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The Use of Microbial Activity Indicators for a Quality Assessment of Highly Crude Oil Contaminated Soils in the Russian Subpolar Tundra at the Arctic Circle.

by Lothar Beyer[1], Wiebke Huyke[2], Stephan Hüttmann[2], Inna Archegova[3] & Tatiana V. Titarenko[4]

Summary: Oil polluted and not oil polluted soils (crude oil hydrocarbons contents: 20-92500 mg kg^{-1} dry soil mass) under natural grass and forest vegetation and in a bog in the Russian tundra were compared in their principal soil ecological parameters, the oil content and the microbial indicators. CFE biomass-C, dehydrogenase and arylsulfatase activity were enhanced with the occurrence of crude oil. Using these parameters for purposes of controlling remediation and recultivation success it is not possible to distinguish between promotion of microbial activity by oil carbon or soil organic carbon (SOC). For this reason we think that these parameters are not appropriate to indicate a soil damage by an oil impact. In contrast the metabolic quotient (qCO_2), calculated as the ratio between soil basal respiration and the SIR biomass-C was adequate to indicate a high crude oil contamination in soil. Also, the β-glucosidase activity (parameter β-GL/SOC) was correlated negatively with oil in soil. The indication of a soil damage by using the stress parameter qCO_2 or the specific enzyme activities (activity/SOC) minimizes the promotion effect of the recent SOC content on microbial parameters. Both biomass methods (SIR, CFE) have technical problems in application for crude oil-contaminated and subarctic soils. CFE does not reflect the low C_{mic} level of the cold tundra soils. We recommend (I) to test every method for its suitability before any data collection in series as well as application for cold soils and (II) the application of ecophysiological ratios as R_{mic}/C_{mic}, C_{mic}/SOC or enzymatic activity/SOC instead of absolute data.

Zusammenfassung: Ölverunreinigte Böden und nicht verunreinigte Böden (Rohölgehalte von 20-92500 mg kg^{-1} TS) unter Gras- und Waldvegetation sowie in einem Moor in der russischen Tundra wurden in ihren bodenökologischen Eigenschaften, dem Ölgehalt und mikrobiellen Parametern untersucht. CFE-Biomasse-C (CFE-C_{mic}), Dehydrogenase (DHA) und Arylsulfataseaktiviät (ARYL) korrelierten positiv mit dem Rohölgehalt. Bei der Erfolgskontrolle von Sanierungs- und Rekultivierungsmaßnahmen kann der Effekt von Öl-C und bodenbürtigem C (SOC) nicht unterschieden werden, d.h. CFE-C_{mic}, DHA und ARYL sind nicht geeignet die Bodenqualität gereinigter Tundraböden zu dokumentieren. Der metabolische Quotient (qCO_2) errechnet aus Basalatmung (R_{mic}) und SIR-C_{mic} ist dagegen ein Indikator für hohe Ölgehalte. Auch die SOC-bereinigte β-Glucosidaseaktivität (β-GL/SOC) war negativ mit dem Ölgehalt korreliert. Die Indikation einer Bodenschädigung durch Verwendung des Stressparameters qCO_2 bzw. der spezifischen Enzymaktivitäten (Aktivität/SOC) minimiert den Einfluss des aktuellen Bodenhumusgehaltes auf die mikrobiellen Aktivitätsparameter. Beide Biomassemethoden (SIR, CFE) könnten bei der Anwendung auf rohölhaltige Böden der Subarktis noch methodische Probleme in sich bergen. Beim Einsatz der CFE reflektiert diese nicht das niedrige Niveau der kalten Tundraböden. Aus den Erfahrungen der vorliegenden Untersuchung schlagen wir vor (I) jede Methode vor ihrer serienmäßigen Anwendung auf ihre grundsätzliche Eignung zur Bodenqualitätsindikation in extremen Klimaten zu überprüfen und (II) grundsätzlich ökophysiologische Verhältnisse wie R_{mic}/C_{mic}, C_{mic}/SOC oder Enzymaktivität/SOC zu bevorzugen anstatt direkte bzw. absolute Messdaten auszuwerten.

