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Long-term impact of acid resin waste deposits on soil quality of forest areas II. Biological indicators.

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Abstract

In this study, we evaluated the effects of two acid resin deposits on the soil microbiota of forest areas by means of biomass, microbial activity-related estimations and simple biological ratios. The determinations carried out included: total DNA yield, basal respiration, intracellular enzyme activities (dehydrogenase and catalase) and extracellular enzyme activities involved in the cycles of C (β-glucosidase and chitinase), N (protease) and P (acid-phosphatase). The calculated ratios were: total DNA/total N; basal respiration/total DNA; dehydrogenase/total DNA and catalase/total DNA. Total DNA yield was used to estimate soil microbial biomass. Results showed that microbial biomass and activity were severely inhibited in the deposits, whilst resin effects on contaminated zones were variable and site-dependant. Correlation analysis showed no clear effect of contaminants on biomass and activities outside the deposits, but a strong interdependence with natural organic matter related parameters such as total N. In contrast, by using simple ratios we could detect more stressful conditions in terms of organic matter turnover and basal metabolism in contaminated areas compared to their uncontaminated counterparts. These results stress that developed ecosystems such as forests can buffer the effects of pollutants and preserve high functionality via natural attenuation mechanisms, but also that acid resins can be toxic to biological targets negatively affecting soil dynamics. Acid resin deposits can therefore act as contaminant sources adversely altering soil processes and reducing the environmental quality of affected areas despite the solid nature of these wastes.

Keywords: acid resin; enzyme activities; heavy metals; hydrocarbons; trace elements; total DNA

1. Introduction

Anthropogenic activities can disturb normal soil functioning and have deleterious effects on environmental quality. Physical and chemical soil properties such as texture, aggregate structure, pH, organic matter content, etc., are all involved in the behaviour of soils and their response to external changes (Parr et al., 1992). However, biochemical and microbiological properties such as enzyme activities, microbial biomass and respiration have been outlined as particularly appropriate for evaluation of soil quality (Pankhurst et al., 1995), due to the key role of microorganisms in the cycling of nutrients, the metabolic capacity and the functional integrity of soils (Nannipieri et al., 2003).

Various studies have shown that inorganic and organic contaminants can have negative effects on soil microbial properties (Benítez et al., 2004; Pérez-de-Mora et al 2005, 2006; Dawson et al., 2007). Heavy metals are known to cause long-term toxic effects within ecosystems and can have a negative influence on soil biological processes (Lee et al., 2002; Kizilkaya et al., 2004). They can also affect microbial proliferation and enzyme activities by masking catalytically active groups, altering protein conformation or competing with other metals involved in the formation of enzyme-substrate complexes (Eivazi and Tabatabai, 1990). However, long-term exposure to heavy metals may also enhance microbial tolerance in soil (Baath et al., 1998; Del Val et al., 1999). In this case, no net effect on broad microbial indices such as soil respiration or microbial biomass may be observed (Khan and Scullion, 2000).

Hydrocarbons can exert a negative impact on soil quality and soil biology. Short n-alkanes can act as solvents for cellular fats and membranes (Sikkema et al., 1995), whereas long chain n-alkanes may contribute to the formation of oil films and slicks, which may in turn block the exchange of water, nutrients and gases (Leahy and Colwell, 1990). Polycyclic aromatic hydrocarbons are known to be carcinogenic, teratogenic and mutagenetic (Miller and

1 Ramos, 2001) and negative effects on the soil microbiota have been also reported (Dechsel et
2 al., 1996; Smreczek et al., 1999). On the other hand, specialized microorganisms can use
3 hydrocarbons as energy and C source and thus proliferate on sites contaminated with such
4 compounds (Coulon et al., 2007; Wentzel et al., 2007).

5 Numerous investigations have assessed the effects of particular contaminants or specific
6 groups of contaminants on soil biochemical and biological properties in agricultural
7 ecosystems. However, there are few studies of interactions between microbiological
8 properties and complex contaminations in forest ecosystems, which still dominate the
9 landscape of many areas in Europe. Knowledge of such interactions and their consequences in
10 the long-term are important to the ecotoxicological assessment of contaminated soils. In this
11 work, we studied various microbiological and biochemical properties and calculated simple
12 ratios to evaluate the effects of acid resin wastes on soil functionality and overall microbial
13 activity in affected areas. General chemical and contaminant data presented in Pérez-de-Mora
14 et al. (in press) was used to interpret activity patterns in soil.

15

16 ***2. Material and methods***

17 A description of the sites (Schlangenburg = site A and Seelacher Berg = site B), the
18 sampling and the general chemical properties, hydrocarbon and trace element concentrations
19 of the soils can be found in Pérez-de-Mora et al. (in press).

