Ecotoxicology, 15, 187–197, 2006

DOI: 10.1007/s10646-005-0050-4

Functional Stability of Microbial Communities in Contaminated Soils Near a Zinc Smelter (Budel, The Netherlands)

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Accepted 9 August 2005/Published online 24 January 2006

Abstract. Environmental pollution causes adverse effects on many levels of ecosystem organization; it might affect the use efficiency of available resources which will make the system more sensitive to subsequent stress. Alternatively the development of community tolerance may make the system more resistant to additional stresses.

In this study we investigate the functional stability, measured in the terms of resistance and resilience, of microbial populations inhabiting contaminated soils near a zinc smelter. With functional stability we mean that we look at processes rather than at population dynamics. We measure changes in respiration and bacterial growth rate in response to addition of stress (lead, salt) or disturbance (heat). We used soils that differ in the level of pollution with zinc and cadmium originating from an adjacent smelter.

Our results showed, with regard to respiration, that the most polluted soils have the lowest stability to salt (stress) and heat (disturbance). This confirms the hypothesis that more stressed systems have less energy to cope with additional stress or disturbance.

However, bacterial growth rates were affected in a different way than respiration. There was no difference between the soils in resistance and resilience to addition of lead. In case of salt treatment, the least polluted soils showed highest stability. In contrast, the least polluted soils were the least stable to increased temperature, which supports the hypothesis that more stressed soils are more stable to additional stress/disturbance due to properties they gained when exposed to the first stress (pollution by the smelter).

Thus, the responses of microbial processes to stress, their nature and size, depend on the kinds of stress factors, especially whether a subsequent stress is similar to the first stress, in terms of the mechanism with which the organisms deal with the stress.

Keywords: stress; disturbance; functional stability; microbial community; respiration; growth rate

Introduction

Emission of toxic substances from metal smelters not only causes air pollution but also soil contamination. These substances constitute stress for soil ecosystems; they affect the physiology of individual organisms, the community structure and community tolerance (Bååth, 1989; Calow, 1991; Pennanen et al., 1996; Bååth et al., 1998b; Kelly and Tate, 1998; Díaz-Raviña and Bååth, 2001). Stress affects the functioning of an organism

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by changing its energy allocation. Detoxification and reparation of damages increase the costs of maintenance. In non-stressed environments maintenance represents more than 80% of organisms' energy use (Congdon et al., 2001). An increase of energy required for maintenance will cause a decrease of energy available for growth and reproduction (Sibly and Calow, 1989). Therefore growth and reproduction are considered to be very sensitive to stress (Bloem and Breure, 2003).

The environmental stress may change the community structure of microbial populations. Stress negatively affects the more sensitive species and decreases their competition ability. Therefore, the more resistant species become more abundant. Many authors showed that environmental stress e.g. metals or organic contaminants can provoke such changes in community structure and successively cause development of community tolerance (Frostegård et al., 1996; Pennanen et al., 1996; Bååth et al., 1998a, b; Kelly et al., 1999; Macnaughton et al., 1999; Gong et al., 2000; Witter et al., 2000).

Exposure to one type of stress may cause a development of community tolerance towards this particular stress but may also cause a development of co-tolerance to another type of stress or disturbance (Díaz-Raviña et al., 1994; Vinebrooke et al., 2004). This happens when detoxification of different stresses relies on similar physiological processes (Giller et al., 1998; Bruins et al., 2000). However, when another way of detoxification is required an organism will have to develop or generate new mechanisms of detoxification which will demand extra energy. Such additional burden may affect the energy budget and functioning of the organism. Each species has a different way of managing its energy budget and thus the response of an entire community to stress is the sum of all individual reactions. The sum of such alterations, together with effects of stress on interactions between organisms and between organisms and their environment, will bring changes in the community and finally in ecosystem functioning. Such effects can be measured as changes in the rates of processes like growth and respiration.

The knowledge that stress alters soil communities raises the question: how stressed communities, for example in long term polluted ecosystems, will react to a next stress or disturbance? Are stressed communities less or more stable to environmental stress or disturbances than not-stressed ones?

Griffiths et al. (2000) defined stability as the ability of the system to withstand disturbance and maintain its normal state. Such defined stability has two measurable components: resistance – which is the inherent capacity of the system to withstand stress/disturbance, and resilience – which is the ability to recover after stress/disturbance.

To define "stress" and "disturbance" we use the classification of Degens et al. (2001). They characterize stress as an environmental condition (chemical, physical or biological) that has a continuous adverse impact on microbial communities. Disturbance they define as an event constrained in time which nevertheless may have a lasting positive or negative impact on microbes.

