

Bioindicative value of eco-physiological indices in routine evaluation of soils – a pilot monitoring study in the Czech Republic

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Der bioindikative Wert öko-physiologischer Indizes für Routine-Bodenbeurteilungen – Eine Pilot-Monitoring-Studie in Tschechien

1. Introduction

Microbial parameters appear a very useful tool in monitoring of soil quality (BROOKES, 1995). Microorganisms growing under given conditions in soil are those the best adapted to the environment. The changes in the microbial community during adaptation can function as early indicators of changes.

There are several model approaches to the routine bio-monitoring of soils that have attracted scientific attention in recent years. Most of them are based on evaluation of correlation links between the selected biomarkers and soil abiotic properties, because the microbial properties of soil are the result of chemical, physical and biological soil properties (OBERHOLZER et al., 1999). There is no single microbial parameter which can be used as a biological index of soil

quality, because of the high complexity of soil microbial community (BROOKES, 1995). BROOKES (1995) proposed the criteria which should be fulfilled by bioindicators of soil quality. Validity of various microbial parameters in the system of monitoring of soil quality was discussed (YAKOVCHENKO et al., 1996; KANDELER et al., 1999).

Relevant set of microbial parameters, however, enabled evaluation of so-called eco-physiological quotients, i.e. derived ratio indices of two or more original measures that are typically related to some aspects of energetical metabolism of soil microbial communities. They contain an internal control that helps to overcome difficulties arising from interpretation of measurements made outside carefully controlled field experiments (BROOKES, 1995). The microbial quotient $C_{\text{bio}}/C_{\text{org}}$ reflects dynamics of the carbon flow through soil microbial biomass and indicates changes of

Zusammenfassung

1999 wurde die laufende Beobachtung mikrobieller Eigenschaften in das Boden-Monitoring-Programm Tschechiens aufgenommen. Der Kohlenstoff- und Stickstoffgehalt der mikrobiellen Biomasse, Grund- und substratinduzierte Atmung, N-Mineralisation und potentielle Nitrifikation wurden in 60 Bodenstichproben gemessen, welche im April, Juli und Oktober 1999 von 27 Grünlandparzellen sowie 33 Ackerparzellen gezogen wurden.

Die Beziehungen zwischen abiotischen und mikrobiellen Parametern waren auf Grünlandböden eindeutiger als auf Ackerböden.

Die Menge an mikrobieller Biomasse war hauptsächlich von der Konzentration der organischen Substanz im Boden bestimmt.

Die mikrobielle Biomasse besaß einen starken positiven Einfluss auf die C und N Mineralisation.

Sechs ökophysiologische Kenngrößen ($C_{\text{bio}}/C_{\text{org}}$, $N_{\text{bio}}/N_{\text{org}}$, $q\text{CO}_2$: Atmungs CO_2 -C/ C_{bio} ; qN: N mineralisiert/ N_{bio} , potentielle Atmung/Grundatmung, $C_{\text{bio}}/N_{\text{bio}}$) wurden aus mikrobiellen Grundeigenschaften errechnet. Sie zeigten jahreszeitliche Schwankungen und unterlagen signifikanten Einflüssen durch die Bodentextur. Das Verhalten der Kennzahlen $N_{\text{bio}}/N_{\text{org}}$ und N mineralisiert/ N_{bio} zeigte das gleiche Muster wie die entsprechenden Kohlenstoffquotienten.

Schlagerwörter: Biomonitoring, Bodeneigenschaften, öko-physiologische Kennzahlen, saisonale Schwankungen, Textureinflüsse.

Summary

Monitoring of microbial properties was introduced to the soil monitoring program in the Czech Republic in 1999. Microbial biomass C and N, basal and substrate-induced respiration, N mineralization and potential nitrification were measured in 60 soil samples taken from 27 plots on grasslands and from 33 plots on arable soil in April, July and October 1999. Relationships between abiotic and microbial parameters were clearer in grasslands than in arable soils. Amount of microbial biomass was mainly determined by concentration of soil organic matter. Microbial biomass had a strong positive influence on C and N mineralization. Six eco-physiological quotients ($C_{\text{bio}}/C_{\text{org}}$, $N_{\text{bio}}/N_{\text{org}}$, $q\text{CO}_2$; respired $\text{CO}_2\text{-C}/C_{\text{bio}}$, $q\text{N}$: N mineralization/ N_{bio} , potential respiration/basal respiration, $C_{\text{bio}}/N_{\text{bio}}$) were calculated from basic microbial properties. They showed seasonal fluctuations and were significantly influenced by texture. Behaviour of the quotients $N_{\text{bio}}/N_{\text{org}}$ and N-mineralization/ N_{bio} showed the same pattern as the parallel carbon quotients.

Key words: biomonitoring, soil quality, eco-physiological quotients, seasonal variability, textural influences.

carbon availability in soil. This quotient increases if organic carbon is being accumulated in soil and declines if soil is being used exploitively (SPARLING, 1997). The metabolic quotient $q\text{CO}_2$ (respired $\text{CO}_2\text{-C}/C_{\text{bio}}$) reflects physiological status of the microbial community. Higher values of $q\text{CO}_2$ indicate less mature ecosystem or a high concentration of mineralizable substrate or stress (ANDERSON and DOMSCH, 1990; SPARLING, 1997). Mutual comparability of sites and very limited availability of control sites are crucial problems of wide-ranged monitoring studies. This is usually solved by either time-related monitoring of individual sites or by biological assessment based on probable values that could be reached in defined physico-chemical conditions. The eco-physiological indices could substantially increase knowledge of an individual site and its history and, on the other hand, it could decrease variability which occurs due to seasonal fluctuations or changeable soil parameters. Furthermore, derivation process is based on commonly acceptable microbiological parameters and does not increase the cost of biomonitoring programmes.