20

21 *2.1. Total DNA yield*

22 Samples for total nucleic acid extraction were kept in dry ice until stored at -80°C.
23 Total nucleic acids from soil (0.5g dw) were extracted using the method of Griffiths et al.
24 (2000). Cells were lysed via mechanical shaking in Precellys-Keramik-Kit Tubes (PeqLab,

Erlangen, Germany) with a Precellys 24® Lysis and Homogenisation Automated Equipment (Bertin technologies, France). Extracted nucleic acids were resuspended in 50mL miliQ water (pH=6.8) and concentration of total DNA was measured via a Nanodrop® ND-1000 spectrometer (Nanodrop Technologies, Wilmington, DE ,US) at 260nm. Extractions were carried out in duplicate. The quality of the DNA extracted was checked by comparing the ratios OD 260/280 and OD 260/230 between samples. Control and contaminated samples did not differ in this regard. Additionally, viability of DNA was examined via PCR amplification of 16S *rRNA* and 18S *rRNA* fragments.

2.2. Soil basal respiration

Soil samples (3-5g and 60% WHC) were incubated up to three days at 25°C in closed glass jars (120mL) (Isermeyer, 1952). Concentrations of CO_2 produced were determined using a Gas Chromatograph (GC-14B, Shimadzu Corporation, Kyoto, Japan) equipped with an Electron Capturer Detector (280°C). Separation of CO_2 from other gases in the sample was achieved through a Porapack Q column (80-100µm Mesh, Millipore). Column temperature was 60°C and the carrier gas was nitrogen (ECD quality, Linde); a flow of 20mL min^{-1} was used.

2.3. Soil enzyme activities

Dehydrogenase activitity was estimated after incubating soil samples with 0.5% 2-*p*-iodophenyl 3-*p*-nitrophenyl-5 tetrazolium chloride (INT) solution and determination of the reduced product iodonitrotrezolium formazan (INTF) via a colorimetric assay at 490nm (Cary Elipse UV/visible Spectrophotometer, Varian, Australia) (von Mersi and Shinner, 1991).

Catalase activity was assessed after incubation of soil samples with H_2O_2 and

estimation of the remaining H_2O_2 via colorimetric determination (λ = 505nm) (Trasar-Cepeda et al., 1999).

Protease activity was estimated by quantifying colorimetrically (λ=700 nm) the release of aromatic amino acids after incubation of soil samples with a buffered casein solution (Ladd and Butler, 1972).

The activities of acid-phosphatase, β-d-glucosidase and chitinase were measured using a microplate fluorometric assay (Marx et al., 2001). Soil suspensions were incubated with the appropriate substrate at pH=6 (800µM of 4-MUB-phosphate for 20min; 400µM 4-MUB-β-D-glucoside for 40min and 400µM 4-MUB-N-acetyl-β-D-glucosaminide for 40min). Determination of the 4-methyl umbelliferone (4-MUB) released after the incubation was carried out with a fluorescence spectrophotometer (Cary Elipse Fluorescence Spectrophotometer, Varian, Australia) at an excitation wavelength of 340nm and emission at 450nm. Controls with water or substrate instead of soil suspension were also performed. A calibration curve for each zone was prepared to minimize the quenching effect due to differences in organic matter quality and quantity of soil samples.

2.4. Statistical analysis

Univariate statistical analyses were performed using the program SPPS 15.0 for Windows. A normality test was carried out for all variables prior to analysis of the variance. The chemical and microbiological data was analysed by ANOVA, considering the sampling zone as the independent variable. Significant statistical differences of all variables between the different zones were established by Tukey´s test when there was homogeneity of the variance and by Games-Howell's test in the opposite case. Correlation matrixes for each site between microbiological properties and biochemical and chemical properties were also calculated. The significance level reported (α=0.01 and α=0.05) is based on Pearson`s

coefficients. Correlations were performed separately for each site as combining the two datasets changed some of the local interdependencies. In order to evaluate the effects of contaminants on biological properties in the surroundings, deposits were excluded from the correlation analysis, as results from ANOVA analysis clearly showed that biological activity was severely reduced in the latter area.

3. Results

3.1. Total DNA

Total DNA yield in samples from the deposits were extremely poor (0.4 μg DNA g^{-1}) and this material could not be amplified via PCR. In the surroundings, DNA concentrations were significantly higher and 16S *rRNA* and 18S *rRNA* amplicons could be obtained from all samples, independently from the degree of contamination. In site A, DNA yields were about 10 times higher in control than in contaminated zones, whereas no significant differences were found in site B (Figure 1a).

3.2. Basal respiration

Basal respiration in the deposits was either not detectable or extremely low in comparison with contaminated and control areas (Figure 1b). In surrounding zones of site A, respiration rate was found to be markedly higher in control (1.8-6 times) than in contaminated areas (Figure 1b). In the latter, significant differences were also reported between zones X_{A1} and X_{A3} (Figure 1b). A different situation was observed in site B, where contaminated zones showed larger C-CO_2 production than the control area (approx. 10 times) (Figure 1b). Significant differences were also reported between X_{B1} and the other contaminated zones (Figure 1b).

3.3. Intracellular enzyme activities

In site A, dehydrogenase activity in the control zone was 5 fold higher than in contaminated zones and about 14 fold larger than in the deposits (Figure 1c). In site B, similar results were obtained in control and contaminated areas and mean values in these areas were around 15 fold larger than those found in the deposit (Figure 1c).