The effect of stress on functional stability can be measured by "stress on stress" (or "disturbance on stress") experiments (Griffiths et al., 2000, 2001a, b). In this type of experiments a stressed (polluted) system is subjected to additional stress or disturbance and the systems' response (change in process rate) is measured at different time intervals following the application of stress/disturbance. To assess the resistance and resilience of the stressed system, its process rates are compared to the rates in a non-stressed control.

There are two contrasting hypotheses regarding the stability of ecological processes. The first predicts that non-stressed systems are more stable because they possess larger energetic resources than stressed communities that allow them to maintain function in case of stress (Naeem and Li, 1997; Aarts and Nienhuis, 1999; Loreau, 2000; Stone et al., 2001). The other hypothesis predicts that stressed systems are more stable since due to the first stress they gained abilities (adaptation, physiological changes) to cope with additional stress and thus maintain function (Odum, 1981).

Until now stability measurements were performed mostly on agricultural soils in which the first stress was strongly controlled in experimental set ups. These soils were either stressed by fumigation (Griffiths et al., 2000), underwent reduced biodiversity due to dilution (Griffiths et al., 2001b) or were stressed with elevated copper concentration and/or low pH (Tobor-Kapłon et al., 2005, submitted). In such experiments, beside differences in the applied stresses/disturbances, soils are treated

in the same way e.g. they receive the same external input of nutrients, are usually situated very close to each other so there is no influence of spatial variation in soil characteristics. This is usually not the case in uncontrolled field situations. To our knowledge, there has been hardly any research done on stability of field soils exposed to uncontrolled stress (pollution). Such work could show whether the functional stability of polluted field soils show similar results as experiments performed with experimental soils.

In the present study we investigate functional stability of soil microbial communities from forest soils that have been exposed for a long time to increased concentrations of zinc and cadmium originating from an adjacent zinc smelter. This stress could affect the functioning of soil microbial communities in terms of their physiology, community structure and tolerance. With functional stability we mean that we look at processes rather than at population dynamics. We measured changes in the soil respiration which is assumed to be mainly due to bacteria. Respiration reflects decomposition of organic matter and is a major ecological process. We also measure the changes in bacterial growth rate, which is a more specific and very sensitive parameter. The microbial community in these soils is dominated by bacteria (Koopmans et al., 1993). To allow comparison between the results of the present study and former ones (Tobor-Kapłon et al., 2005, submitted) we have chosen for stresses and disturbance as used in our previous work i.e. lead, salt and heat.

Materials and methods

Description of area and sampling

The sampling area is situated in the south of Netherlands in the vicinity of a zinc smelter in Budel. The first factory was established there in 1892. In 1973 for environmental reasons a new cleaner technology was introduced. Nowadays the smelter produces 230,000 tons zinc per year.

Sampling sites were situated along a gradient of pollution going from the smelter to the North-East. All sites were covered by Scotch pine (*Pinus silvestris* L.). Samples were collected from three locations that were situated at intervals of approx. 1, 2 and 6 km from the smelter (further referred to as soils A, B, and C). The locations have a similar soil structure: sandy with approximately 2.5% of clay (Koopmans et al., 1993). Samples were taken from the upper 10 cm of the soil mineral layer (litter was removed). On either location four replicate plots were sampled. From each replicate 15 soil cores, 4 cm in diameter and 10 cm depth were taken and then mixed and sieved (0.5 cm mesh).

Preparation of samples

After sieving, field moist soil from each plot was divided into subsamples of approx. 250 g and each of them was placed in a 300 ml plastic jar. The samples were divided into four sets, each containing four samples. One set was treated with lead nitrate (1000 mg Pb kg^{-1} dw), the second was treated with sodium chloride (6.67 g NaCl kg^{-1} dw), the third was heated for 18 h at 50 °C and the fourth one served as a non-stressed control. After treatment samples were kept in darkness at a temperature of 20 °C until measurement. From each jar samples for respiration, bacterial growth rate measurement and chemical analyses were taken.

Respiration

Twenty four hours before each measurement 200 g of soil from each jar was placed into ± 600 ml glass bottles and dry lucerne meal (alfalfa (*Medicago sativa*)) was added and mixed thoroughly. Lucerne was added in amounts corresponding to 3.2 g C kg^{-1} soil, which was sufficient to induce respiration and offset nutrient limitation. Without substrate addition the respiration is usually too low to measure significant differences within 24 h. Carbon dioxide production was measured with a GC apparatus (Carlo ERBA Instruments). For the first measurement samples were prepared as described above directly after application of stress and termination of disturbance.