The aims of this work were:

1. To present multivariate set of parameters incorporated in biomonitoring strategy for soils in the Czech Republic.
2. To define mutual interrelationships between abiotic and biotic parameters with special emphasis on derived indices.
3. To document information value of six derived eco-physiological indices for routine biomonitoring, namely with respect to seasonal changes and some basic soil categories.

2. Materials and Methods

2.1 Sites, soil sampling and storage

Sixty plots belonging to the network of basal soil monitoring plots of Czech Republic were chosen (fig. 1).

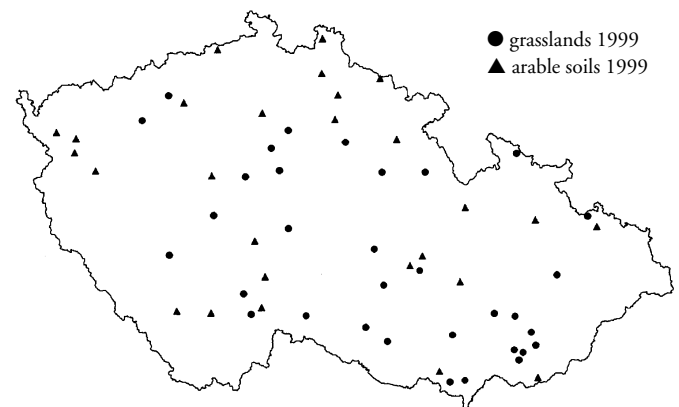


Figure 1: Location of sampling sites in the Czech Republic
Abbildung 1: Lage der Stichprobenparzellen in der Tschechischen Republik

Samples were taken from 33 plots on arable soils and 27 plots on grasslands during the first week of April, July and October 1999. 18 subsamples were taken from an area 40×25 m from the depth of 0–15 cm and a pooled sample was prepared. The samples were sieved ($< 2\text{mm}$), stored at 4°C and all microbial analyses were carried out during the period of three months after sampling.

The soil samples were then dried at laboratory temperature to determine pH, cation exchange capacity, texture and total nitrogen. C_{org} determination required pulverization to get particles that would pass through a 0.25 mm sieve.

2.2 Physical and chemical parameters

Physical and chemical parameters were estimated in samples taken in April 1999. Moisture was assessed as a weight decrease during 6-hour-drying at 105° C. Maximum water-holding capacity was determined according to ISO 14240–2 (1997). Granulometric analyses of soils were carried out by means of the pipette method (HONSA, 1997). Twenty grams were weighed for determination of pH. 50 ml of an extractant KCl ($c = 0.2 \text{ mol l}^{-1}$) were added and pH was recorded using a glass electrode after 24 hours. Organic carbon was determined by sulfochromic oxidation according to ISO 14235 (1998). 13 ml of a mineralizing mixture were added to 5 g of soil before estimation of total nitrogen. The mineralizing mixture contained 10 g of selenium mixture for determination of total nitrogen (Merck) and 10 g salicylic acid in concentrated H_2SO_4 . After 12 hours H_2O_2 (30 %) was added and the samples were mineralized for 2h at 420° C. Soil nitrogen was determined after distillation into boric acid (Kjeltec Auto 1030 Analyzer, Tecator). Soil samples were saturated with Ba^{2+} for estimation of cation exchange capacity ($\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$, $c = 5 \%$, pH adjusted at 8.1 with triethanolamine) and the concentrations of H^+ in extract were estimated by titration. Then Ba^{2+} was removed using the solution of MgCl_2 (0.2 mol.l^{-1}) and the concentration of Ba^{2+} in the extract was estimated by means of AAS. CEC was calculated from the concentrations of H^+ and Ba^{2+} (ZBÍRAL, 1995).

2.3 Biological parameters

Anaerobic N mineralization (ammonification) was measured by incubating 5 g of soil samples under waterlogged conditions in an closed tube for 9 days (BUNDY and MEISINGER, 1994, adjusted). The released ammonium was measured after 2 and 9 days colorimetrically (FORSTER, 1995). N mineralization was expressed as a net increase of $\text{NH}_4^+\text{-N}$.

Samples were preincubated two days at 25° C prior to estimation of a short-term nitrification activity (SNA). Potential nitrification was estimated in a shaken soil suspension amended with ammonium sulfate at pH 7.2. Sodium chlorate was used to inhibit NO_2^- -oxidation (ISO/CD 15685, 1998). Samples were taken after two and six hours. Nitrite was determined photometrically with sulfanilamide and N-(1-naphthyl-ethylenediamine) dihydrochloride (FORSTER, 1995).

Determinations of the soil microbial C and N biomass were performed by the fumigation-extraction method according to ISO 14240–2 (1997). The samples were preincubated one day prior to fumigation at 25° C. Organic C was estimated photometrically by the dichromate-oxidation method (YAKOVCHENKO et al., 1998) and N as nitrate after alkaline persulfate oxidation (CABRERA and BEARE, 1993; KANDELER, 1993). Coefficients $k_c = 0.38$ and $k_N = 0.45$ were used to calculate C and N microbial biomass (JOERGENSEN, 1995).

Substrate-induced respiration (potential respiration, PR) was determined after the addition of glucose (0.5 % d.w). Samples were incubated 6 hours at 22° C. Released carbon dioxide was measured by means of gas chromatograph with TCD detector. Basal respiration rate (BR) was measured during the period of 24 hours at 22° C. Samples were preincubated 6 days at 22° C before measuring of BR and PR.

