.7	330	242	316	331	1471	2053

Table 3: The relative coefficient of variance CV_r (%) for microbial biomass (SIR) and enzyme activities of the arable and grassland site. Given are the spring (first) and autumn (second) values of each year.

Tabelle 3: Relative Variationskoeffizienten CV_r (%) für die mikrobielle Biomasse (SIR) und die Enzymaktivitäten der Acker- und Grünlandflächen. Angegeben sind die jährlichen Frühjahrs- und Herbstwerte.

	CV_r					
	Grassland			Arable land		
	1995	1996	1997	1995	1996	1997
SIR	7.0-6.0	8.5-7.0	8.4-9.3	3.4-2.8	3.5-8.9	3.3-1.7
N-mineralization	6.9-5.6	8.5-7.8	7.3-7.4	5.8-5-7	1.7-2.6	2.6-2.0
Urease	6.9-5.1	6.9-9.0	6.6-8.6	5.1-2.7	9.3-3.2	1.8-5.4
DEA	4.1-4.0	6.2-9.8	14.6-7.4	4.8-7.7	6.6-9.1	10.9-4.1
APA	12.9-12.8	13.5-13.3	13.0-16.4	2.3-3.1	1.9-4.2	0.9-13.8
ASA	7.1-5.6	4.6-8.4	6.5-6.5	2.4-3.3	1.6-0.5	2.2-1.2
Xylanase	6.0-6.8	8.1-6.2	7.5-8.4	3.7-14.1	3.0-5.2	1.5-9.3
n	36	24-32	32	4	4	4

microbial biomass, C- and N-mineralization. These findings provided that the within-spatial variability of the microbial properties was encompassed by sampling spaces of 5–10 m.

3.4 Influence of site conditions

The influence of abiotic soil conditions upon the microbial properties has been simply estimated by correlation analyses. A significant relationship to the climatic conditions, characterized by the water supply (BOTTNER, 1985; SARATHCHANDRA et al., 1988) and air temperature (WARDLE, 1992), was only determined for the soil moisture (r = 0.48–0.88). No correlation was found with C_{org} and pH. Similiar results were observed by other authors (KOWALZCYK et al., 1987, VON STEIGER et al., 1996). In contrast, ZANTUA et al. (1977) and WARDLE and GHANI (1995) reported a moderate correlation between C_{org} and microbial processes at locations with high spatial variation of C_{org}, whereas other authors (DUTZLER-FRANZ, 1976a, b; LYNCH, 1984; JAGNOW, 1986; GEHLEN and SCHRÖDER, 1989) reported a close correlation between microbial variables, C_{org} and pH-value.

The sensitivity of the microbial properties to the soil type and land use was assessed by discriminant analysis, which revealed 4 significant *DF*s. *DF*1 explained 61 to 98.5 % of the total variance of the data (1995–1997) and was domi-

nated by either APA or ASA (Fig. 6). There was a highly significant discrimination between the soil types along axis 1 (DF dominated by ASA) and axis 2 (DF dominated by APA). *DF* 1 and *DF* 2 explained 97–99 % of the variance, and the variables ASA and APA contributing to *DF* 1 and *DF* 2 are the most important in relation to the other discriminant functions. *DF*s 3 and 4 covered 1–3 % of the variance and were poor discriminators. Data for 1997 are shown in Table 4; comparable results were found in 1995 and 1996. In all cases, at least 97 % of the data points were correctly classified. The results show that the soil type, as a summarized expression of complex site conditions (organic matter, nutrients, cation exchange capacity, acidity, soil texture, water/air supply), governs the microbial performance.