Catalase activity in site A was found to be 3-6 times higher in the control than in the other areas, but there were no differences between the deposit and contaminated zones (Figure 1d). In site B, there were no significant differences between control and contaminated zones, but catalase activity in these areas was substantially higher than in the deposits (Figure 1d).

3.4. Extracellular enzyme activities

In general, potential extracellular activities followed a similar trend to intracellular enzymes with higher activity values outside than inside the deposits, where some enzymes were even inhibited (Figure 2). As a rule higher activity patterns were observed in control than in contaminated zones of site A, whilst similar or even higher enzymatic values were recorded in contaminated zones of site B compared with the control area (Figure 2).

No β-glucosidase activity was detected in the deposits. In site A, mean activity was between 2.5-10 fold higher in the control than in contaminated zones (Figure 2a). The lowest activity values were recorded in zone X_{A2} (Figure 2a). By contrast, enzymatic activity in contaminated zones of site B was about 10 fold larger than that in the control area (Figure 2a). No activity differences were recorded among contaminated zones in site B.

In contrast to β-glucosidase, chitinase activity was not inhibited in the deposits (Figure 2b). Nonetheless, the lowest activity values in both sites were recorded here. In site A, chitinase activity was highest in the control area, but differences with contaminated zones were not as inherent as for β-glucosidase (Figure 2b). In site B, chitinase activity in

contaminated zones was 2-4 times higher than in the control (Figure 2b). The highest values were recorded in zone X_{B1}.

There was no protease activity in deposit A and mean values in deposit B were extremely low (Figure 2c). In site A, protease activity in control was 4-38 fold higher than that in contaminated zones (Figure 2c). There were also significant differences among contaminated zones: potential activity in X_{A1} was more than twice than in the remaining zones (Figure 2c). In site B, however, there were no significant differences between control and contaminated zones (Figure 2c). Here, protease activity was more than 30 fold larger than in the deposit.

No acid-phosphatase activity was recorded in deposit B, while in deposit A, although low, enzymatic activity was similar to some of the contaminated zones (Figure 2d). In site A, the highest activity was recorded in the control area. Here, potential activity was 2.5-7 fold higher than in contaminated zones (Figure 2d). In contrast, in site B enzymatic response was larger in contaminated areas than in the control (Figure 2d), but no significant differences between contaminated zones were observed.

3.5. Microbiological ratios

The total DNA/total N ratio showed inherent differences between control, deposit and contaminated zones in the following order: deposit < contaminated < control (Figure 3a). Although there were no significant differences between contaminated zones in none of the sites, an increasing trend was observed from more contaminated to less contaminated zones in site B (Figure 3a).

Ratios related to respiration/total DNA, including those based on intracellular enzymes, were generally highest in the deposits followed by contaminated zones (Figures 3b, c and d). As a rule there were significant differences between controls, deposits and contaminated zones, but not among contaminated zones in the same site, except in the case of zone X_{B3},

where lower ratios were observed compared to the other contaminated areas (Figures 3b, c and d).

4. Discussion

4.1. Total DNA yield

The soil microbial biomass plays a decisive role in the cycling of nutrients, the degradation of organic compounds and other xenobiotics, and the immobilisation/release of trace elements (Nannipieri et al., 2002). Commonly, microbial biomass in soil is estimated by the chloroform fumigation-extraction method (Vance et al., 1987). However, as we determined the C_{mic} and N_{mic} contents of soil samples by this procedure abnormally elevated C_{mic}/N_{mic} ratios (above 20) were found in contaminated areas and the deposits (data not shown). Since soil microorganisms have typical ratios of 5-10, this bias seemed to be caused by the dissolution of hydrocarbons in chloroform. To have an estimation of the soil microbial biomass, we employed a well-known DNA extraction procedure (Griffiths et al., 2000). This approach is less time consuming than microscopic counting of microorganisms and gives an overall estimation of microbial biomass (bacteria, fungi and archaea). Although plant and animal material may be co-extracted, the highly positive correlations between total DNA yield and respiration and enzyme activities in both sites support the utilization of this approach in our study (all above 0.600 $p<0.01$; data not shown). Despite a period of 60 years since dumping of the waste, extraordinary low values of total DNA were found in the two deposits. Here, microbial colonization was likely to be limited by the extreme nature of the resin (acid, hydrophobic and enriched with contaminants). Outside the deposits, DNA yields differed between control and contaminated zones in site A, but not in site B. These results could be highly influenced by differences in natural organic matter between control and contaminated zones of site A, in contrast to their respective counterparts in site B. This hypothesis is based on the positive correlation between total DNA and total N (Tables 1 and 2), which can be

considered more indicative of the natural organic matter content in our soils than total organic C, since N was not a major component of the resin (Pérez-de-Mora et al., in press). The higher clay content of soil B could also account for higher DNA yields in contaminated zones of site B compared to those of site A, since microorganisms are mainly associated with the finer soil fractions (Kandeler et al., 2000). It should be noted that proliferation of hydrocarbon degrading communities or metal resistant populations in contaminated zones of site B could also contribute to higher biomass and hence DNA yields. Results also suggest that there was no clear effect of contaminants on biomass outside the deposits. At least, no strong negative correlations were reported (Tables 1 and 2). Furthermore, no patent effect of soil pH was observed on DNA yields in site A in spite of the acidity of the resin (Table 1). It is possible, however, that less acidic conditions in some contaminated parts of site B, could have stimulated microbial development. The buffering capacity of the soil (naturally acid) was apparently sufficient to attenuate the acidity of the resin quite effectively or else the acidity generated by the resin was no greater than that of the soil.