2.4 Statistical data analysis

Standard techniques were used for the inspection of the distribution of examined variables (histograms, goodness of fit test, Kolmogorov-Smirnov test). Log-normally distributed variables were log-transformed ($Y = \text{Ln} [X + 1]$) and confidence limits for the geometric mean were evaluated using standard procedures (ZAR, 1984; PARKIN et al., 1990; PARKIN and ROBINSON, 1992). Mutual interrelations among the variables were evaluated on the basis of Spearman's rank order correlation coefficient. Under verified assumptions for the analyses (goodness-of-fit test for the normality, Bartlett's test for the homogeneity of variance), the parameters categorized according to different criteria (seasonality, soil type) were compared in one-way ANOVA models, followed by Tukey multiple – range test.

An array of 60 rows representing the individual sites described by 18 parameters that are characterized by median value computed throughout the sampled seasons were considered for the multivariate analysis. The analyses were carried out under verified assumptions of multivariate normal distribution (GARRETT, 1986; KRZANOWSKI, 1988) and homogeneity of variance-covariance matrices in the groups of soils to be separated by discriminant analysis (Box's test; KRZANOWSKI, 1988).

The principal component analysis (PCA) was used to characterize the correlations and balance among biotic and abiotic parameters through linear transformation of original variables (JOLLIFFE, 1986). In order to prevent certain

variables from having more influence than others due to the scaling effect, the data were standardized prior to PCA (i.e. original variables were centered to obtain a zero average and normalized in order to obtain variance of 1). Data pretreated in this way provided the most informative and meaningful bilinear projections showing the relation between the soils and the descriptors (ARNOLD and COLLINS, 1993).

The multivariate discriminant analysis (DA) was carried out in order to separate soil groups with a priori known texture type or soil samples taken in different seasons. The raw discriminant (canonical) function (DF) coefficients were used to compute the raw canonical scores (D_i) for each of $i = 1, 2, \dots, n$ soil samples.

3. Results and discussion

Soil and microbial properties

Sixty experimental plots present a representative set of soils of the Czech Republic. Characterization of the set of soils according to soil types (ISSS-ISRIC-FAO, 1998) and texture is given in Tables 1 and 2. The soil physico-chemical and biological characteristics are shown in Tables 3–5.

Soil organic matter content, C and N microbial biomass (C_{bio} resp. N_{bio}), basal and substrate induced respiration and anaerobic N mineralization were higher in permanent grasslands. Measured values of microbial parameters are in

Table 1: Characterization of the set of soils according to soil types
Tabelle 1: Anzahl der Proben in den verschiedenen Bodentypen

	Arable soils	Grasslands
Regosol	1	1
Leptosol	–	2
Chernozem	5	–
Luvisol	5	1
Albeluvisol	4	–
Cambisol	6	11
Podzol	–	1
Planosol	3	5
Gleysol	4	5
Fluvisol	5	1

Table 2: Characterization of the set of soils according to textural categories
Tabelle 2: Anzahl der Proben in den Texturkategorien der Böden

	Arable soils	Grasslands
loamy sand	3	–
sandy loam	7	9
loam	18	14
clayey loam	3	4
clay	2	–

a range found in literature (YAKOVCHENKO et al., 1996; KANDELER et al. 1999). Only the ratio between C_{bio} and N_{bio} is too low in our study. ANDERSON and DOMSCH (1980) observed the average soil microbial biomass $C_{\text{bio}}/N_{\text{bio}}$ ratio 6.7. The medians of eco-physiological parameters showed lower differences between arable soils and grass-

Table 3: Summary statistics of soil chemical and physical properties. *A* means arable soils, *G* means grasslands

Tabelle 3: Zusammenfassende Darstellung der bodenchemischen und -physikalischen Eigenschaften (*A* = Ackerland, *G* = Grünland)

	Median		5th percentile		95th percentile	
	A	G	A	G	A	G
C_{org} (%)	1.5	3.0	1.0	1.4	2.7	10.4
N_{tot} (%)	0.16	0.30	0.12	0.15	0.25	1.14
C/N	9.4	9.7	8.0	9.0	13.6	11.8
Clay (< 0.001 mm)	14.4	6.6	3.8	4.1	31.7	20.4
pH (KCl)	6.2	5.7	4.5	3.6	7.4	7.1
CEC (mmol.kg ⁻¹)	195.0	250.0	135.0	170.0	330.0	835.0

Table 4: Summary statistics of soil microbial properties. *A* means arable soils, *G* means grasslands. Abbreviations: C_{bio} – carbon microbial biomass, N_{bio} – nitrogen microbial biomass, SNA means short-term nitrification activity

Tabelle 4: Statistische Übersicht der mikrobiellen Bodeneigenschaften (*A* = Ackerland, *G* = Grünland; Abkürzungen: C_{bio} – Mikrobieller Biomasse-Kohlenstoff; N_{bio} – Mikrobieller Biomasse-Stickstoff; SNA – kurzfristige Nitrifizierungsaktivität)

	Median		5th percentile		95th percentile	
	A	G	A	G	A	G
C_{bio} ($\mu\text{g C g}_{\text{d.w.}}^{-1}$)	158.5	453.7	118.9	170.6	287.3	1709.6
N_{bio} ($\mu\text{g N g}_{\text{d.w.}}^{-1}$)	49.8	150.2	33.3	46.4	112.2	475.3
Basal respiration ($\mu\text{g CO}_2\text{-C h}^{-1}\text{g}_{\text{d.w.}}^{-1}$)	0.23	0.51	0.15	0.20	0.43	1.15
Potential respiration ($\mu\text{g CO}_2\text{-C h}^{-1}\text{g}_{\text{d.w.}}^{-1}$)	3.42	7.64	2.07	2.67	4.74	15.09
N mineralization ($\mu\text{g NH}_4^+\text{-N d}^{-1}\text{g}_{\text{d.w.}}^{-1}$)	3.59	10.26	1.58	2.60	7.62	19.52
SNA ($\text{ng NO}_2^-\text{-N.h}^{-1}\text{g}_{\text{d.w.}}^{-1}$)	377.5	121.2	42.3	2.1	1354.6	2701.8