Bacterial growth rate

Bacterial growth rate was measured as the rate of incorporation of radioactively labelled thymidine into bacterial DNA, according to the microcentrifugation method described by Bååth et al.

(2001). Thymidine is incorporated only by bacteria and not by eukaryotes like fungi.

Measurement was performed as follows: 5 g of soil was shaken with 40 ml of milliQ-water in plastic tubes, then centrifuged and filtered through glass wool. Supernatants (1.5 ml) were decanted into eppendorf tubes. Samples were incubated for 2 h at 20 °C with 3.75 μl radioactively labelled thymidine (methyl[^{3}H]thymidine, 925 GBq mmol^{-1}, Amersham, U.K) diluted1:3 with milliQ-water. Incorporation, washing of excess tracer, and measurement of radioactivity incorporated by actively growing bacterial cells are described in detail by Bååth et al. (2001). Radioactivity was measured using a Beckman liquid scintillation spectrometer. Because a well established conversion factor is lacking for this method, the results are expressed in DPM units (decays per minute). DPM values are proportional to the amount of cells produced during 2 h of incubation.

Functional stability

For stability analysis we used the approach of Griffiths et al. (2000). Due to absolute differences in the investigated parameters (respiration rate and bacterial growth rate) between soils and sampling dates, resistance was normalised to the value of the control (soils without addition of stress or disturbance), i.e.

Resistance = % change from control = $[(CO_{2\ treated} - CO_{2\ contr.})/CO_{2\ contr.}] \times 100\%$

All measurements were done four times: 24 h, 2 weeks, 1 and 2 months after disturbance. The response after 24 h was used as a measure of resistance, while the change over time was adopted as a measure of resilience. The overall effect of a stress/disturbance was the average response over the time of the experiment.

Chemical analyses

For each measurement, 10 g samples were placed into 50 ml plastic tubes. Samples were shaken (30 rev min^{-1}) with 40 ml of 0.01 M $CaCl_2$ for 48 h. After shaking, pH was measured with a pH-meter (Φ 34 Beckman). Tubes with soil slurry were centrifuged at 3000 rev min^{-1} for 10 min; samples were filtered through a 0.45 μm syringe-filter (Total Organic Carbon free). Measurement of heavy metal concentrations was performed using an ICP-AES (Parkin Elmer Optima 3300 DV).

Soil acidity (pH-KCl) was measured only once in non-treated samples. Aliquots of field moist soil (10 g) were placed into plastic tubes and 50 ml of 1 M KCl was added. Samples were shaken for 3 h on the shaker at 180 rev min^{-1}. After shaking, pH was measured with a pH-meter (Φ 34 Beckman).

Dry mass was measured as a loss of weight after drying at 105 °C for 24 h, whereas organic matter content was determined as a loss on ignition (550 °C, 6 h).

Statistical analysis

For analysis of resistance to stress and disturbance (response 24 h after stress addition) we used the Univariate General Linear Model (SPSS) procedure with site as fixed factor. For analyses of resilience and overall effect, using all four time points (24 h, 2 weeks 1 and 2 months), we used also the Univariate General Linear Model procedure with full factorial design with site and time as fixed factors. All analyses were performed using SPSS 11.5.0 statistical software. Because the results of respiration of control soils were not normally distributed a log-transformation was applied.

Results

Soil properties

The concentration of zinc was highest in soils from site A (1 km distance from the smelter) and lowest in site C soils (6 km from the smelter) ($p < 0.0001$). Similarly, the concentrations of cadmium were different at each site and were lowest on location C and highest on location A ($p < 0.0001$). The concentration of lead was significantly lower in site C soils than in the soils from the two other sites ($p < 0.0001$) (Table 1).

The soils differed significantly in pH (KCl) ($p < 0.0001$) and organic matter content ($p < 0.0001$). The most acid were the C soils with pH 3.0 whereas A soils had a pH of 3.9. Highest organic matter content was found in C soils ($8.3 \pm 0.9\%$) and lowest in A soils ($3.0 \pm 0.3\%$) ($p < 0.0001$).