[1] Institute of Polar Ecology, University of Kiel, Wischhofstraße 1-3, Building 12, 24148 Kiel, Germany, present adress: Soil Science Germany, Juister Straße 10, 26802 Moormerland, Germany, <soil_science@web.de>
[2] Fa. Groth & Co. (GmbH & Co.), Geotechnology Section, Feldmannstraße 1, 25524 Itzehoe, Germany
[3] Institute of Biology, Komi Scientific Center, Ural Branch of the Russian Academy of Sciences, 167610 Russia, Komi Republic, Syktyvkar, Kommunisticheskaya St. 28
[4] KomiArcticOil JSC, Vozeyskaya St. 21-A, Usinsk, 169706, Komi Republic, Russia

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INTRODUCTION

The subpolar Upper Vozoy oil field is located in the Timan Pechora region (latitude: 67° 30'N, longitude: 57° 30'E) in Russia close to the Barents Sea and nearly 1000 miles northeast of Moscow (Fig. 1). This oil field is provided with one of the most prominent oil resources in Russia west of the Ural mountains. Defect pipeline systems and obsolete oil exploitation techniques during the Soviet era have induced a tremendous extent of oil pollution: up to 30 % crude oil in soil and oil lakes on the soil surface everywhere (REES et al. 1999). These contaminations on the drilling fields of the KomiArcticOil, a Canadian-Russian joint venture, are proposed to be removed, the soils to be remediated and the sites to be recultivated. In order to control the efficiency of these treatments selected environmental indicators should be applied to characterize soil quality or soil health. In the first period it was tested if the commonly used enzymatic activity tests and microbial biomass determinations (BEYER 1998) using substrate induction method (SIR) as well as chloroform fumigation extraction technique (CFE) are appropriate to characterize the microbial soil conditions after remediation and recultivation in the subpolar regions.

MATERIALS AND METHODS

Soils

All soils are characterized by a high water table, having a cryic temperature regime (<8 °C), but no permafrost. Therefore, they were not classified as typical permafrost-affected soils, the Gelisols (SOIL SURVEY STAFF 1998). Soils no.1, 2, 5 and 6 are weakly developed mineral soils with aquic conditions and a shallow organic layer. According to the recent Soil Taxonomy they were classified as Histic Cryaquepts (SOIL SURVEY STAFF 1998). Occasionally the soils are buried under a shallow sandy deposit derived from road constructions (Tab. 1). Both oil-spilled soils are covered by a thick crude oil film mixed with mineral materials. Soil 3 and 4 are typical peatland soils in a bog. The organic materials are weakly humified and sapric materials were not found. Therefore they have to be classified as Cryofibrists (SOIL SURVEY STAFF 1998). Sampling was carried out in August 1998 with three field replicates.

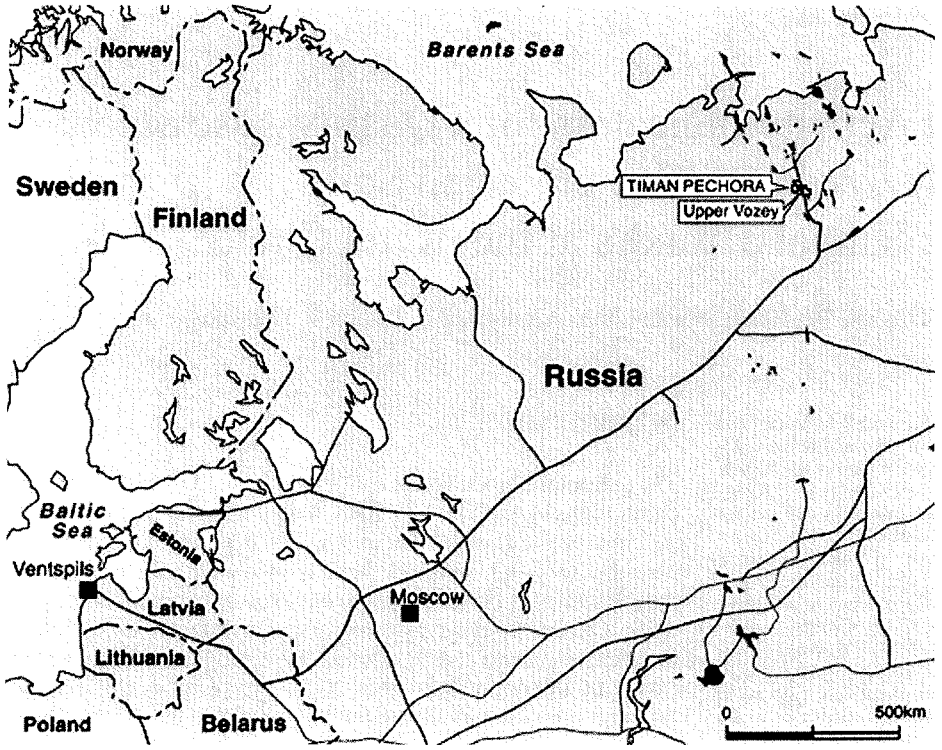

Fig. 1: Location of the Upper Vozoy Oil Field of the KomiArcticOil in the Russian arctic tundra.