Table 5: Summary statistics of soil eco-physiological indices. A means arable soils, G means grasslands. Abbreviations: PR – potential respiration, BR – basal respiration, $q\text{CO}_2$ – specific respiration, $q\text{N}$ – specific N mineralization
 Tabelle 5: Statistische Zusammenfassung öko-physiologischer Indizes (A = Ackerland; G = Grünland; Abkürzungen: PR – Atmungspotential, BR – Grundatmung, $q\text{CO}_2$ – spezifische Atmung, $q\text{N}$ – spezifische Stickstoffmineralisierung

	Median		5th percentile		95th percentile	
	A	G	A	G	A	G
$C_{\text{bio}}/C_{\text{org}}$ (%)	1.11	1.58	0.79	0.66	1.99	2.31
$C_{\text{bio}}/N_{\text{bio}}$	3.55	3.27	2.25	2.34	5.48	5.41
$q\text{CO}_2$ ($\mu\text{g CO}_2\text{-C h}^{-1} \text{mg C}_{\text{bio}}^{-1}$)	1.44	1.16	0.85	0.53	2.00	1.89
PR/BR	13.53	13.67	8.92	8.72	22.93	19.51
$q\text{N}$ ($\mu\text{g NH}_4^+\text{-N d}^{-1} \text{mg N}_{\text{bio}}^{-1}$)	74.8	66.1	55.2	31.6	109.7	112.3
$N_{\text{bio}}/N_{\text{tot}}$ (%)	3.08	4.57	1.90	2.24	4.87	6.78

lands than basic microbial properties (Tables 4 and 5) because these quotients present “normalized” microbial data from soils with different contents of organic matter (BRELAND and ELTUN, 1999).

Relationships between C_{bio} , N_{bio} and C resp. N mineralization in grasslands and arable soils are displayed in fig. 2. Basal respiration and N mineralization positively correlated with C_{bio} resp. N_{bio} . Respiration reflects the overall activity of aerobic heterotrophs and strongly depends on the concen-

tration of easily mineralizable carbon substrate (TATE, 2000). N released during anaerobic incubation presents the easily decomposable fraction of soil organic nitrogen and originates partially from aerobic organisms killed by the anaerobic conditions (BUNDY and MEISINGER, 1994). This value reflects previous immobilization of N to microbial biomass and therefore is closely connected with N_{bio} . The described correlations were weaker in arable soils than can be explained by a narrower range of biomass values than in grasslands.

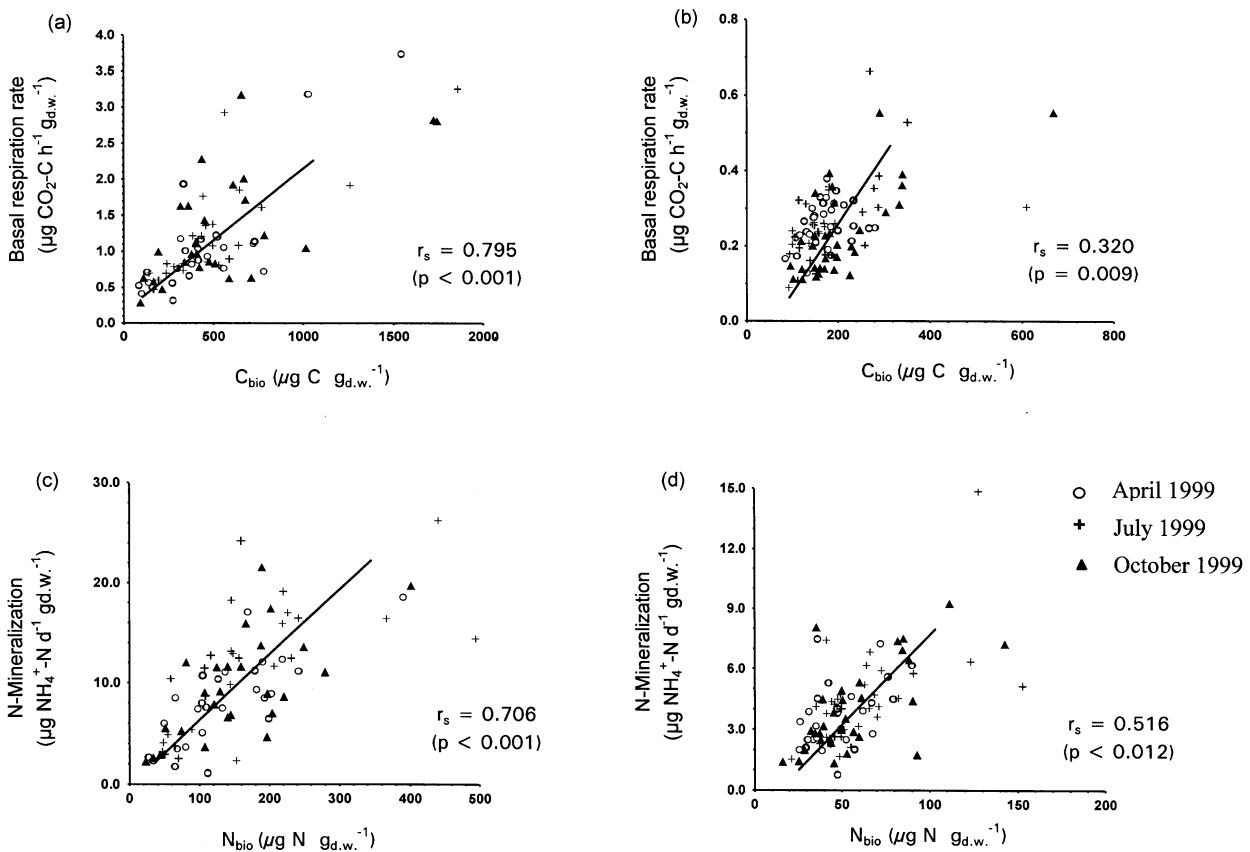


Figure 2: Regression between C_{bio} and basal respiration in grasslands (a) and arable soils (b) and regression between N_{bio} and N mineralization in grasslands (c) and arable soils (d)