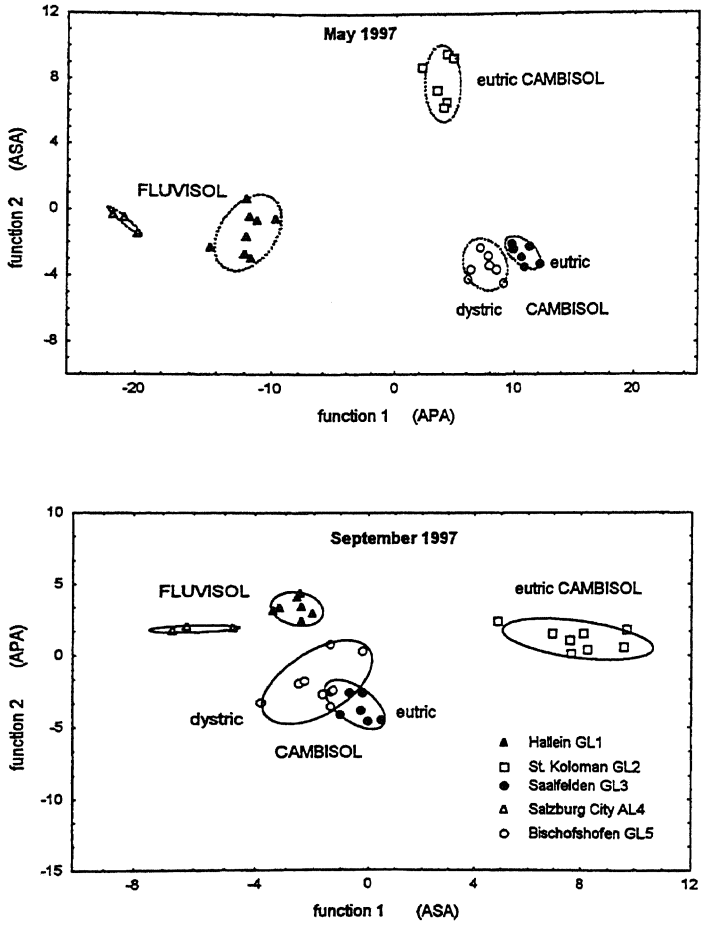

Figure 6: Two-dimensional plot (*DF* 1, *DF* 2) of the discriminant analysis including all microbial variables. The discriminant analyses included data of 5 sites, 2 depths, 4 replicates. Data are labelled by the soil type. Lines include data within the 95 % confidence interval of each site.

Abbildung 6: Zweidimensionale Darstellung (*DF* 1, *DF* 2) der Dauerbeobachtungsflächen im Diskriminanzraum im Frühjahr und Herbst 1997. Datenpunkte einer Fläche sind einheitlich symbolisiert. Kreise stellen das 95 % Vertrauensintervall dar.

3.5 Classification design

In order to define a classification concept for microbiological variables, we differentiated activity classes by applying the 30 and 70 percentile of the data set of the specific microbial variable. Using 30 and 70 percentile gives similar widths of the 3 categories, which were attributed as low, normal and high. Within the site the measurements of each parameter were classified in one of the 3 activity categories. The frequency, with which the data values occured in one activity class, was summed up over all parameters. Then the site was classified in the respective activity category in which the highest total frequency of measurements was observed. The results showed that the sites could be classified according to the land use (Fig. 7). While 50–70 % of the activities of the *Cambisol GL2* were classified as high, activities of *Cambisol GL3* and *GL5*, and *Fluvisol GL1* were in the low and normal range. The arable *Fluvisol AL4* had zero frequencies in the high activity class. While the results of the classification in spring and autumn were identical for the *Fluvisol sites*, the frequencies in the medium and high activity class of the *Cambisol sites* raised at the end of vegetation period. Single parameter classification designs have been reported by HÖPER et al. (1997), who defined the activity categories by using the lower and upper quartile and JÖRGENSEN (1997) introduced a 5 class design with the interquartile range representing the normal activity class. ÖHLINGER et al. (1993) delimited 4 classes of equal range of the data values before the frequencies were calculated. The above researchers all outlined single parameter classification systems. In contrast, the main feature of this concept is that the width of the activity categories are similar and that all single frequencies are summarized in order to assess the microbial properties of a site.

4. Conclusion

These investigations demonstrate that microbial properties including biomass and soil enzyme processes are potentially able to indicate changes in the soil state. The spatial variability of most of the microbial variables within the sites was small, fulfilling one of the basic criteria of soil monitoring. The temporal amplitudes reflected the sensitivity of the

Table 4: Results of discriminant analyses of the microbial variables (SIR, N-mineralization, APA, ASA, DEA, urease and xylanase activity) from 5 different sites. Data are shown for spring 1997.

Tabelle 4: Ergebnisse der Diskriminanzanalyse für die Probenahme April 1997. Alle Flächen (Gruppierungsvariable) und mikrobiellen Parameter (Merkmalsvariablen) wurden miteinbezogen.