Although the same amount of $Pb(NO_3)_2$ (1000 mg Pb kg^{-1} dw) and NaCl (6.67 g kg^{-1})

Table 1. Soil acidity, organic matter content and bioavailable concentrations of heavy metals, (expressed in mg kg^{-1} organic matter and in mg l^{-1} of 0.01 M $CaCl_2$ extract) in not treated soils

			pH (0.01 M $CaCl_2$)		pH (1 M KCl)		Zn		Cd		Pb		Na		Organic matter	
	Site	N	Mean	S.E.M	Mean	S.E.M	Mean	S.E.M	Mean	S.E.M	Mean	S.E.M	Mean	S.E.M	Mean	S.E.M
mg kg^{-1} o.m	A	16					1297	273 A	8.42	0.82 A	90.4	9.14 A	496	203 A	30.0	2.67 A
	B	16					533	35.4 B	6.88	0.86 A	88.9	5.99 A	199	8.36 A	32.5	3.13 A
	C	16					185	10.6 C	2.60	0.14 B	29.5	3.25 B	115	7.27 B	82.5	9.40 B
mg l^{-1}	A	16	3.76	0.03 A	3.86	0.08 A	8.97	2.05 A	0.06	0.01 A	0.57	0.06 A	3.04	1.17 A		
	B	16	3.77	0.01 A	3.82	0.02 A	3.99	0.35 B	0.05	0.01 A	0.62	0.02 A	1.42	0.05 A		
	C	16	3.09	0.02 B	3.04	0.05 B	3.24	0.20 B	0.05	0.00 A	0.48	0.03 B	2.00	0.11 A		

Means marked with different letters are statistically different (Tukey's HSD, $p < 0.05$)

was added to each soil, the interplay between metals, soil pH and organic matter content caused changes in the concentrations of the bioavailable fraction of lead and sodium (Table 1 & 2). Thus the available doses of applied stress were different among treatments. The concentrations of heavy metals (Pb, Zn and Cd) remained on the same level over time following addition of stress.

Primary stress (contamination by the smelter)

The respiration rate was significantly different between the sites ($p = 0.003$; calculated on the basis of log-transformed data) and was higher in the C soils than in the other ones. The original values of soil respiration were: 8.8 ± 3.1 in A soils, 6.2 ± 0.3 in B and 10.1 ± 0.5 mg CO_2 kg^{-1} h^{-1} in C soils.

In contrast to respiration rate, [^{3}H]thymidine incorporation (indication of bacterial growth rate) was not significantly different between the sites ($p = 0.2$). The incorporation rates (DPM incorporated during 2 h of incubation) were: $1.4 \pm 0.1 \times 10^4$ in A, $1.3 \pm 0.1 \times 10^4$ in B and $1.1 \pm 0.1 \times 10^4$ DPM in C soils.

Secondary stress (applied in pot experiments)

Effects of lead (stress)

Respiration. The different sites showed no significant difference in the resistance of respiration to lead ($p = 0.12$). ANOVA showed significant ($p = 0.03$) recovery of respiration, from on average $7.4 \pm 5.7\%$ reduction 1 day after stress addition to $23.7 \pm 11.1\%$ increase 2 months later. There was no difference in the recovery between the soils ($p = 0.2$) (Fig. 1a).

Bacterial growth rate. The bacterial growth rate was greatly reduced in all soils after stress addition. No difference in the resistance of bacterial growth between soils was found. There was no effect of time nor interaction between time and site ($p = 0.2$

Table 2. Acidity and bioavailable concentrations of heavy metals (expressed per liter of 0.01 M $CaCl_2$ extract) in soils to which stress, in the form of lead (1000 mg Pb kg^{-1}) or salt (6.67 g NaCl kg^{-1}) was added (averages of all time points)

			pH (0.01 M $CaCl_2$)		Zn mg l^{-1}		Cd mg l^{-1}		Pb mg l^{-1}		Na mg l^{-1}	
Stress	Site	N	Mean	S.E.M	Mean	S.E.M	Mean	S.E.M	Mean	S.E.M	Mean	S.E.M
Lead	A	16	3.69	0.01 A	8.18	1.48 A	0.07	0.01 A	60.9	5.71 A	1.32	0.09 A
	B	14	3.73	0.02 A	4.55	0.41 B	0.07	0.01 A	56.2	5.68 A	1.33	0.05 A
	C	16	3.05	0.02 B	3.56	0.19 B	0.05	0.00 A	36.7	4.48 B	2.13	0.28 B
Salt	A	16	3.82	0.01 A	7.09	1.17 A	0.07	0.01 A	0.83	0.03 AB	588	6.06 A
	B	16	3.90	0.04 A	7.39	3.44 A	0.06	0.01 A	0.72	0.03 AB	572	7.89 AB
	C	16	3.14	0.02 B	3.06	0.17 A	0.05	0.00 A	0.60	0.05 BC	510	35.4 BC

Means marked with different letters are statistically different (Tukey's HSD, $p < 0.05$).