Abbildung 2: Regression zwischen C_{bio} und Grundatmung in Grünland (a) und Ackerböden (b) sowie Regression zwischen N-Mineralisierung in Grünland (c) und Ackerböden (d)

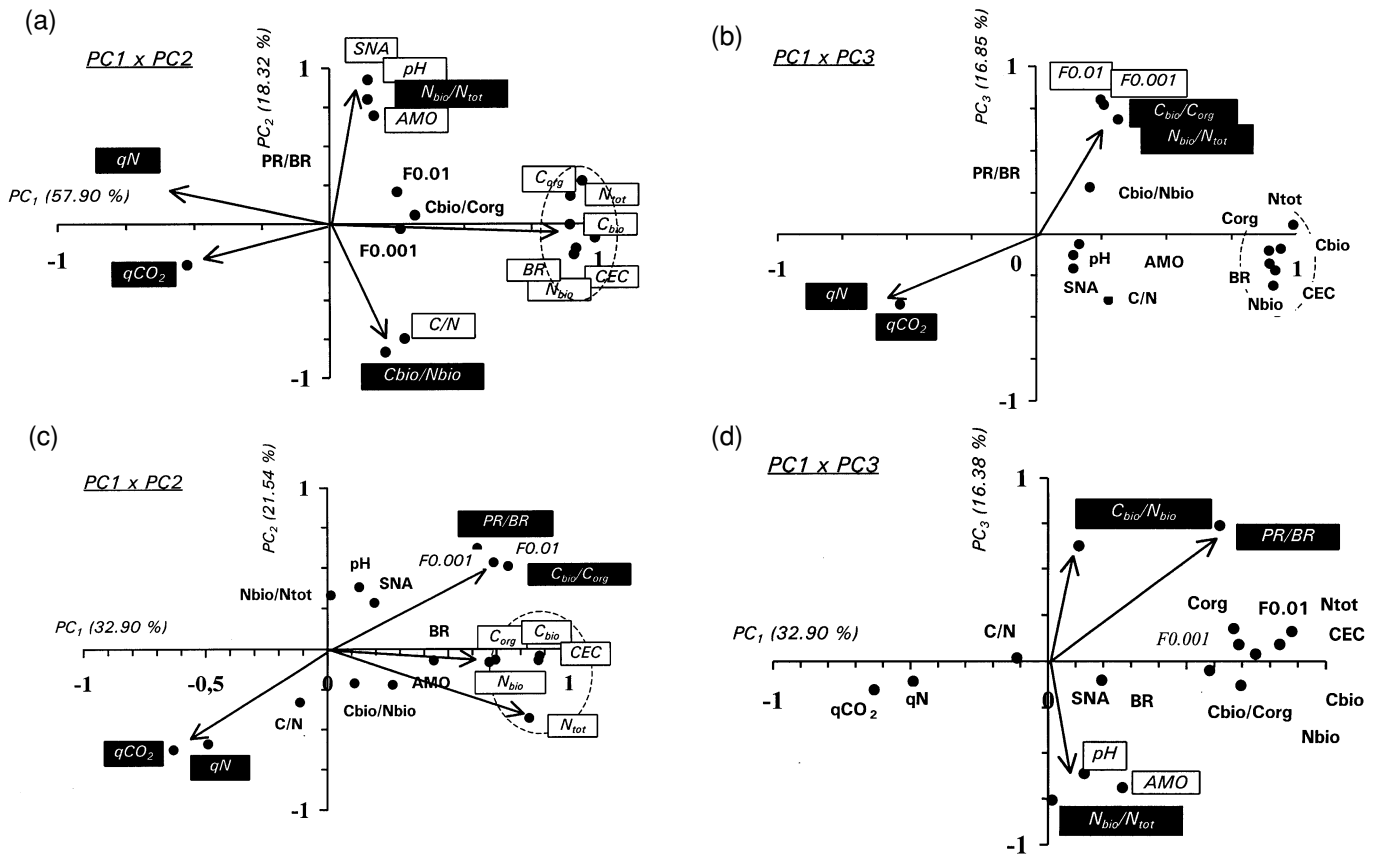


Figure 3: Principal component weight plots expressing correlation relation between eco-physiological microbial indices and other examined parameters in grasslands (a, b) and in arable soils (c, d). Abbreviations: AMO – anaerobic N mineralization, F 0.01 – content of soil particles < 0.01 mm, F 0.001 – content of soil particles < 0.001 mm, qCO_2 – specific respiration, qN – specific N mineralization, BR – basal respiration, PR – potential respiration, SNA – short-term nitrification activity, CEC – cation exchange capacity

Abbildung 3: Faktorenanalyse zur Erklärung der Korrelation zwischen ökophysiologicalen mikrobiellen Indizes und anderen geprüften Parametern in Grünland- (a, b) und Ackerböden (c, d). Abkürzungen: AMO – anaerobe N Mineralisation, F 0.01 – Korngrößenfraktion < 0,01 mm, F 0.001 – Korngrößenfraktion < 0,001 mm, qCO_2 – spezifische Atmung, qN – spezifische N Mineralisation, BR – Grundatmung, PR – Atmungspotential, SNA – kurzfristige Nitrifizierungsaktivität, CEC – Kationenaustauschkapazität

Eco-physiological quotients

Principal component analysis revealed that total amount of C and N in microbial biomass in grassland soils were mainly determined by amount of soil organic matter and by CEC (fig. 3a). Relative amount of microbial biomass related to C_{org} or N_{tot} content (C_{bio}/C_{org} , N_{bio}/N_{tot}) partially negatively correlated with qCO_2 (fig. 3b). The higher level of qCO_2 may indicate unfavorable conditions for microbial growth when more substrate is utilized for maintenance metabolism and less for the synthesis of new microbial biomass that results in the negative relationship between qCO_2 and C_{bio}/C_{org} .