	Spring 1997				Autumn 1997			
	DF 1	DF 2	DF 3	DF 4	DF 1	DF 2	DF 3	DF 4
Wilks' lambda	0.0001	0.0156	0.3362	0.7301	0.002	0.055	0.439	0.819
Eigenvalue	139.2	20.59	1.17	0.37	23.1	7.4	0.8	0.2
Degree of freedom	28	18	10	4	28	18	10	4
Cummulative variance %	86	99	99.7	100	73.3	96.9	99.3	100
Canonical correlation coefficient	0.996	0.977	0.735	0.519	0.98	0.94	0.66	0.43
Correlation coefficient[a]								
APA	-0.267	-0.024	0.235	-0.232	0.057	0.195	-0.050	-0.399
ASA	0.037	0.530	0.098	-0.063	0.313	0.014	-0.123	-0.175
N-mineralization	0.031	0.056	0.348	-0.089	0.242	0.263	-0.158	0.313
Urease activity	0.026	0.065	0.163	-0.310	0.157	0.033	-0.145	-0.063
Xylanase activity	0.019	-0.016	0.045	-0.236	0.033	-0.093	-0.102	0.016
SIR	-0.012	0.104	0.113	-0.304	0.131	0.104	0.150	-0.083
DEA	0.000	0.061	0.125	-0.616	0.201	0.259	-0.503	0.664

[a] denotes pooled within-groups correlation between the discriminating variables and the canonical discriminant functions.

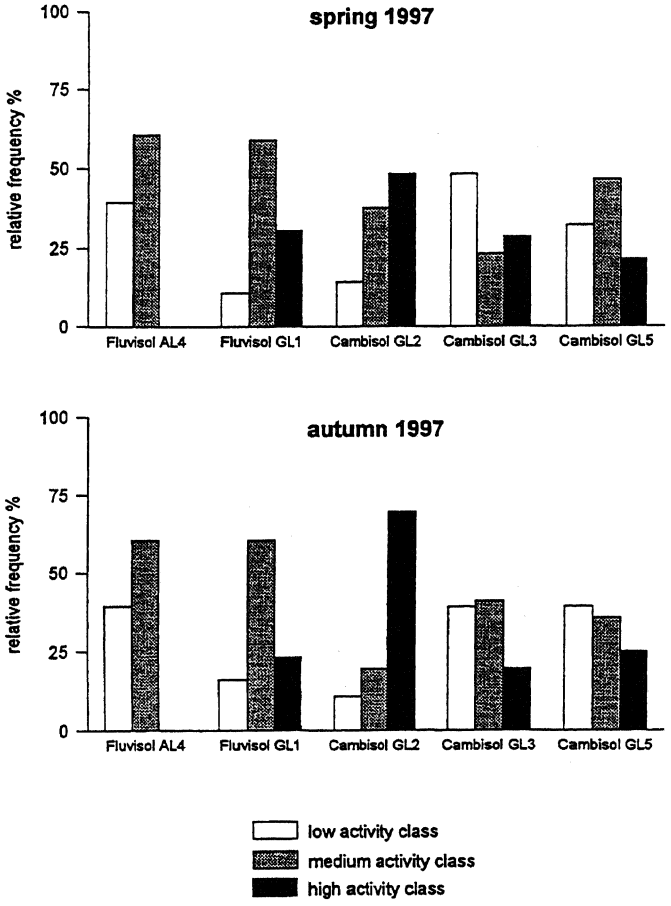

Figure 7: Summarized frequency (%) of the measured data in the 3 activity classes for each site. Data are shown for 1997. n = 56 (grassland), n=28 (arable land).

Abbildung 7: Aufsummierte Häufigkeitsverteilung (%) aller gemessen Daten einer Fläche auf die 3 Aktivitätsklassen. Ergebnisse für 1997. n = 56 (Grünland), n = 28 (Ackerland).

microbial properties to varying seasonal soil conditions (e.g. water, temperature and substrate availability). Relatively high temporal amplitudes were detected for APA, xylanase activity and DEA, indicating the sensitivity of these enzymes to available substrate. In conclusion, the temporal fluctuations of the microbial parameters reduce the chance to detect changes by successive sampling. Hence, focusing on a single sampling in spring to exclude natural factors (e.g. plant-specific seasonal influences) in soil monitoring programs, is suggested. New points of emphasis will also concentrate on microbial community structure (e.g. signature molecules such as ergosterol, phospholipid fatty acids, nucleic acids), as it seems to be more robust to fluctuating seasonal soil conditions (VAN BEELEN and DOELMAN, 1997; BENTHAM et al., 1992; BRÜGGEMANN et al., 1995).

By delimiting 3 microbial activity categories, the monitoring sites were clearly classified according to the land use (grassland, arable field). The activities of the *Cambisols* were classified as high (*GL2*) on the one hand and low to normal (*GL3, GL5*) on the other hand. The *Fluvisol sites* were categorized as exhibiting normal activitiy, with *Fluvisol AL4* recording no data in the high activity class. The classification for each site was consistent over time. Discriminant analyses significantly differentiated all sites according to the soil type and land use, elucidating the factors that determine the microbial performance.