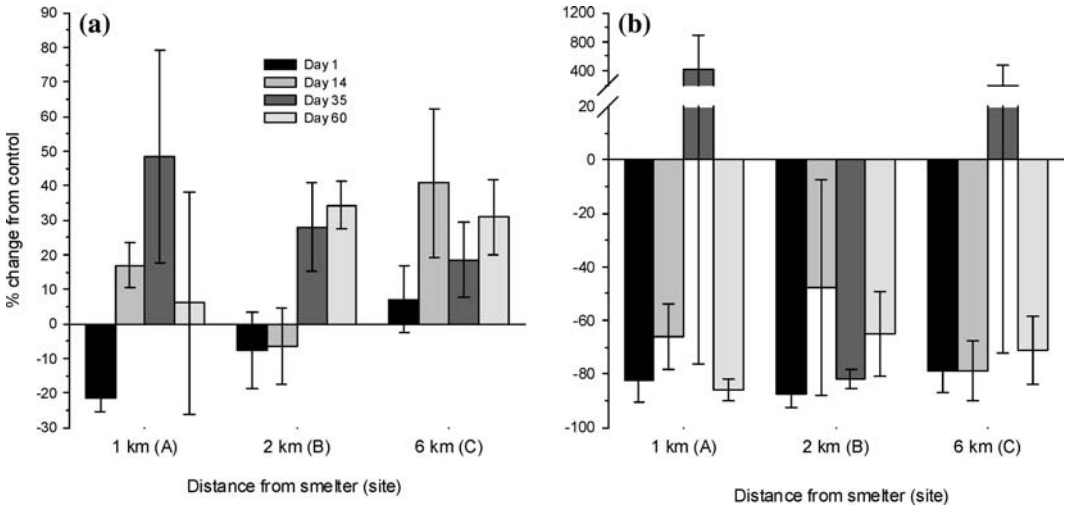

Figure 1. Effect of stress (lead) at increasing time intervals following application of stress (mean ± SEM) on (a) the ability of soil microbial communities to decompose lucerne (alfalfa) residues and (b) on the growth rate of bacteria. The soils at increasing distance from a zinc smelter differ in level of pollution.

and $p = 0.7$ respectively). Neither was the effect of site significant ($p = 0.8$). The results of effects of lead on bacterial growth rate should be taken with caution since extremely high variation was found in the measurements of day 35 (Fig. 1b).

Effects of salt (stress)

Respiration. Salt treatment caused a reduction of respiration in all soils. The soils from the least contaminated site (C) were, initially, significantly more resistant than the soils from the most polluted site (A) (15.8 ± 15.3% versus 57.1 ± 3.5% reduction for sites C and A, respectively) ($p = 0.03$). Soils with different levels of contamination differed in the overall effect of salt on their respiration ($p = 0.002$). The most affected were soils from site A (40.1 ± 7.8% reduction) whereas respiration at the most remote site (C) was hardly affected (on average 1.1 ± 8.2% increase). There was no recovery from the salt stress ($p = 0.1$). The interaction between time and site was also insignificant ($p = 0.9$) (Fig. 2a).

Bacterial growth rate. There were no differences in resistance between soils from different sites ($p = 0.9$). In all cases salt reduced bacterial growth rate by approx. 90%. The statistically significant effect of time ($p = 0.01$) suggests that bacterial growth rates were able to recover from this stress. The interaction of time and site was not significant ($p = 0.3$), which means that the recovery was statistically not different among soils (Fig. 2b).

Effects of heat (disturbance)

Respiration. The resistance of the investigated soils to heat (disturbance) was different between sites ($p = 0.03$); the strongest resistance was in the cleanest soils (C: 48.9 ± 2.4% reduction) and the weakest resistance was in the most polluted one (A: 71.9 ± 20.7% reduction). The highly significant effect of time ($p < 0.0001$) indicates the resilience of respiration going from on average from 62% reduction after 1 day to 61% increase after 2 months from disturbance) (Fig. 3a).

Bacterial growth rate. The soils did not differ in the resistance of bacterial growth rate to heat ($p = 0.6$). The significant interaction between time and site ($p = 0.02$) indicates different patterns of recovery between the soils; the bacterial growth rates in C soils recovered most rapidly and strongly overshoot the control values (Fig. 3b).