Abb. 1: Lokalität der untersuchten Gebiete im oberen Vozoy-Ölfeld der KomiArcticOil in der arktischen Tundra Russlands

Methods

The principal soil properties were determined according to SCHLICHTING et al. (1995). The dry matter content was determined gravimetrically at 105 °C in dry-oven. The total organic carbon (TOC) was determined after dry combustion at 600 °C and measurement of the evolved CO_2. The crude oil was extracted with 1,1,2-trichlortrifluorethan and measured using IR spectroscopy (DIN 38409 1981). The crude oil carbon (Oil-C) was calculated as oil x 0.86 $[CH_2)_n]$. In order to distinguish within the TOC, Oil-C and native soil organic carbon (SOC), SOC was calculated as TOC, Oil-C. Total nitrogen (Nt) was measured after a Kjeldahl extraction in a flow injection analyzer. All enzyme tests and biomass carbon estimations were carried out according to ALEF (1991) and SCHINNER et al. (1996) based on µg or mg g^{-1} soil dry mass (DM): arginine ammonification activity (ARG) in µg NH_4-N g^{-1}, dehydrogenase activity (DHA) in µg TPF g-1, β-glucosidase activity β(-GL) in µg saligenin g^{-1}, arylsulfatase activity (ARYL) in µg glucose g^{-1}. Biomass-C (C_{mic}) was estimated by using two methods: (I) the substrate induced technique by using glucose as microbial food substrate (SIR) and measurement of O_2 consumption in a Sapromat and (II) the chloroform fumigation technique (CFE) according to VANCE et al. (1987). The results are given in µg Cmic g_{-1} dry soil mass. The basal respiration (Rmic) measurement is equivalent to the SIR determination but without the glucose amendment. Rmic is given in µg CO_2-C h^{-1} g^{-1} dry soil mass. The Cmic/SOC ratio reflects C_{mic} in percent of SOC. The metabolic quotient (qCO_2) is an indicator for detection of microbial stress and defined as $R_{mic}/SIR\text{-}C_{mic}$ x 10^3. The pH value was measured in 0.02 *N* CaCl2 with a simple glass electrode. All microbial lab measurements were carried out in four replicates.

RESULTS AND DISCUSSION

Typical soils of the landscape were investigated, which had a highly different level of crude oil pollution. Selected data are summarized in Table 1. Compared to the German remediation threshold value of 0.5 mg g^{-1} or 500 mg kg^{-1} soil dry mass, already the "weakly polluted" soils were significantly contaminated (Tab. 1, Oil-C). Surprisingly, we found considerable amounts of oil in soils, which had nor oily smell neither have been visibly classified as contaminated (Tab. 1). In the extremely polluted Cryaquept under grass in the both surface horizons the oil carbon content (Oil-C) was higher as the soil organic carbon (SOC), means that the SOC/Oil-C ratio was <1. In the mineral Histic Cryaquepts this ratio was lower with higher oil contaminations (Tab. 1). This was not the case for the organic Cryofibrists. The very wide SOC/Nt ratios in the organic layers (Oi/Oe) suggest that the used oil extraction method (H18) is not appropriate for quantitative estimations of the crude oil. This is also a matter of general discussion within the soil remediation analytics (HÜTTMANN 1999). However, for this study the problem is of minor concern because at the soil colloids highly adsorbed hydrocarbons are environmentally less bioavailable as the extractable ones (ALEXANDER 1994, RISER-ROBERTS 1992).