We conclude that soil microbial monitoring is an important instrument in soil monitoring programs. However, the determination of significant soil changes is often hampered by the high variability of the data due to soil heterogeneity,

by the lack or small number of replicates and the temporal variation of the parameters. Increasing the numbers of replicates and sampling events is often restricted by financially considerations. In order to overcome these restrictions the linkage to other instruments such geoinformation systems and geostatistical methods is suggested.

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References

ANDERSON, J. P. E. and K. H. DOMSCH (1978): A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biology and Biochemistry, 10, 215–221.

BEELEN, P. VAN and P. DOELMAN (1997): Significance and application of microbial toxicity tests in assessing ecotoxicological risks of contaminants in soil and sediments. Chemosphere, 34, 455–499.

BENTHAM, H., J. A. HARRIS, P. BIRCH and K. C. SHORT (1992): Habitat classification and soil restoration assessment using analysis of soil microbiological and physicochemical characteristics. Journal of Applied Ecology, 29, 711–718.

BONMATI, M., B. CECCANTI and P. NANNIPERI (1991): Spatial variability of phosphatase, urease, protease, organic carbon and total nitrogen in soil. Soil Biology and Biochemstry, 23, 391–396.

BOTTNER, P. (1985): Response of microbial biomass to alternate moist and dry conditions in a soil incubated with ^{14}C- and ^{15}N-labelled plant material. Soil Biology and Biochemistry, 17, 329–337.

BREMER, E. and C. VAN KESSEL (1992): Seasonal microbial biomass dynamics after addition of lentil and wheat residues. Soil Science Society America Journal, 56, 1141–1146.

BROOKES, P. C. (1993): The potential of microbiological properties as indicators in soil pollution monitoring. In: SCHULIN, R., A. DESAULES, R. WEBSTER and B. VON STEIGER (Eds.): Soil Monitoring. Birkhäuser Verlag, Basel, Boston, Berlin, pp. 229–253.

BRÜGGEMANN, R., L. ZELLES, Q. Y. BAI and A. HARTMANN (1995): Use of Hasse diagram technique for evaluation of phospholipid fatty acids distribution as biomarkers in selected soils. Chemosphere, 30, 1223–1236.

BUCHANAN, M. and L. D. KING (1992): Seasonal fluctuations in soil microbial biomass carbon, phosphorus and activity in no-till and reduced-chemical-input maize agroecosystems. Biology and Fertility of Soils, 13, 211–217.

CAMPBELL, C. A. and V. O. BIEDERBECK (1976): Soil bacterial changes as affected by growing season weather conditions: a field and laboratory study. Canadian Journal of Soil Science, 56, 293–310.

CAMPBELL, C. D., S. J. CHAPMAN and F. URQUHART (1995): Effect of nitrogen fertilizer and temporal and spatial variation of mineral nitrogen and microbial biomass in a silvopastoral system. Biology and Fertility of Soils, 19, 177–184.

DESAULES, A. and B. VON STEIGER (1993): Gaps of current Concepts in Soil Monitoring: Potential and Drawbacks of other Concepts. In: SCHULIN, R., A. DESAULES, R. WEBSTER, and B. VON STEIGER (Eds.): Soil Monitoring. Birkhäuser Verlag, Basel, Boston, Berlin, pp. 49–52.

DOMSCH, K. H. (1980): Interpretation and evaluation of data. Weed Research Organization Technical Report, 59, pp. 6–8.

DOMSCH, K. H., G. JAGNOW and T. H. ANDERSON (1983): An ecological concept for the assessment of side-effects of agrochemicals on soil micro-organisms. Residue Review, 86, 65–105.

DUTZLER-FRANZ, G. (1976a): Der Einfluß einiger chemischer und physikalischer Bodenmerkmale auf die Enzymaktivität verschiedener Bodentypen. Zeitschrift für Pflanzenernährung und Bodenkunde, 140, 329–350.

DUTZLER-FRANZ, G. (1976b): Beziehungen zwischen der Enzymaktivität verschiedener Bodentypen, der mikrobiellen Aktivität, der Wurzelmasse und einigen Klimafaktoren. Zeitschrift für Pflanzenernährung und Bodenkunde, 140, 351–374.