Comparison of effects of different stresses

Respiration. Analysis of variance showed that there was significant difference between resistance (effect on day 1) to the different stresses and the

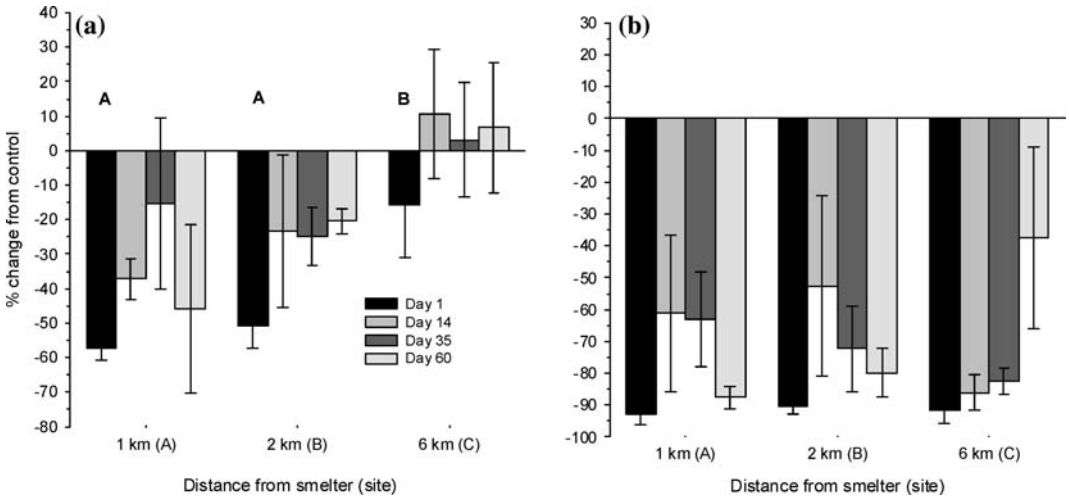

Figure 2. Effect of stress (salt) at increasing time intervals following application of stress (mean ± SEM) on (a) the ability of soil microbial communities to decompose lucerne (alfalfa) residues and (b) on the growth rate of bacteria. The soils at increasing distance from a zinc smelter differ in level of pollution. Bars representing resistance (day 1) and marked with different letters are significantly different (Tukey's HSD, $p < 0.05$).

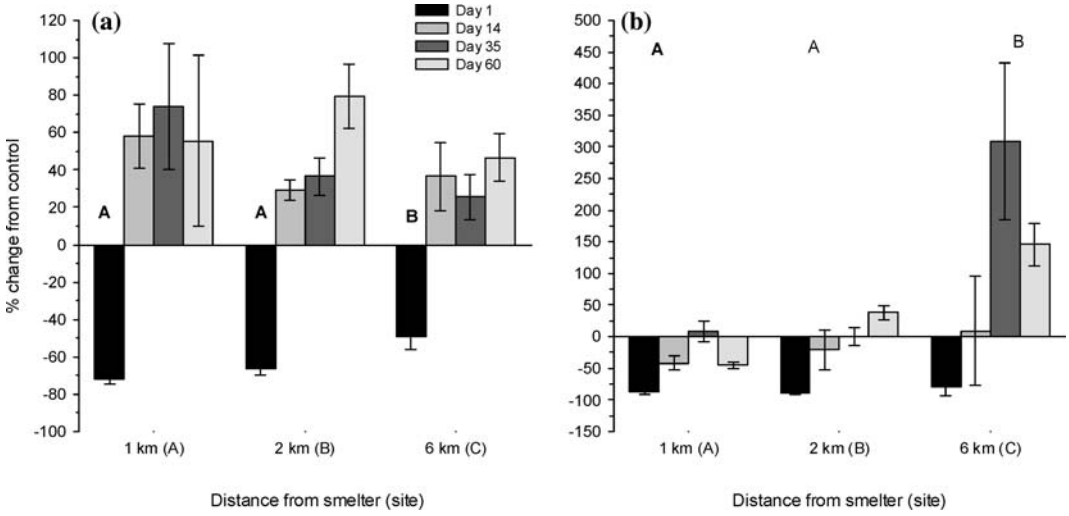

Figure 3. Effect of disturbance (heat) at increasing time intervals following application of stress (mean ± SEM) on (a) the ability of microbial communities to decompose lucerne (alfalfa) residues and (b) on the growth rate of bacteria. The soils at increasing distance from a zinc smelter differ in level of pollution. Bars representing resistance (day 1) (Fig 3a) and marked with different letters are significantly different (Tukey's HSD, $p < 0.05$). Sites marked with different letters (Fig 3b) have a different pattern of recovery (Tukey's HSD, $p < 0.05$).

disturbance ($p < 0.0001$). Respiration was most resistant to lead and least resistant to heat. Considering the average effect of the whole incubation period, salt was the only treatment that imposed a negative effect on respiration rate. In contrast, the two other treatments despite initial decrease of respiration caused on average an increase of this process. The effect of treatment (lead, salt or heat) was different at each site ($p = 0.04$) and recovery followed different patterns in different treatments ($p < 0.0001$) and at different sites ($p = 0.07$).