Soil organic matter content as a source of nutrients and energy presented a key factor controlling microbial growth. The negative relationships between C_{bio} and qCO_2 (fig. 3a) may be explained by supposed higher microbial species diversity at increased content of soil microbial biomass. The

systems with higher diversity were able to more efficiently utilize substrate that resulted in lower qCO_2 . The negative correlation between qCO_2 and C_{bio}/C_{org} is in accord with this finding. More groups of microorganisms were able to utilize organic substrates resulting in more C entering to microbial biomass in the soils with higher diversity of the microbial community and the lower levels of qCO_2 . The relationships among C_{bio} , C_{bio}/C_{org} and qCO_2 have already been described (BRELAND and ELTUN, 1999). We introduced parallel eco-physiological quotients for nitrogen: N_{bio}/N_{tot} as a relative N content in microbial biomass and the “specific N mineralization potential” qN , as potentially mineralizable nitrogen per unit of soil microbial nitrogen. Our results show that relationships between N_{bio} and qN and between N_{bio}/N_{tot} and qN were similar as for C (fig. 3 a, b).

The positive influence of CEC on C_{bio} may be caused by higher capacity to adsorb organic compounds on negative charged surfaces of soil particles with increased CEC. Microsites with high concentration of substrate create good conditions for microbial growth (TATE, 2000). Microbial quotients ($C_{\text{bio}}/C_{\text{org}}$, $N_{\text{bio}}/N_{\text{tot}}$) increased with increasing amount of clay particles. The opposite dependencies were found between clay and metabolic quotients $q\text{CO}_2$ and $q\text{N}$ (fig. 3b). This can be explained by ability of clay particles to protect soil microorganisms against stress conditions. Clay protects microorganisms against faunal grazing and lowers the moisture fluctuations (FRANZLUEBBERS et al., 1996). Another possible explanation is that microorganisms living inside clay particles have limited access to nutrients, that results in lower part of active microbial biomass, thus in lower $q\text{CO}_2$ (HASSINK, 1994). The results of PCA analysis described above were less clear in arable soils (fig. 3 c, d) and revealed partially different regulative associations than in the case of grassland soils. Namely significance of the ratio of basal and potential respiration (PR/BR) which reflected availability of mineralizable substrate (PARKINSON and COLEMAN, 1991) was higher in arable soils than in grasslands.

Potential nitrification showed positive correlation with pH (fig. 3a), confirming well known fact that nitrification is sensitive on pH. Nitrification is commonly slower in acid soils (TATE, 2000). The similar relationship with pH was found for N mineralization (fig. 3a, d). The ratio $C_{\text{bio}}/N_{\text{bio}}$ is often used to characterize the structure of the microbial community in the terms of contribution of bacteria and fungi. A higher $C_{\text{bio}}/N_{\text{bio}}$ ratio indicate that microbial biomass contain a higher proportion of fungi, whereas a low value can mean that bacteria are dominant (MOORE et al.,

2000). A negative correlation between the ratio $C_{\text{bio}}/N_{\text{bio}}$ and pH (fig. 3a, d) supports this conclusion, because favourable conditions for fungi growth having the higher ratio $C_{\text{bio}}/N_{\text{bio}}$ are at lower pH (TATE, 2000). The fact that the C/N ratio had only little influence on microbial properties was caused by a very narrow range of this parameter.

Textural effects

The sensitivity of eco-physiological indices to texture categories and seasonal changes that represent substantial “among sites” variability component masking microbiological differences was assessed by discriminant analysis. All specified texture categories are significantly distinguished only in grassland soils (fig. 4). Grassland clayey soils were characterized by increased both C and N content in microbial biomass. In addition to it, increased values of $q\text{CO}_2$ and $q\text{N}$ coefficients in sandy grassland soils very significantly contributed to the separation of sandy and clayey soils. Eco-physiological coefficients in arable soils separated only clayey soils as objects with increased ratios $C_{\text{bio}}/C_{\text{org}}$ and PR/BR (fig. 4). The difference between grasslands and arable soils may be explained by differences in soil management. Tillage in arable soils causes aeration of soils, disruption of soil aggregates containing important amount of easily decomposable organic matter and exposition of organic substrates to microbial attack. It results in fast decomposition of soil organic matter. Therefore organic matter accumulation is lower in arable soils and the steady state flow of organic matter through microbial biomass is often disturbed. Because the effects of texture cohere with higher accumulation of organic matter in fine soils and faster decomposition of organic matter in sandy soils, the influence of texture is less profound in arable soils than in grasslands. Fertilization can also shift the balance in nutrient