4.2. Soil basal respiration and enzyme activities

The basal respiration rate can reflect both the rate of mineralization of soil organic C and the activity of microorganisms (Giller et al., 1998). The lack or remarkably low respiration rate of the deposits stresses the severity of the resin as a habitat for microorganisms. The fact that some respiration was measured in deposit B (Figure 1b), suggests that some acidophilic microorganisms may be present here. Further studies should be carried out to find out why this did not happen in deposit A. Outside the deposits respiration rate was apparently influenced by other variables such as microbial biomass rather than by contaminant concentrations (Table 2). Such differences can be interpreted better when normalizing respiration rates through microbial biomass yields. This is further discussed in subsection 4.3.

Soil enzymes are considered to be sensitive indicators of contamination because of their role in organic matter cycling and regulation of nutrient pools (Visser and Parkinson, 1992). For this reason, we evaluated two intracellular enzymes such as dehydrogenase and catalase, which typically reflect general microbial activity in soil (García et al., 1997; Carmiña et al., 1998), and various extracellular enzymes involved in the cycling of C (β-glucosidase and chitinase), N (protease) and P (acid-phosphatase). Results from enzymatic tests were quite consistent with those of total DNA and basal respiration, showing that the resin had a clear negative effect on these properties in the deposits, but not in the surroundings. Here, the soil enzymatic response was more likely influenced by the amount of microbial biomass (total DNA) and natural organic matter (total N) rather than the degree of contamination. This is supported by the strong positive correlations between microbiological properties and total N, including enzymatic activities of the C and P cycles, and the lack of high negative correlations with contaminants in both sites (Tables 1 and 2). This would explain for instance why in contaminated zones of site B similar or even higher intra- and extra-cellular enzyme activities were recorded compared to the control. Soil organic matter plays a dual role as a source for enzyme production and energy reservoir and can therefore promote microbial activity and development. Furthermore, the higher natural organic matter and clay content of contaminated zones of site B compared to those of site A, could also enhance adsorption of extra-cellular enzymes with inorganic complexes or those associated with organic colloids. Such complexes are characterised by a marked resistance to thermal and proteolytic degradation and allows activities to persist in harsh conditions inhibiting microbial activity (Nannipieri et al., 2002). In addition, less acidic conditions in contaminated zones of part B, could also account for high enzymatic activity in this zone, since soil pH is crucial for enzymatic survival and functioning (Acosta-Martínez and Tabatabai, 2000).

4.3. Respiration and enzymatic ratios

The potential of absolute enzyme activities to respond to environmental stress such as pollutants has been questioned (Trasar-Cepeda et al., 2000). In agreement with this, we did not find a consistent response of biochemical properties to contaminant concentrations outside the deposits. Several authors have proposed that the limitations of individual biochemical properties may be overcome by using simple indicators such as the ratio between two biochemical properties (Aoyama and Naguno, 1997; Dalal, 1998).

One of these indices is the microbial biomass/total organic C ratio, which has been proposed as a useful indicator of soil pollution by heavy metals (Brookes, 1995) and organic matter turnover (Insam and Mershack, 1997). Due to the interferences observed with these two properties, we calculated an alternative ratio based on total DNA yields and total N estimations. As it is depicted in Figure 3a, higher ratios were observed in controls than in contaminated zones and, in turn, in contaminated zones than in the deposits. A higher ratio indicates that soil microorganisms can use organic matter more efficiently and thus environmental conditions are less stressful for microbial development (Spargling, 1992). Although the ratio was not able to discriminate significantly between different pollution levels in contaminated areas, negative correlations were observed for most contaminants, particularly in site B (Table 2). Here, the ratio augmented as distance from the deposit increased (Figure 3a).

Another simple indicator commonly used to evaluate microbial stress and soil disturbance is the basal respiration/soil microbial biomass ratio (qCO_2) (Insam and Domsch, 1988; Anderson and Domsch, 1993). The ratio is generally higher in distorted systems compared to stable systems, since survival under stress conditions requires additional energy, which cannot be utilized for growth (Haynes, 1999). As we did for biomass/total organic C, we calculated an alternative ratio based on basal respiration and total DNA yields. The ratio was significantly higher in all contaminated areas than in controls and correlated positively with many of the contaminants in both sites (Tables 1 and 2). Although no consistent response was

observed for deposit B in relation to affected zones, significant differences were observed between contaminated zones in both sites, with higher ratios in areas closer to the deposits (Figure 3b).