Bacterial growth rates. The bacterial growth rate was less resistant to the applied treatments than respiration. Salt had the strongest effect on the average response of bacterial growth ($p < 0.0001$). Caution, however should be taken when comparing the effect of lead with the other treatments. The extremely high variation of the day 35 might obscure the effect of this treatment.

Discussion

The aim of this study was to examine the functional stability of microbial populations inhabiting soils that differ in level of pollution caused by heavy metals. Aerial deposition of Zn, Cd and Pb emitted from the adjacent smelter caused an increase in heavy metal concentrations in the investigated soils. The highest concentrations were observed in the A soils (site closest to the smelter) and the lowest in C soils (the most remote site). Heavy metals cause, usually, an inhibition of decomposition of organic matter, which leads to an increase of the organic matter layer in contaminated areas (Bååth, 1989; Giller et al., 1998). Here we have, apparently, a reversed situation; the organic matter content was the highest in the most remote area, which was the least contaminated by the heavy metals. It has to be mentioned that we used soils from the mineral horizon after removal of the litter layer. Increased organic matter content in the mineral layer may indicate that the soil fauna inhabiting this environment is more active and thus able to transfer more organic material into the soil profile. This difference might also be due to the natural spatial variation, partly dependent on (historical) land use and vegetation.

In this study we examined whether increased zinc and cadmium concentrations affect the stability (i.e. resistance and resilience) of respiration and bacterial growth rate to environmental stress or disturbance.

Respiration

Respiration reflects decomposition of organic matter which is a major ecological process, and thus reflects the condition of the system (Giller et al., 1998). In the present study measurements in the untreated control soils showed that the respiration rate was the highest in the most remote (clean) locations. This may indicate that non-stressed environments constitute better conditions for the functioning of soil microorganisms (Giller et al., 1998). However, it may also reflect the higher content of organic matter in the C soils.

Immediately after application of a second stress/disturbance in addition to the long-term contamination, the system undergoes a shock which reveals as a change in process rates. The stronger the change, the less resistant is the process to the particular stress/disturbance. Later, the mechanisms of recovery, responsible for resilience, start to work.

The second stress (lead or salt) and disturbance (heat) caused changes in respiration (decomposition of lucerne meal (alfalfa)) in all soils but each stress/disturbance induced a different pattern of response. In the cases of salt and heat, the cleanest soils (C) were the most resistant and the most polluted soils (A) were the least resistant.

Although the average values of responses measured 24 h after the stress addition suggest that the C soils were most resistant to lead, the statistical analysis did not reveal significant differences. There was strong recovery in all soils after addition of lead. The initial respiration rate was regained, and even exceeds the control level, in all soils already 2 weeks after the stress addition. Lack of significant differences in resistance and strong recovery of respiration after lead application is different from the results of Griffiths et al. (2000) and of our former work (Tobor-Kapłon et al., 2005). In both studies no recovery from heavy metal stress was found and the less stressed soils were more stable to additional stress.

In contrast with our former results, one could also argue that the communities close to the smelter (A soils), that have already been exposed to zinc and cadmium for a long time, may have developed co-tolerance to lead (Díaz-Raviña et al., 1994) and therefore may be expected to be more stable than the communities in less polluted (C) soils. Indeed, lead had the weakest effect on respiration compared to salt and heat. However, the relatively weak effect of lead was found also at the least polluted site. These most remote (C) soils are also the richest in organic matter which is known to immobilise heavy metals, and indeed are

characterized by the lowest $CaCl_2$-exchangeable metal concentrations. Therefore the addition of lead resulted in smaller increase of bioavailable lead in C soils than in A soils. Thus, the difference in organic matter content between the sites may be the reason that no significant differences in stability were found.

The mechanism behind recovery, observed after application of lead, probably relied on the lessened competition caused by the death of the sensitive organisms (Sardinha et al., 2003) and associated with this the sudden release of bioavailable carbon from dead bacterial cells. Such rapid food supply could stimulate the activity of resistant organisms that could then start to compensate for the loss of others. Such a mechanism may also explain the recovery of respiration and bacterial growth rate after application of the two other treatments.

Also in the case of heat (disturbance), respiration showed recovery in all soils. Although the resilience was similar in all soils, the most polluted ones due to their low resistance were the least stable to heat. The cleanest soils, as the most resistant, showed the highest stability of respiration rate. In this case, changes in the energy budget of organisms inhabiting the examined soils probably are responsible for different levels of stability to additional stress. The organisms from highly polluted soils have lowered resources due to the allocation of energy to detoxification and reparation of damages caused by the first stress, which makes an additional stress more severe for them (Calow, 1991; Kuperman and Carreiro, 1997).