The level of the CFE biomass carbon (CFE-C_{mic}) was comparable to this of well-known data obtained in temperate regions (Tab. 2). The data range of the organic Cryofibrists was similar to the observations of CHENG & VIRGINIA (1993) made for organic surface horizons in the Alaskian tundra (68° 38´N, 149° 25´W), whereas in the mineral Cryaquepts the level was much lower. This suggest a certain influence of SOC on CFE-C_{mic} (r = 0.545*, n = 17). The effect of Oil-C on the CFE- C_{mic} was not directly detectable due to an essential positive effect of SOC on CFE- C_{mic} (BEYER 1995), which was observed excluding the organic Cryofibrists (r = 0.745**, n = 13). The CFE-

no.	horizon	depth cm	DM	Oil-C	TOC	SOC	SOC / Oil-C	N_t mg g^{-1}	SOC / N_t	pH[A]
			---------- mg g^{-1} DM ----------							
Soil 1: Histic Cryaquept under grass vegetation and with visible crude oil contamination										
1.0	Oil/C[B]	0-2	946	92.5	150.7	58.2	0.63	ND	ND	ND
1.1	C[C]	-15	687	46.5	76.9	30.4	0.65	1.32	23.0	7.26
1.2	Oi/Oe	-30	255	33.5	363.8	330.5	9.87	5.83	56.7	5.79
1.3	Bg	-55	872	0.4	3.3	2.9	7.25	0.26	11.2	5.86
Soil 2: Histic Cryaquept under grass vegetation without visible crude oil contamination										
2.1	C[C]	0-10	616	0.9	48.3	47.4	52.7	1.98	23.9	7.06
2.2	Oi/Oe	-30	107	1.8	393.8	392.0	217	6.03	65.3	5.83
2.3	ABg	-42	818	0.03	10.5	10.5	350	0.59	17.8	4.59
Soil 3: Sphagnic Cryofibrist in a bog without visible crude oil contamination										
3.1	Oi	0-30	215	2.8	499.5	496.7	177	4.69	105.8	3.73
3.2	Oe	-60	136	4.3	493.4	489.1	113	20.57	23.8	4.06
Soil 4: Hydric Cryofibrist in a bog with visible crude oil contamination										
4.1	Oi	0-17	261	1.3	361.5	360.2	359	11.19	32.1	3.78
4.2	Oe	>17[D]	126	3.1	439.7	436.6	141	15.16	28.8	3.55
Soil 5: Histic Cryaquept under open pine/birch forest with visible crude oil contamination										
5.0	Oil[B]	0-5	134	3.8	349.3	345.5	90.9	9.53	35.1	5.47
5.1	Oi/Oe	-18	118	1.8	481.7	479.9	266	4.01	119.7	5.55
5.2	A	-38	574	0.1	96.7	96.6	966	4.93	19.6	5.68
5.3	Bg	-50	856	0.03	15.0	15.0	500	0.98	15.3	5.81
Soil 6: Histic Cryaquept under open pine/birch forest without visible crude oil contamination										
6.1	Oi/Oe	0-15	177	2.9	389.0	386.1	133	8.32	46.4	4.42
6.2	A	-24	827	0.1	3.9	3.8	38.0	0.34	11.2	5.76
6.3	Bg	-47	856	0.02	2.9	2.9	145	0.34	8.5	5.96

Tab. 1: Selected principal soil properties in the oil exploitation region of KomiArcticOil (Usinsk) in the Russian tundra (soil classification according to Soil Taxonomy, SOIL SURVEY STAFF 1998). ND: not determined, DM: dry mass; TOC: total organic carbon; Oil-C: crude oil carbon; SOC: soil organic carbon (TOC – Oil-C); N_t: total nitrogen in mg g^{-1} DM; [A]: in 0.02 *N* $CaCl_2$; [B]: crude oil mixed with sandy materials; [C]: sandy deposit; [D]: oil-polluted water table 10 cm below the soil surface.

Tab. 1: Ausgewählte Bodeneigenschaften in der Ölförderregion KomiArcticOil (Usinsk) in der russischen Tundra (Bodensystematik gemäß Soil Taxonomy, SOIL SURVEY STAFF 1998). ND: nicht bestimmt, DM: Trockenmasse, TOC: gesamter organischer Kohlenstoff, Oil-C: Rohöl-Kohlenstoff, SOC: organischer Boden-Kohlenstoff, N_t: Gesamtstickstoff in mg g^{-1} DM, [a]: in 0,02 *N* $CaCl_2$, [B]: Rohöl gemischt mit sandigem Material, [C]: sandige Ablagerung, [D]: Öl verunreinigter Wasserspiegel 10 cm unter der Bodenoberfläche.