EDWARDS, C. A., S. SUBLER, S. K. CHEN and D. M. BOGOMOLOV (1996): Essential Criteria for selecting Bioindicator Species, Processes, or Systems to assess the environmental Impact of Chemicals on Soil Ecosystems. In: STRAAHLEN, N. M. and D. A. KRIVOLUTSKY (Eds.): Bioindicator Systems for Soil Pollution. Kluwer Adademic Publishers, Dordrecht, London, pp. 67–84.

FAO-UNESCO (1994): Soil Map of the World. Revised Legend with corrections. ISRIC Wageningen

FRIEDEL, J. K., J. C. MUNCH and W. R. FISCHER (1996): Soil microbial properties and the assessment of available soil organic matter in a haplic luvisol after several years of different cultivation and crop rotation. Soil Biology and Biochemistry, 28, 479–488.

GEHLEN, P. (1987): Bodenchemische, bodenbiologische und bodenphysikalische Untersuchungen konventionell und biologisch bewirtschafteter Acker-, Gemüse-, Obst- und Weinbauflächen. Dissertation, Universität Bonn.

GEHLEN, P. and D. SCHRÖDER (1989): Variabilität mikrobiologischer Eigenschaften von Böden aus gleichem Substrat und ähnlicher Bewirtschaftung. Kali-Briefe, 19, 545–556.

HÖPER, H., O. HEINEMEYER and B. KLEEFISCH (1997): Erfassung bodenmikrobiologischer Parameter im Rahmen der Bodendauerbeobachtung in Niedersachsen: Methodische Aspekte und Ergebnisse. VDLUFA-Schriftenreihe, 46, 763–766.

HOFFMANN, G. (1968): Eine photometrische Methode zur Bestimmung der Phosphatase-Aktivität in Böden. Zeitschrift für Pflanzenernährung, Düngung und Bodenkunde, 118, 161–172.

JAGNOW, G. (1986): Mikrobieller Abbau bestimmter Stoffgruppen in der Buchenstreu. In: ELLENBERG, H., R. MAYER and J. SCHAUERMANN (Hrsg.): Ökosystemforschung – Ergebnisse des Solling-Projekts. Kap. 5.5, pp. 292–298.

JÖRGENSEN, R. G. (1995): Die quantitative Bestimmung der mikrobiellen Biomasse in Böden mit der Chloroform-Fumigation-Extraktions-Methode. Institut für Bodenwissenschaft, Universität Göttingen.

JÖRGENSEN, R. G. (1997): Die Beurteilung von Böden mit mikrobiologischen Parametern. VDLUFA-Schriftenreihe, 46, 767–770.

JURITSCH, G. (1995): Einrichtung von Bodendauerbeobachtungsflächen im Bundesland Salzburg (BDF) Konzept 7/1995. Amt der Salzburger Landesregierung, Abt. Land- u. Forstwirtschaft.

JURITSCH, G. and L. WIENER (1993): Salzburger Bodenzustandsinventur. Amt der Salzburger Landesregierung.

KANDELER, E. (1996): N-Mineralization under waterlogged conditions. In: SCHINNER, F., R. ÖHLINGER, E. KANDELER and R. MARGESIN (Eds.): Methods in Soil Biology. Springer Verlag, Berlin, Heidelberg, New York, pp. 141–143.

KANDELER, E. and H. GERBER (1988): Short-term assay of soil urease activity using colorimetric determination of ammonium. Biology and Fertility of Soils, 6, 68–72.

KANDELER, E. and K. BÖHM (1996): Temporal dynamics of microbial biomass, xylanase activity, N-mineralization and potential nitrification in different tillage systems. Applied Soil Ecology, 4, 181–191.

KANDELER, E., R. MARGESIN, R. ÖHLINGER and F. SCHINNER (1993): Bodenmikrobiologisches Monitoring – Vorschläge für eine Bodenzustandsinventur. Die Bodenkultur, 44, 357–377.

KEENEY, D. R. (1982): Nitrogen-availability indices. In: PAGE, A. L., R. H. MILLER and D. R. KEENEY (Eds.): Methods of soil analysis, Part 2. American Society of Agronomy, Soil Science Society America, Madison WI, p. 711.

KOWALZCYK, T., H. KARNATH and D. SCHRÖDER (1987): Standörtliche, jahreszeitliche und tiefenabhängige Variabilität bodenbiologischer Eigenschaften. VDLUFA-Schriftenreihe, 23, 913–924.