Enzyme/total DNA ratios were also calculated for intracellular and extracellular enzymes, but only those of intracellular activities (dehydrogenase and catalase) showed a consistent response in both sites (Figures 3c and d). This may be attributed to the fact that intracellular enzymes are closely related to microbial activity and respiration in contrast to extracellular enzymes, whose activity is usually independent from the state of the organism (active, inactive or dead) that produces it (Nannipieri et al., 2002). Both ratios decreased significantly following the order deposit > contaminated zones > control. For site B, both ratios were significantly lower in X_{B3} than in other contaminated zones and similar to those found in control (Figures 3c and d).

Some authors have proposed the use of complex indicators involving various biochemical properties to estimate changes in soil quality (Beck, 1984; Stefanic, 1994; Trasar-Cepeda et al., 2000). However, there is no consensus at present among soil scientists about a universal indicator that may be used in all situations, probably because of the complexity of many soils, particularly of multi-element contaminated sites, where many biotic and abiotic factors interact. As proposed by Nannipieri et al., (2002) we tried to assess changes in soil quality by means of various microbiological and biochemical properties and the utilization of simple ratios. With this approach we could show that: a) acid resins are toxic wastes for soil microorganisms, b) there is a higher stress for microbial populations in contaminated areas compared to controls, and c) outside the deposits, soil functional diversity seems to be more related to biological and abiotic properties such as microbial biomass and total N content rather than to contaminant concentrations.

5. Conclusions

Acid resin deposits are extreme habitats offering little chances for microbial colonization. The contamination of surrounding areas as a result of contaminant release and transport of acid resin fragments had a negative effect on soil microbial populations, decreasing organic matter turnover and metabolic efficiency. Nonetheless, the natural attenuation potential of developed systems such as forests, as reflected by its natural organic matter and other soil constituents, can buffer the toxicity of such wastes maintaining soil functionality even at extreme levels of contamination. Given the toxicity of such wastes and the degree of contamination of surrounding areas, deposits should be ideally isolated or removed to prevent further deterioration of these sites, as long as it is technically possible. Depending on end-use strategies and transport of contaminants to groundwater, natural attenuation mechanisms may be an economic and feasible option for affected zones.

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Caption of figures

Figure 1. Mean values and standard errors of a) total DNA, b) soil basal respiration, c) dehydrogenase and d) catalase. Columns with the same letter do not differ significantly, $P \leq 0.01$. Units are referred to dry weight of soil.

Figure 2. Mean values and standard errors of a) β-glucosidase, b) chitinase, c) protease and d) acid-phosphatase. Columns with the same letter do not differ significantly, $P \leq 0.01$. Units are referred to dry weight of soil.

Figure 3. Mean values and standard errors of simple microbiological ratios; a) total DNA/total N, b) basal respiration/total DNA, c) dehydrogenase/total DNA and d) catalase/total DNA. Columns with the same letter do not differ significantly, $P \leq 0.01$. Units are referred to dry weight of soil.