C_{mic}/SOC ratio and the Oil-C showed considerable strong correlations over all soil samples ($r = 0.766$***, n = 17). Obviously weak oil pollutions promote the production of CFE-Cmic, what can be seen by the comparison of the weakly contaminated organic Cryofibrists. A similar observation has been made by JÖRGENSEN et al. (1995) with respect to the biological impact of fuel hydrocarbons in soil on microbial biomass. On the other hand it is highly probable that within the CFE-C_{mic} extraction with chloroform oil compounds will be extracted. This would lead (I) to a "pseudo" correlation between CFE-C_{mic} and Oil-C and (II) an overestimation of the current biomass levels. In addition the current data suggest that the CFE lab method did not reflect the well-known low level of C_{mic} for the cold arctic soils compared to the temperate soils (Table 2), despite it is clear that the mean annual ambient and soil temperatures are much lower (BEYER 1998, SCHMIDT 1999).

Compared to CFE-C_{mic} the SIR-C_{mic} were extremely low (Tab. 2). INSAM & HASELWANDTER (1989) found somewhat higher values close to the Columbus icefield (52° 20'N, 117° 20'W), what was in accordance with the lower latitude of nearly 10°. However, CHENG & VIRGINIA (1993) found nearly the same level of CFE- and SIR-data in soil of the Alaskian tundra (68° 38'N, 149° 25'W), which location is comparable to our soils. However, these authors investigated soils with a much higher content of organic carbon (168-431 g kg^{-1} DM). The low SIR level was also found compared to natural soils of the temperate climate region in North Germany (Tab. 2, last three lines) and contradicts the frequently very high SOC values, which would suggest the occurrence of much higher SIR-C_{mic} levels (BEYER 1995). However, we found a strong correlation between SIR-C_{mic} and SOC ($r = 0.85$***, n = 16), which was much more significant as for the CFE-C_{mic} data (see above). In the cold (cryic temperature regime!) and subarctic soils the microorganisms are obviously not capable to use the applied glucose as food source within the given six hours of measuring time (ALEF 1991, SCHINNER et al. 1996), because they are not adapted to the high temperatures in the laboratory. In this case the SIR in the commonly applied procedure would not be appropriate for the cold arctic soils. This is in line with observations made by SCHMIDT (1999), who concluded that the SIR method is inappropriate to estimate microbial biomass in tun-

	CFE		SIR			R_{mic}	qCO_2	CAI
	C_{mic}	C_{mic}/SOC	C_{mic}	C_{mic}/SOC	R_{mic}			
Soil 1: Histic Cryaquept under grass vegetation and with visible crude oil contamination								
1.1	1424	4.68	14.1	0.046	0.18	0.15	10.6	0.83
1.2	480	0.15	47.0	0.014	0.61	0.46	9.8	0.75
1.3	2	0.07	2.0	0.068	0.03	0.01	5.2	0.38
Soil 2: Histic Cryaquept under grass vegetation without visible crude oil contamination								
2.1	327	0.69	13.1	0.027	0.17	0.06	4.6	0.35
2.2	1462	0.37	207.0	0.053	2.71	0.61	2.9	0.23
2.3	27	0.26	0.7	0.006	0.009	0.004	5.7	0.44
Soil 3: Sphagnic Cryofibrist in a bog without visible crude oil contamination								
3.1	231	0.05	7.8	0.002	0.10	0.11	14.1	1.10
3.2	340	0.07	10.7	0.002	0.14	0.16	14.9	1.14
Soil 4: Hydric Cryofibrist in a bog with visible crude oil contamination								
4.1	NF	NA	19.7	0.005	0.25	0.17	8.6	0.68
4.2	1933	0.44	56.0	0.013	0.73	0.33	5.9	0.45
Soil 5: Histic Cryaquept under open pine/birch forest with visible crude oil contamination								
5.0	2704	0.78	ND	ND	ND	ND	ND	ND
5.1	1536	0.32	103.8	0.022	1.36	0.40	3.9	0.29
5.2	182	0.19	6.9	0.007	0.09	0.08	11.6	0.88
5.3	33	0.22	0.8	0.005	0.01	0.03	3.8	3.00
Soil 6: Histic Cryaquept under open pine/birch forest without visible crude oil contamination								
6.1	1587	0.41	103.2	0.027	1.35	0.53	5.1	0.39
6.2	11	0.28	0.5	0.013	0.006	0.003	5.6	0.50
6.3	7	0.24	0.3	0.010	0.004	0.002	6.1	0.50
Selected References								
P^A	1402	1.99	ND	ND	ND	ND	ND	ND
A^A	168	1.14	311	1.97	4.07	0.42	1.45	0.10
F^A	942	0.60	ND	ND	ND	ND	ND	ND