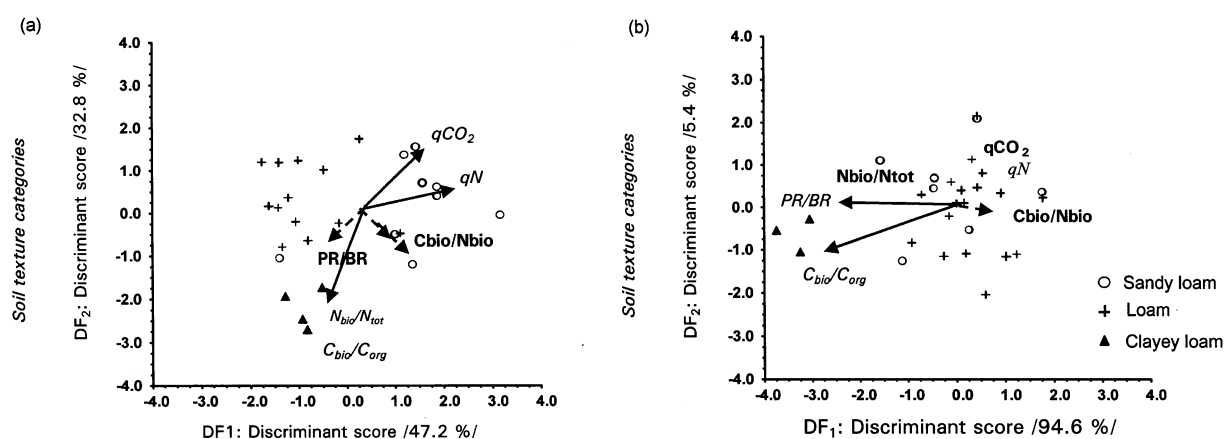


Figure 4: Contribution of ecophysiological indices to the multivariate discrimination of soil texture types in grasslands (a) and in arable soils (b). Abbreviations as in Fig. 3

Abbildung 4: Multivariate Diskriminantenanalyse der Bodentexturen in Grünland- (a) und Ackerböden (b) mit ökophysiologicalen Kenngrößen

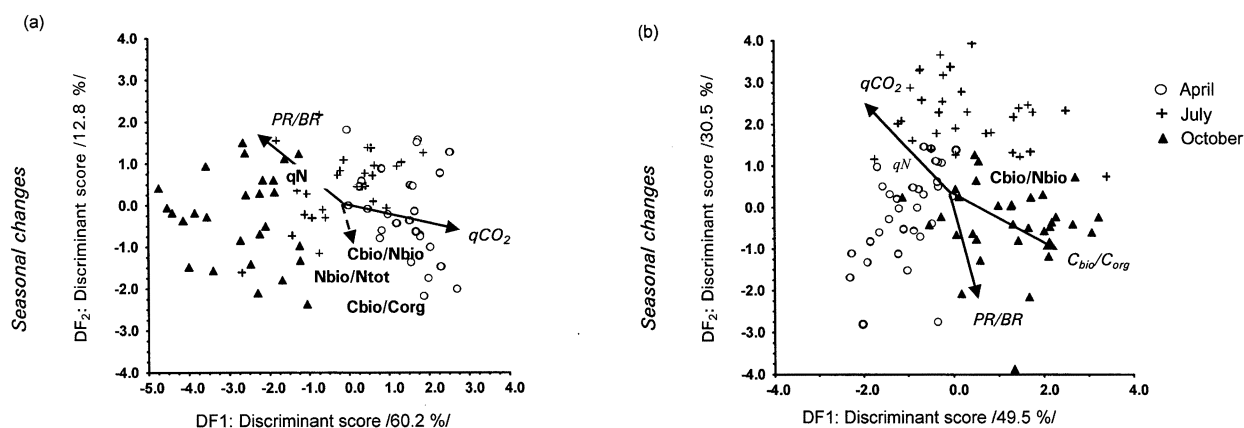


Figure 5: Contribution of ecophysiological indices to the multivariate discrimination of seasonal changes in grasslands (a) and in arable soils (b). Abbreviations as in Fig. 3

Abbildung 5: Multivariate Diskriminantenanalyse der Saisonschwankungen in Grünland- (a) und Ackerböden (b) mit ökophysiologicalen Kenngrößen

cycling that contribute to masking of texture effects on the microbial properties in arable soils.

Seasonal effects

Activities of microbial communities in arable soils appeared to be more seasonally influenced than in grassland soils (fig. 5). Autumn samples are apparently separable from early spring ones by increased C_{bio} content and the increased ratio between potential and basal respiration. July samples (early summer) were successfully separated by strongly increased qCO_2 levels. On the other hand, only autumn samples were separated from the others in grassland soils due to increased PR/BR values and decreased qCO_2 values. Seasonal fluctuations of microbial properties are strongly influenced by life cycle of plants. In spring, the developing root system provides enough of easily mineralizable substrate to microorganisms and has a positive effect on their activities. In arable soils, the higher input of organic matter from plant residues after the harvest promote microbial growth that caused the higher microbial quotient C_{bio}/C_{org} in October. Because fluctuations of organic matter inputs are higher in arable soils during season, the seasonal variability of microbial properties is larger in arable soils than in grasslands.

4. Conclusions

Eco-physiological quotients derived from basic microbial properties are potential sensitive indicators of soil quality,

which are convenient for soil biomonitoring where control plots are often lacking. Besides already validated quotients qCO_2 and C_{bio}/C_{org} , parallel quotients for nitrogen, were introduced. Dependency of qN and N_{bio}/N_{tot} on physicochemical and biological properties showed the same pattern as the qCO_2 resp. C_{bio}/C_{org} indices. The quotients qCO_2 , C_{bio}/C_{org} , qN and N_{bio}/N_{tot} present useful tools for characterization of the flow of energy and nutrients through soil microbial biomass. The indices reasonably contributed to the recognition of seasonal changes and texture categories of arable and grassland soils. Seasonal variability was higher in arable soils where spring, summer and autumn samples were discriminated. Sensitivity to texture was more profound in grasslands where sandy loam, loam and clayey loam were discriminated.

References

- ANDERSON, J. P. E. and K. H. DOMSCH (1980): Quantities of plant nutrients in the microbial biomass of selected soils. *Soil Science* 130, 211–216.
- ANDERSON, T. H. and K. H. DOMSCH (1990): Application of eco-physiological quotients qCO_2 and qD on microbial biomasses from soils of different cropping histories. *Soil Biology and Biochemistry* 22, 251–255.
- ARNOLD, G. M. and A. J. COLLINS (1993): Interpretation of transformed axes in multivariate analysis. *Applied Statistics* 42, 381–400.