Heat imposed the strongest changes in respiration when compared with the other treatments, while in our former experiments (Tobor-Kapłon et al., 2005, submitted) we found heat (disturbance) to be less harmful than lead and/or salt. The earlier experiments, however, were performed on agricultural soils which are exposed to higher variation in temperatures. This could increase their tolerance against heat. Soils used for the present experiment are covered by a litter layer and moreover shielded by forest which protects them very well against temperature fluctuations.

The respiration showed no resilience after the addition of salt. The most polluted soils remained the most affected, thus the least stable, and the cleanest ones the most stable. In this case the resistance of respiration determined the stability of the soils to the addition of salt.

Griffiths et al. (2000) found a similar pattern of stability of respiration to stress (copper); the weaker stability (resistance) of the more stressed soils was interpreted as a confirmation of the "insurance hypothesis of biodiversity" (Naeem and Li, 1997; Aarts and Nienhuis, 1999; Loreau, 2000). This hypothesis says that more diverse systems are more stable. In later work, however, Griffiths et al. (2001b) proved that reduced biodiversity per se does not reduce stability, but merely the stress applied to reduce biodiversity in the earlier experiments (Griffiths et al. 2000). Also our present results indicate effects on the physiology of the organisms. In our former work (Tobor-Kapłon et al., 2005) we have also found that not contaminated soils were more stable (resilient) to salt than contaminated soils. Though the energy-budget-explanation also holds in the present study, an increased tolerance to an acidic environment can also have an impact on stability to salt. It is known that development of tolerance towards low pH involves changes in cell surface properties that makes the exposed organisms more resistant to changes in osmotic pressure (Leyer and Johnson, 1993; Faleiro et al., 2003). The C soils have the lowest pH of all examined soils, which might make them more tolerant to salt.

Bacterial growth rate

Bacterial growth rate was generally more sensitive to the applied stresses and disturbance than respiration. Immediately after treatment bacterial growth rates were reduced by more than 80%.

Lead treatment imposed a very strong stress for bacterial growth rate; in all soils the growth was initially inhibited by approx. 80%. No difference in the resistance, neither in resilience to lead was found. This suggests that the bacterial growth in all these soils has a very low stability to the addition of lead.

There was no difference between the soils in the resistance of bacterial growth to salt. In A and B soils after initial recovery a reduction in growth occurred. The constant recovery of bacterial growth in C soils (cleanest ones) makes them the most stable to this stress.

There was hardly any difference between the soils in their resistance to heat. The bacterial growth rate was resilient in all soils; however, in the C soils the recovery was the strongest. These soils, due to their higher organic matter content, are probably the richest in food sources. Thus, organisms that survived the heat shock were able to strongly increase their growth rate. The C soils were thus the most affected by heat and therefore the least stable to this disturbance.

The effects on bacterial growth rate cannot be directly compared with effects on respiration because of differences in the treatments of the soils used for the two types of measurements. Lucerne meal was added only to the soils in which respiration was measured. This was necessary to ensure a sufficient response within 24 h. Because not all organisms can utilise this material with the same efficiency, only the activity of responsive microorganisms is measured. The relatively high amount (3.2 mg C g^{-1} soil) of plant residues added may therefore have caused a shift in community composition. Bacterial growth rate was measured within 2 h after adding a radioactive tracer ([^{3}H]thymidine) to subsamples of the soil. The low concentration of thymidine (0.1 μM) and the short incubation time prevent changes in growth rate during the incubation. So the measured growth rate reflects the actual growth rate of the total soil bacterial community.

Conclusions

We found that soils that differ in the level of pollution have different stability to applied stresses and disturbance. The results give support to both concepts of stability: (1) non-stressed systems are more stable because they possess larger energetic resources, and (2) stressed systems are more stable since they gained abilities (adaptation, physiological changes) to cope with additional stress. This implies that there is no ''general'' stability to stresses/disturbance but rather stability of a particular process to a given stress/disturbance. The responses of microbial processes to stress, their nature and size, depend on the kinds of stress factors, especially whether a subsequent stress is similar to the first stress, in terms of the mechanism with which the organisms deal with the stress.

Acknowledgments

We thank M.R. Veninga, A. Vos, S. Racamy and W. Schuurmans for technical assistance. This research was funded by the TRIAS Programme (TRIpartite Approach to Soil system processes) of Delft Cluster, SKB (Foundation for development and transmission of soil knowledge) and NWO (the Netherlands Organisation for Scientific Research), as project 835.80.083.

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