Tab. 2: Microbial biomass carbon (C_{mic}), respiration (R_{mic}) and ecophysiological parameters of the investigated tundra soils. P^A: pasture; A^a: arable land; F^a: forest, unpublished data from surface soils in the temperate climate region (North Germany); ND: not determined; NF: not found, under detection limit; NA: not available.; SOC: soil organic carbon (in mg g^{-1} soil dry mass, for data see Tab. 1), C_{mic}: microbial biomass carbon; CFE: chloroform fumigation extraction method; SIR: substrat induced method (both in µg g^{-1} soil dry mass); R_{mic}: basal respiration SIR-R_{mic}: substrat induced respiration (both in µg CO_2-C h^{-1} g^{-1} soil dry mass); qCO_2: metabolic quotient: basal respiration/biomass-C (R_{mic}/SIR-C_{mic} x 10^3); CAI: carbon availability index: basal respiration/substrat induced respiration (without unit).

Tab. 2: Mikrobieller Biomasse-Kohlenstoff (C_{mic}), Basalatmung (R_{mic}) und ökophysiologische Parameter in den untersuchten Tundraböden. P^A: Weideland, A^A: Ackerland, F^A: Wald, unveröffentl. Daten von Boden-Oberflächen temperierter Klimate (Nord-Deutschland), ND: nicht bestimmt, NF: unter Nachweisgrenze, NA: nicht vorhanden, SOC: organischer Boden-Kohlenstoff (in mg g^{-1} Boden-Trockenmasse, Daten siehe Tab. 1), C_{mic}: mikrobieller Biomasse-Kohlenstoff, CFE: Chloroform-Gas-Extraktions-Methode, SIR: Substrat induzierte Methode (beide in µg g^{-1} Boden-Trockenmasse), R_{mic}: Basalatmung, SIR-R_{mic}: Substrat induzierte Basalatmung (beide in µg CO_2-C h^{-1} g^{-1} Boden-Trockenmasse), qCO_2: metabolischer Quotient aus Basalatmung und Biomasse-Kohlenstoff (R_{mic}/SIR-C_{mic} x 10^3), CAI: Kohlenstoff-Verfügbarkeits-Index: Basalatmung/Substrat induzierter Atmung (ohne Einheit).

dra soils. For this reason it would make sense to use a longer incubation time or to apply a more favorable substrate (concerning this matter see also SCHMIDT 1999) as it has been suggested from HÜTTMANN (1999) for diesel-contaminated soils. On the other hand it might be worthwhile to discuss the impact of the incubation temperature. According to ROBINSON & WOOKLEY (1997) in temperate climates the temperature response on microbial biomass, expressed by the Q_{10} coefficient, is 2.0 between 5 and 15 °C, whereas it is nearly 4 between 0 and 10 °C for arctic soils. For this reason already NADELHOFFER et al. (1991) made their lab experiments at the relevant in-situ temperature of the investigated habitat. We urgently recommend to focus future research work in soil microbiology in cold soils on this matter. However, to say it again, comparing SIR data with CFE data one should keep in mind the already discussed and highly probable overestimation due to faults within the extraction procedure. In any case currently none of the both biomass carbon estimation techniques, nor the SIR neither the CFE method, will deliver quantitative results. But congruent maxima and minima ($r_{CFE/SIR}$ = 0.712**, n = 17) suggest a similar indication tendency of both methods, e. g. the reaction against crude oil impact.

The metabolic quotient (qCO_2) calculated from the basal respiration (R_{mic}) and SIR-C_{mic} was mostly lower with less crude oil contamination. These results confirm the suitable application

of qCO_2 as stress indicator. On the other hand from the CFE data it might be doubtful, if an oil pollution induces always stress for the microorganisms. Very impressive the indication by using the qCO_2 was found with the soils under grass vegetation (Tab. 2: Cryaquepts). CHENG et al. (1996) recently introduced a new approach by using the ratio between the basal respiration (R_{mic}) and the substrate induced respiration. This index is supposed to reflect the carbon availability (CAI) in soil (Tab. 2). If CAI is close to 1 it suggest that carbon is no limiting factor for microbial respiration (CHENG et al. 1996). For the Cryaquepts under grass a high carbon availability was correlated to an oil impact. This suggest that Oil-C is used as carbon source from the microbes. This would be in line with the high CFE-C_{mic} values of the polluted soils (Tab. 2: Cryaquepts under grass). However, this was not found for the other soils. Nevertheless, an excessive interpretation of the qCO_2 values should be limited, because with the extremely low levels of R_{mic} and SIR-C_{mic} already small and commonly observed variations within the measurement deviations would lead to considerable calculation errors (AALEF 1991, SCHINNER et al. 1996, HÜTTMANN 1999, BEYER 1995).