Figure
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Figure 1

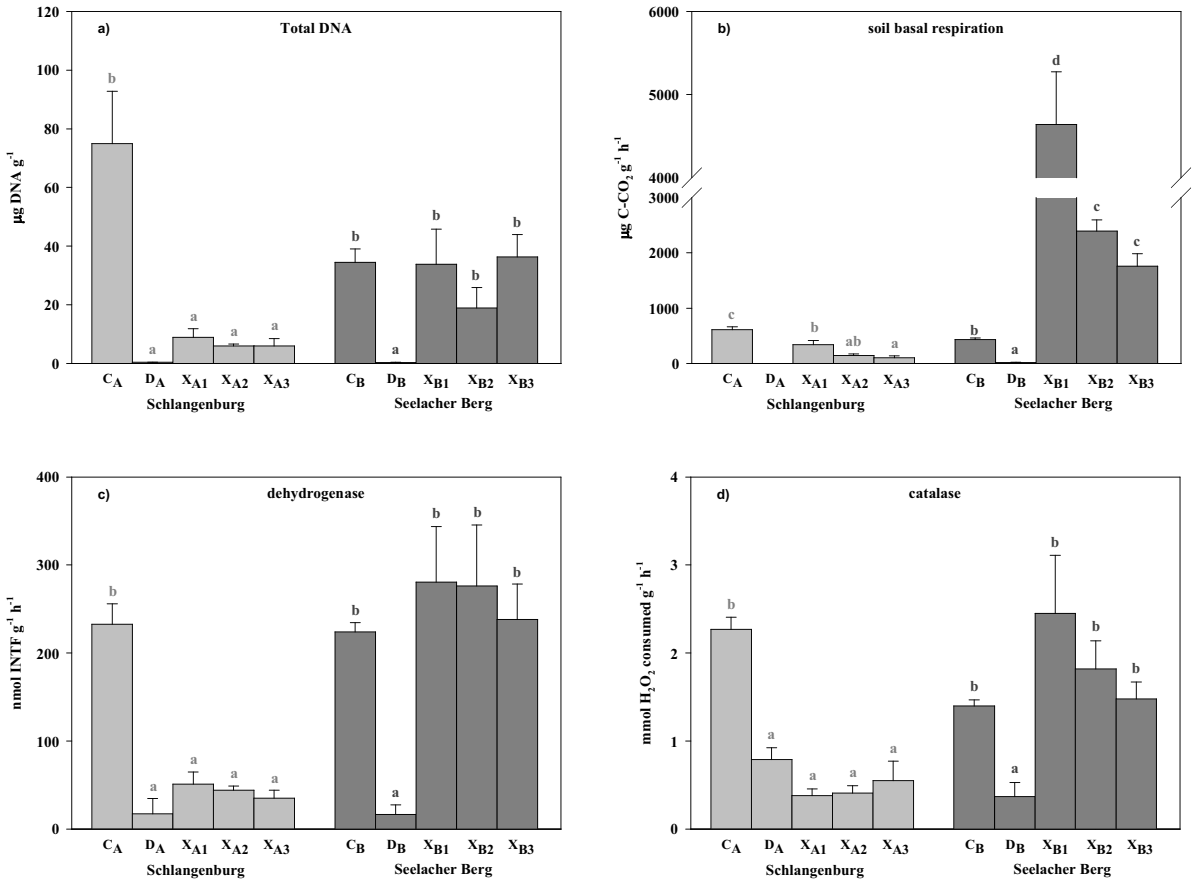