LÜTZOW, V. M. (1993): Jahreszeitliche Fluktuationen der mikrobiellen Biomasse und ihres Stickstoff-Gehaltes in konventionell und biologisch-dynamisch bewirtschafteten Parabraunerden der Friedberger Wetterau. Dissertation Universität Gießen, Verlag Shaker.

LYNCH, J. M. (1984): Interactions between biological processes, cultivation and soil structure. Plant and Soil, 76, 307–318.

MIRTL, M. (1996): Kurzbeschreibung des Integrated Monitoring in Österreich 1995/96. Integrated Monitoring Serie: IM – Rep – 001. Umweltbundesamt Wien.

ÖHLINGER, R. (1995): Oberösterreichische Bodenzustandsinventur – Mikrobielle Biomasse, anaerobe N-Mineralisation, Phosphatase. VDLUFA-Schriftenreihe, 40, 603–606.

ÖHLINGER, R., A. EIBELHUBER and M. VINZENZ (1993): Die Verteilung und Streuung von Bodenenzymaktivitäten in einer einheitlichen Fläche und deren Einfluß auf Probenahme und Analyse. Die Bodenkultur, 44, 103–117.

PHILIPP, B., F. MUTSCH, E. KANDELER and R. MAIER (1994): Enzymaktivitätsuntersuchungen bei der Österreichischen Waldboden-Zustandsinventur – Arylsulfatase. Mitteilungen der Österreichischen Bodenkundlichen Gesellschaft, 48/49, 139–153.

SARATHCHANDRA, S. U., K. W. PERROTT, M. R. BOASE and J. E. WALLER (1988): Seasonal changes and the effects of fertilizer on some chemical, biochemical and microbio-

logical characteristics of high-producing pastoral soil. Biology and Fertility of Soils, 6, 328–335.

SCHINNER, F. and M. VON MERSI (1990): Xylanase-, CM-cellulase- and invertase activity in soil, an improved method. Soil Biology and Biochemistry, 22, 511–515.

SCHINNER, F., R. ÖHLINGER, E. KANDELER and R. MARGESIN (1996): Methods in Soil Biology. Springer Verlag, Berlin, Heidelberg, New York.

SMITH, J. L., J. JONATHAN, J. HALVORSON and J. R. HARVEY BOLTON (1994): Spatial relationships of soil microbial biomass and C and N mineralization in a semi-arid shrub-steppe ecosystem. Soil Biology and Biochemistry, 26, 1151–1159.

SPEIR, T. W., D. J. ROSS and V. A. ORCHARD (1984): Spatial variability of biochemical properties in a taxonomically-uniform soil under grazed pasture. Soil Biology and Biochemistry, 16, 153–160.

STEIGER, VON B., K. NOWACK and R. SCHULIN (1996): Spatial variation of urease activity measured in soil monitoring. Journal of Environmental Quality, 25, 1285–1290.

STRAALEN, VAN N. M. and D. A. KRIVOLUTSKY (1996): Bioindicator Systems for Soil Pollution. NATO ASI Series. Kluwer Academic Publisher, London.

TABATABAI, A. and J. M. BREMNER (1970): Arylsulfatase activity of soils. Soil Science Society America Proceedings, 34, 225–229.

WARDLE, D. A. (1992): A comparative assessment of factors which influence microbial biomass carbon and nitrogen levels in soil. Biological Revue, 76, 321–358.

WARDLE, D. A. and A. GHANI (1995): Why is the strength of relationships between pairs of methods for estimating soil microbial biomass often so variable? Soil Biology and Biochemistry, 27, 821–829.

ZANTUA, M. I., L. C. DUMENIL and J. M. BREMNER (1977): Relationships between soil urease activity and other soil properties. Soil Science Society America Journal, 41, 350–352.

ZECHMEISTER-BOLTENSTERN, S. (1996): Denitrification Enzyme Activity (DEA). In: SCHINNER, F., R. ÖHLINGER, E. KANDELER and R. MARGESIN (Eds.): Methods in Soil Biology. Springer Verlag, Berlin, Heidelberg, New York, pp. 179–184.

Address of authors

Dr. Dagmar Tscherko, Federal Office and Research Centre for Agriculture, Institut of Applied Soil Science, Department of Soil Microbiology, Spargelfeldstraße 191, A-1226 Wien. Present address: University Hohenheim, Institute of Soil Science, Emil-Wolff-Straße 27, D-70593 Stuttgart, e-mail: tscherko@uni-hohenheim.de

Univ. Prof. Dr. Ellen Kandeler, University Hohenheim, Institute of Soil Science, Emil-Wolff-Straße 27, D-70593 Stuttgart.

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