For the enzymes we found similar disappointing results as found for the microbial biomass estimations. Dehydrogenase (DHA) und arylsulfatase activity (ARYL) were inappropriate for the Cryofibrists because of the interferences within the photometric measurements due to the soil organic matter induced color of the extracts (Tab. 3). This was also observed for recultivated soils on brown coal mine sites (KOLK et al. 1996). However, the obtained data from mineral soils suggest that this is obviously not an effect deriving directly from the crude oil. DHA reacted positive on oil, e.g. comparison of surface horizons of the Cryaquept under forest (Tab. 3, 5.1 and 6.1): with a low SOC and a high Oil-C content DHA was significantly higher. A similar pattern was observed comparing the subsurface horizons of the Cryaquept under grass (Tab. 3, 1.2 and 2.2). Only when the Oil-C content reached very high levels a negative impact on DHA was detectable (Tab. 3, 1.1

	DHA	DHA / SOC	β-GL	β-GL / SOC	ARG	ARG / SOC	ARYL	ARYL / SOC
Soil 1: Histic Cryaquept under grass vegetation and with visible crude oil contamination								
1.1	313	10.3	4	0.13	1.1	0.04	7	0.23
1.2	412	1.2	18	0.05	2.6	0.008	ND	ND
1.3	7	2.4	1	0.34	NF	ND	5	1.72
Soil 2: Histic Cryaquept under grass vegetation without visible crude oil contamination								
2.1	431	9.1	19	0.40	11.7	0.25	39	0.82
2.2	131	0.3	82	0.21	39.4	0.11	7	0.02
2.3	2	0.2	2	0.19	1.4	0.13	NF	NF
Soil 3: Sphagnic Cryofibrist in a bog without visible crude oil contamination								
3.1	NA	NA	14	0.03	11.0	0.02	2	0.004
3.2	NA	NA	20	0.04	11.7	0.02	NA	NA
Soil 4: Hydric Cryofibrist in a bog with visible crude oil contamination								
4.1	NA	NA	49	0.14	11.6	0.03	NA	NA
4.2	NA	NA	17	0.04	9.5	0.02	NA	NA
Soil 5: Histic Cryaquept under open pine/birch forest with visible crude oil contamination								
5.0	522	1.5	96	0.28	63.2	0.18	226	0.65
5.1	195	0.4	50	0.10	40.8	0.09	7	0.02
5.2	132	1.4	18	0.19	1.8	0.02	35	0.36
5.3	6	0.4	3	0.20	3.1	0.21	3	0.20
Soil 6: Histic Cryaquept under open pine/birch forest without visible crude oil contamination								
6.1	277	0.7	118	0.31	18	0.05	ND	ND
6.2	51	13.1	2	0.51	0.9	0.23	4	1.03
6.3	<1	<0.3	<1	<0.3	3.4	1.17	3	1.03
Selected References[A]								
pasture	197	2.8	127	1.80	6.6	0.09	ND	ND
arable land	72	4.3	68	4.10	4.7	0.28	455	27.4
forest	6	0.03	138	0.78	8.9	0.05	ND	ND

Tab. 3: Selected enzyme activities of the investigated tundra soils . ND: not determined; NF: not found, under detection limit; NA: not available; SOC: soil organic carbon, data see Table 1; DHA: dehydrogenase activity in µg TPF g^{-1} soil DM; DHA/SOC: DHA in µg TPF mg^{-1} SOC; β-GL: β-glucosidase in µg saligenin g^{-1} soil DM; β-GL/SOC: β-GL in µg saligenin mg^{-1} SOC; ARG: arginine ammonification in µg NH_4-N g^{-1} soil DM; ARG/SOC: ARG in µg NH_4-N mg^{-1} soil SOC; ARYL: arylsulfatase in µg glucose g^{-1} soil DM; ARYL/SOC: ARYL in µg glucose mg^{-1} SOC; [A] legend see Table 2