Figure 2

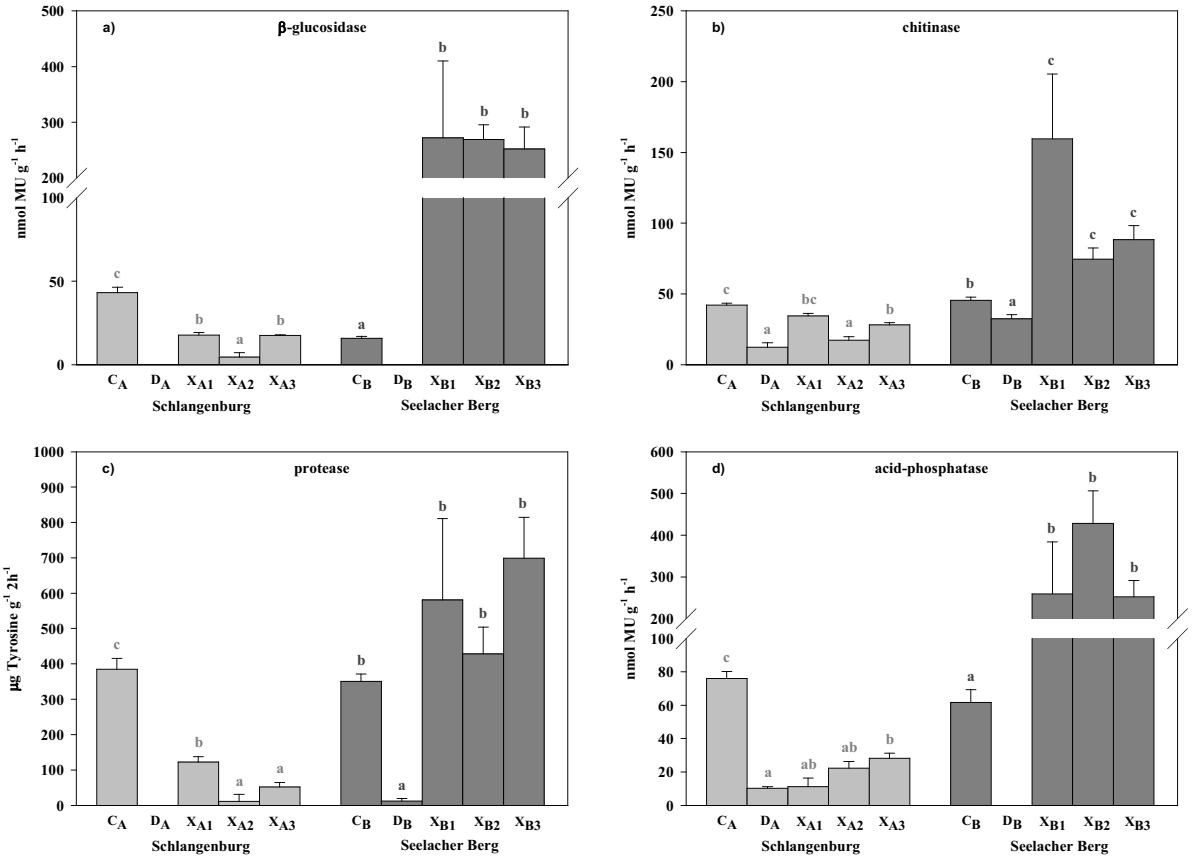
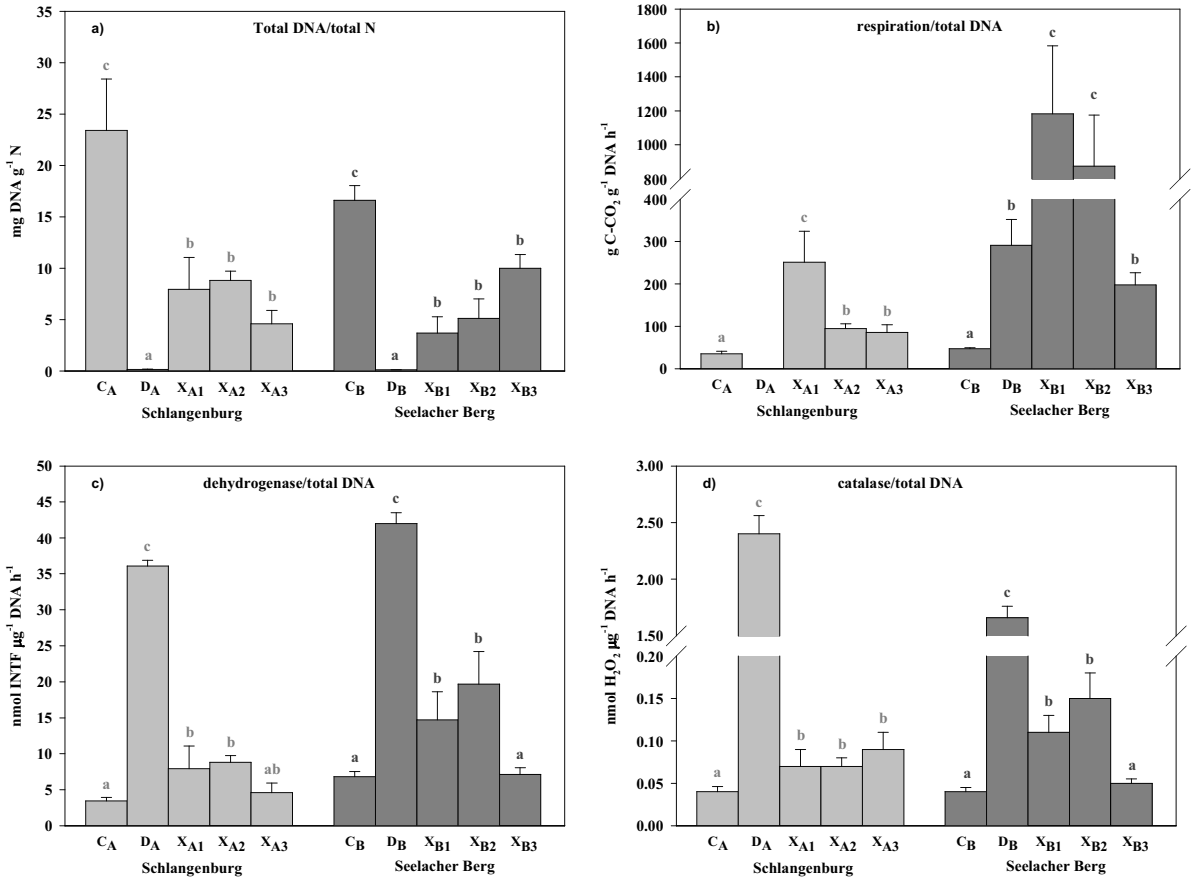