- BRELAND, T. A. and R. ELTUN (1999): Soil microbial biomass and mineralization of carbon and nitrogen in ecological, integrated and conventional forage and arable cropping system. *Biology and Fertility of Soils* 30, 193–201.
- BROOKES, P. C. (1995): The use of microbial parameters in monitoring soil pollution by heavy metals. *Biology and Fertility of Soils* 19, 269–279.
- BUNDY, L. G. and J. J. MEISINGER (1994): Nitrogen availability indices. In: WEAVER R. W., S. ANGLE, P. BOTTOMLEY, D. BEZDICEK, S. SMITH, A. TABATABAI and A. WOLLUM (Eds.): *Methods of Soil Analysis: Part 2, Microbiological and Biochemical Properties*. Soil Science Society of America, Madison, 951–984.
- CABRERA, M. L. and M. H. BEARE (1993): Alkaline persulfate oxidation for determining total nitrogen in microbial biomass extracts. *Soil Science Society America Journal* 57, 1007–1012.
- FORSTER, J. C. (1995): Soil nitrogen. In: ALEF K. and P. NANNIPIERI (Eds.): *Methods in Applied Soil Microbiology and Biochemistry*. Academic Press, London, 79–87.
- FRANZLUEBBERS, A. J., R. L. HANEY, F. M. HONS and D. A. ZUBERER (1996): Active fractions of organic matter in soils with different textures. *Soil Biology and Biochemistry* 28, 1367–1372.
- GARRETT, R. G. (1989): The chi-square plot: a tool for multivariate outlier recognition. *Journal of Geochemical Exploration* 32, 319–341.
- HASSINK, J. (1994): Effect of soil texture on the size of the microbial biomass and on the amount of C and C mineralized per unit of microbial biomass in Dutch grassland soils. *Soil Biology and Biochemistry* 26, 1573–1581.
- HONSA, I. (1997): Stanovení zrnitostního složení. In: ZBÍRAL J., I. HONSA and S. MALÝ (Eds.): *Analýza půd III. Ústřední kontrolní a zkušební ústav zemědělský*, Brno, 100–106.
- ISO 14240–2 (1997): Soil quality – Determination of soil microbial biomass – Part 2: Fumigation-extraction method. International Organization for Standardization, Geneva.
- ISO/CD 15685 (1999): Soil quality – Ammonium oxidation, a rapid method to test potential nitrification in soil. International Organization for Standardization, Geneva.
- ISO 14235 (1998): Soil quality – Determination of organic carbon by sulfochromic oxidation. International Organization for Standardization, Geneva.
- ISSS-ISRIC-FAO (1998): World reference basis for soil resources. *World Soil Resources Reports*. FAO, Rome.
- JOERGENSEN, R. G. (1995): Microbial Biomass. In: ALEF K. and P. NANNIPIERI (Eds.): *Methods in Applied Soil Microbiology and Biochemistry*. Academic Press, London, 375–418.
- JOLLIFFE, I. T. (1986): *Principal Component Analysis*. Springer-Verlag, New York.
- KANDELER, E. (1993): Bestimmung von Nitrat. In: SCHINNER F., R. ÖHLINGER, E. KANDELER and R. MARGESIN (Eds.): *Bodenbiologische Arbeitsmethoden*. Springer-Verlag, Berlin, 369.
- KANDELER, E., D. TSCHERKO and H. SPIEGEL (1999): Long-term monitoring of microbial biomass, N mineralisation and enzyme activities of a Chernozem under different tillage management. *Biology and Fertility of Soils* 28, 343–351.
- KRZANOWSKI, W. J. (1988): *Principles of Multivariate Analysis. A User's Perspective*. Clarendon Press, Oxford.
- MOORE, J. M., S. KLOSE and M. A. TABATABAI (2000): Soil microbial biomass carbon and nitrogen as affected by cropping systems. *Biology and Fertility of Soils* 31, 200–21.
- OBERHOLZER, H. R., J. REK, P. WEISSKOPF and U. WALTHER (1999): Evaluation of soil quality by means of microbiological parameters related to the characteristics of individual arable sites. *Agribiological Research* 52, 113–125.
- PARKIN, T. B. and J. A. ROBINSON (1992): Analysis of log-normal data. *Advances of Soil Science* 20, 193–235.
- PARKIN, T. B., S. T. CHESTER and J. A. ROBINSON (1990): Evaluation of methods for calculating confidence intervals about the mean of a lognormally distributed variable. *Soil Science Society America Journal* 54, 321–326.
- PARKINSON, D. and D. C. COLEMAN (1991): Methods for assessing soil microbial populations, activity and biomass. *Microbial communities, activity and biomass. Agriculture, Ecosystems and Environment* 34, 3–33.
- SPARLING, G. P. (1997): Soil microbial biomass, activity and nutrient cycling as indicators of soil health. In: PANKHURST C. E., B. M. DOUBE and V. V. S. R. GUPTA (Eds.): *Biological Indicators of Soil Health*, CAB International, Wallingford. 97–119.
- TATE, R. L. (2000): *Soil Microbiology*, 2nd edn. John Wiley & Sons, New York.
- YAKOVCHENKO, V. and L. J. SIKORA (1998): Modified dichromate method for determining low concentrations of extractable organic carbon in soil. *Communications in Soil Science and Plant Analysis* 29, 421–433.
- YAKOVCHENKO, V., L. J. SIKORA and D. D. KAUFMAN (1996): A biologically based indicator of soil quality. *Biology and Fertility of Soils* 21, 245–251.

ZAR, J. H. (1984): Biostatistical analysis. 2nd edn. Prentice Hall, London.

ZBÍRAL, J. (1995): Stanovení potenciální kationtové výměnné kapacity. In: ZBÍRAL J. (Ed.): Analýza půd I. Ústřední kontrolní a zkušební ústav zemědělský, Brno, 125–129.

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