Tab. 3: Ausgewählte Enzymaktivitäten in den untersuchten Tundraböden. ND: nicht bestimmt, NF: unter Nachweisgrenze, NA: nicht vorhanden, DM: Trockenmasse, SOC: organischer Boden-Kohlenstoff (in mg g^{-1} Boden-DM, Daten siehe Tab. 1), DHA: Dehydrogenase-Aktivität in µg TPF g^{-1} Boden-DM, DHA/SOC: DHA in µg TPF mg^{-1} SOC, β-GL: β-Glucosidase in µg Saligenin g^{-1} Boden-DM, β-GL/SOC: β-GL in µg Saligenin mg^{-1} SOC, ARG: Arginine Ammonifikation in µg NH_4-N g^{-1} Boden-DM, ARG/SOC: ARG in µg NH_4-N mg^{-1} Boden-SOC, ARYL: Arylsulfatase in µg Glucose g^{-1} Boden-DM, ARYL/SOC: ARYL in µg Glucose mg^{-1} SOC, [A]: siehe "selected references" in Tab. 2.

and 2.1). Excluding the organic Cryofibrists, β-glucosidase (β-GL) and arginine ammonification activity (ARG) showed significant correlations to SOC ($r_{\beta\text{-GL/SOC}}$ = 0.817***, $r_{ARG/SOC}$ = 0.753**, n = 13), but not with Oil-C. For β-GL, based on soil dry mass no correlation with Oil-C was found, but based on carbon mass (β-GL/SOC) a weakly negative correlation was found (r = -0.53*, n = 17). In addition (β-GL in the Cryofibrists was highest with the lowest SOC and simultaneously the lowest Oil-C content. This was not the case for ARG. Due to the shortage of sampling substrate the data source for ARYL is minimized (no field replicates) and the statements for ARYL should be considered with caution. But the current data suggest that ARYL was reacting positive on oil contaminations in a similar way as DHA, a negative impact was observed only with the highest oil contaminations.

To use enzyme activities as a regular indicator in contaminated and/or cold soils more informations are required to estimate an impact on soil ecosystems. Our data contradict the statement of DICK (1997) that the most promizing field of application for enzymes is assessing pollution impact on soil. Research is needed across the range of soil types, ecosystems and environment (DICK 1997) and distribution on extracellular and cell-derived enzyme activities (NANNIPIERI et al. 1996, DILLY & NANNIPIERI 1998). The latter concluded that almost all research on soil enzymes has evolved without considering the ecological implications. The approach of plotting enzyme activities versus other microbial parameters (DILLY & NANNIPIERI 1998) might be more promizing than what is recently done. In addition plant-microorganism impact with respect to energy transfer within ecosystems or between the sub-ecosystems (plant, soil surface, surface soil, subsurface soil) might be relevant interactions for the regulation of enzyme activities (DILLY et al. 2000). Arising from this background it sounds questionable if soil enzymes are appropriate to assess soil quality in extreme environments, where such needed soil ecological and environmental interactions are nearly unknown (ROBINSON & WOOKLEY 1997).

CONCLUSIONS

CFE biomass-C, dehydrogenase and arylsulfatase activity were enhanced with the occurrence of crude oil. Negative impacts were observed only with the highest oil contaminations. For this reason we think that these parameters are not appropriate to indicate a soil damage by an oil impact. In contrast the metabolic quotient (qCO_2), calculated as the ratio between soil basal respiration and the SIR biomass-C was adequate to indicate a high crude oil contamination in soil. Also, the β-glucosidase activity (parameter β-GL/TOC) was correlated negatively with oil in soil. However, especially the both biomass methods (SIR, CFE) have technical problems in application for crude oil-contaminated soils. Especially for an application in cold soils the commonly used high incubation temperature of >20 °C should be changed into the relevant in-situ temperature. For the SIR method other substrates as glucose should be discussed. Data interpretation needs to consider (1) the impact of the substrate (organic or mineral) on microbial and enzyme activity, (2) the degree of contamination, (3) the age of the oil compounds due to the chemical modification within time and (4) the advantages and disadvantages of the single method. For this reason we urgently recommend (a) to test every method for its suitability before any data collection in series and (b) the use of ecophysiological ratios as R_{mic}/C_{mic}, C_{mic}/SOC or enzymatic activity/SOC instead of pure data. The amendment of such specific activities in cold soils has been already sucessfully carried out for cold soils in Antarctica (TSCHERKO et al. 2002).

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