a)
β-glucosidase
nmol MU g⁻¹ h⁻¹
b)
chitinase
nmol MU g⁻¹ h⁻¹
c)
protease
µg Tyrosine g⁻¹ 2h⁻¹
d)
acid-phosphatase
nmol MU g⁻¹ h⁻¹
C_A D_A X_A1 X_A2 X_A3 C_B D_B X_B1 X_B2 X_B3
Schlangenburg
Seelacher Berg

Figure 3


a)
Total DNA/total N
mg DNA g-1 N
b)
respiration/total DNA
g C-CO2 g-1 DNA h-1
c)
dehydrogenase/total DNA
nmol INTF µg-1 DNA h-1
d)
catalase/total DNA
nmol H2O2 µg-1 DNA h-1
CA DA XA1 XA2 XA3 CB DB XB1 XB2 XB3
Schlangenburg
Seelacher Berg

Table

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Table 1. Pearson´s correlations between chemical and microbiological properties in site A (N = 20).

	Site A (Schlangenburg)											
	DNA	**resp**	**deh**	**cat**	**β-glu**	**chit**	**prot**	**a-pho**	**resp/ DNA**	**deh/ DNA**	**cat/ DNA**	**DNA/ TN**
pH	-.035	-.537*	-.093	-.034	-.234	-.562**	-.195	.009	-.758**	-.272	-.183	.051
TOC	.406	.810**	.550*	.593**	.615*	.653*	.658**	.415	.322	-.160	-.029	.214
TN	.690**	.794**	.807**	.919**	.879**	.702**	.867**	.799**	-.177	-.423	-.175	.419
Ex-C	.337	.776**	.462*	.428	.471*	.644**	.443	.419	.657**	.314	.209	.173
Ex-N	.483*	.849**	.600**	.538*	.629**	.749**	.576**	.528*	.520*	.178	.060	.305
Ex-P	-.470*	-.606**	-.570**	-.366	-.573**	-.604**	-.628**	-.476*	-.092	-.044	.470*	-.394
HC	-.308	-.165	-.275	-.398	-.547*	-.552*	-.329	-.389	.238	.261	-.021	-.130
As	.037	-.058	.048	.325	.168	-.013	-.047	.162	-.286	-.280	.376	-.088
Cd	-.223	-.308	-.244	-.008	-.105	-.187	-.374	-.097	-.094	.016	.539*	-.289
Cu	-.313	-.142	-.282	-.111	.001	.092	-.318	-.191	.290	.262	.696**	-.425
Pb	-.158	.313	-.110	-.181	-.012	.382	.003	-.325	.707**	.277	.151	-.131
S	-.237	.290	-.182	-.201	-.120	.242	-.140	-.286	.894**	.490*	.428	-.257
Zn	-.101	.392	.018	.124	.240	.490*	.038	.038	.704**	.427	.511*	-.260
Ex-As	-.122	-.184	-.143	-.112	-.211	-.202	-.131	-.035	-.058	-.018	.118	-.048
Ex-Cd	-.197	.350	-.101	-.129	-.058	.244	-.085	-.072	.939**	.647**	.440	-.258
Ex-Cu	-.207	.289	-.146	-.194	-.101	.248	-.153	-.161	.963**	.687**	.473*	-.259
Ex-Pb	-.215	.287	-.198	-.263	-.079	.310	-.067	-.323	.801**	.315	.186	-.213
Ex-S	-.208	.302	-.154	-.198	-.115	.234	-.143	-.174	.958**	.629**	.456*	-.254
Ex-Zn	-.166	.384	-.085	-.110	-.015	.324	-.052	-.091	.939**	.594**	.418	-.235

a-pho = acid-phosphatase; β-gluc = β-glucosidase; cat = catalase; chit = chitinase; deh = dehydrogenase; Ex = extractable; HC = total hydrocarbons; pro = protease; resp = respiration; TN = total N; TOC= total organic C.
*$P \leq 0.05$; **$P \leq 0.01$.

Table 2. Pearson´s correlations between chemical and microbiological properties in site B (N = 20).

	Site B (Seelacher Berg)											
	DNA	**resp**	**deh**	**cat**	**β-glu**	**chit**	**prot**	**a-pho**	**resp/ DNA**	**deh/ DNA**	**cat/ DNA**	**DNA/ TN**
pH	.468*	.044	.486*	.250	.577**	.221	.630**	.606**	-.396	-.373	-.449*	.212
TOC	-.039	.796**	.026	.205	.148	.445*	.058	.031	.721**	.385	.298	-.565**
TN	.363	.950**	.408	.699**	.619**	.839**	.461*	.406	.325	.124	.223	-.507**
Ex-C	-.012	-.147	.112	.212	-.230	-.126	-.284	-.128	-.209	-.067	.082	.149
Ex-N	.316	-.101	.530*	.463*	.148	.052	.101	.268	.536*	-.362	-.208	.386
Ex-P	.300	.466*	.513*	.568**	.672**	.552*	.488*	.588**	-.214	-.145	-.019	-.300
HC	-.233	.530*	-.173	-.119	-.150	.105	-.166	-.166	.805**	.456*	.266	-.449*
As	.079	.865**	.130	.545*	.447*	.739**	.210	.217	.395	.220	.396	-.625**
Cd	.173	.734**	.266	.635**	.764**	.756**	.441	.633**	.231	.297	.529*	-.593**
Cu	-.142	.678**	-.103	.047	.023	.293	-.038	-.041	.813**	.479*	.345	-.531*
Pb	-.188	.588**	-.146	-.060	-.087	.173	-.108	-.119	.821**	.477*	.296	-.470*
S	-.132	.749**	-.070	.134	.068	.378	-.034	-.041	.767**	.440	.360	-.583**
Zn	.379	.718**	.334	.704**	.757**	.835**	.563**	.585**	.140	.143	.338	-.320
Ex-As	-.199	.440	-.078	.245	.000	.290	-.233	-.156	.189	.068	.290	-.423
Ex-Cd	-.446*	.486*	-.275	.108	.003	.200	-.359	-.114	.543*	.530*	.747**	-.743**
Ex-Cu	-.207	.305	-.096	.203	-.044	.139	-.255	-.160	.186	.126	.317	-.388
Ex-Pb	-.294	.443	-.133	.096	-.185	.146	-.344	-.298	.433	.257	.318	-.498*
Ex-S	-.337	.560*	-.169	.184	.030	.285	-.267	-.102	.519*	.463*	.622**	-.678**
Ex-Zn	-.374	.560*	-.229	.173	-.102	.308	-.260	-.037	.529*	.529*	.734**	-.737**

a-pho = acid-phosphatase; β-gluc = β-glucosidase; cat = catalase; chit = chitinase; deh= dehydrogenase; Ex = extractable; HC = total hydrocarbons; pro = protease; resp = respiration; TN = total N; TOC= total organic C.
*$P \leq 0.05$; **$P \leq 0.01$.