

Influence of metal contamination on soil parameters in a lysimeter experiment designed to evaluate phytostabilization by afforestation

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Abstract

Phytostabilization is a possible remediation strategy for metal polluted soils. In this paper, we present an overview of soil-related results from a large lysimeter study designed to evaluate phytostabilization of a metal contaminated agricultural topsoil by afforestation. We report on changes in solid phase metal speciation and availability, water household measurements (e.g. evapotranspiration), root distribution, microbial biomass and activity, and soil solution composition (metals, DOC and nutrients). Elevated metal concentrations in the soil solution mainly affected the community structure of microorganisms and lead to a reduction in root growth. This not only influenced the degradation of soil organic matter and the formation of DOC, but, as a consequence, also the speciation and solubility of metals.

Keywords: heavy metals, phytostabilization, speciation, microbial activity, soil solution, roots

1 Introduction

Phytostabilization is a possible remediation strategy for metal polluted soils (ROBINSON *et al.* 2006). A contaminated site can be afforested to stabilize the soil against erosion and runoff. In addition, uptake of water and elements by plant roots can help to keep the metals within the plant/soil system, and thus prevent their leaching to the groundwater. The total metal content in the soil will therefore not be reduced but transfers into the food chain should be minimized. On the other hand, chemical changes in the soil caused by plant-induced processes at the soil-root interface may increase or decrease metal mobility, and therefore the risk for leaching. Root-induced acidification of the rhizosphere soil for example leads to an increase in metal mobility (HINSINGER *et al.* 2003). Thus, the chemical shifts taking place in the soil after a change in land-use may affect metal bioavailability and leaching and thus have to be assessed to validate the suitability of a phytostabilization. In particular, a change from agriculture, where soil acidification is prevented by fertilization, to forest, may increase metal mobility and availability (STIGLIANI *et al.* 1991).

Plant activity has an annual cycle and therefore the influence of plants on metal concentrations and speciation can be expected to show seasonal fluctuations. The limited number of investigations dedicated to the effect of vegetation on the concentrations and speciation of metals in the soil solution of uncontaminated (TYLER 1981; BERGKVIST 1987; KELLER

and DOMERGUE 1996) and contaminated soils (FEDERER and STICHER 1994; DEROME and NIEMINEN 1998; DENAIX *et al.* 2001; MCLAREN *et al.* 2004) calls for further in-situ studies to better understand the influence of plants on metal solubility in soils.

Bioavailability and mobility of metals depend to a great extent on the speciation in the solid phase and the solution (PLETTE *et al.* 1999). Complexation of metals by organic compounds derived from soil organic matter or plant residues is therefore important for understanding the behavior and the effects of metals in polluted and non-polluted soils. Microbial communities play an important role in soils because they perform many functions in nutrient cycling or decomposition processes (NANNIPIERI *et al.* 2003). Metals are of concern because of toxicity to soil microorganisms and, thus, impairment of ecosystem functions (GILLER *et al.* 1998). At the plant scale, roots are usually the plant organs most severely affected by metals due to their direct exposure (BARCELO and POSCHENRIEDER 1990). This causes reduced root growth and impaired capabilities to extract soil water and nutrients with effects on biomass production and transpiration.

In this paper, we present an overview of soil-related results from a large lysimeter study designed to evaluate phytostabilization of a metal contaminated agricultural topsoil by afforestation. We report on changes in solid phase metal speciation and availability, water household measurements (e.g. evapotranspiration), root distribution, microbial biomass and activity, and soil solution composition (metals, DOC and nutrients). The use of lysimeters with different soils and various treatments and the sampling of soil solution with suction cups allowed both a controlled variation of important parameters and enabled to gain in-situ information on mobilization of metals from the solid phase and the influence of the solubilized metals on soil parameters and plants.

2 Materials and methods

2.1 Description of the lysimeter experiment

The model ecosystems were planted in spring 2000 in Birmensdorf, Switzerland. 32 lysimeters were associated with open-top chambers (OTC) with two lysimeters in each OTC. The lysimeters (Fig. 1) had a surface area of 3 m² and a depth of 1.5 m. A 50 cm drainage layer was made of three sublayers of fire dried quartz gravel (grain size 5–8, 1.5–2.2, and 0.7–1.2 mm). The soil was composed of an 80 cm layer of subsoil and a 15 cm layer of topsoil. The subsoil, filled in 1999, was either an acidic loamy sand from a Haplic Alisol near Eiken, Aargau, Switzerland, or a calcareous sandy loam originating from a Calcaric Fluvisol at Brugg, Aargau, Switzerland. The topsoil was a weakly acid loam from an agricultural field at Birr, Aargau, Switzerland. These situations are representative for an acidic soil, in which the topsoil pH is raised by agricultural practice, and a calcareous soil with a decarbonated topsoil, respectively. Some physical and chemical characteristics of the three soils are given in Table 1.

During the vegetation period (May through October), the roofs of the OTC closed automatically during rain events to exclude natural precipitation, and the lysimeters received irrigation with synthetic rain (pH 5.5) or acid rain (pH 3.5). The ionic composition of the synthetic rain was taken as the 30-year means of the local natural rain (in μM : 70 NH_4 , 70 NO_3 , 3.2 PO_4 , 17 Cl , 3.1 SO_4 , 4.3 Na , 7.7 K , 5 Ca , 1.3 Mg , 0.15 Zn) and the pH was adjusted with HCl. During winter (November through April), the roofs were open all the time, and the lysimeters received natural precipitation.

Table 1. Physical and chemical characteristics of the soil materials used in the lysimeters

	topsoil	acidic subsoil	calcareous subsoil
Texture (% sand ; silt ; clay)	36 ; 49 ; 15	87 ; 8 ; 5	74 ; 16 ; 10
pH (0.01M CaCl ₂)	6.55 ± 0.12	4.2	7.4
C _{inorg} [g/kg]	<1	<1	21
C _{org} [g/kg]	15.1	3.2	11.2
N _{tot} [g/kg]	1.5	<0.3	0.6
C _{org} /N _{tot}	10	> 10	18
P _{extractable} [mg/kg]	1160	298	296
P _{org} [mg/kg]	862	84	54
P _{avail} [mg/kg]	49 ± 5	18	11
Mg _{exch} [mg/kg]	79	14	35
Al _{exch} [mg/kg]	<0.5	205	<0.5
K _{exch} [mg/kg]	283	23	21
Ca _{exch} [mg/kg]	1790	116	2600
Mn _{exch} [mg/kg]	1.9	9.5	2.2
CEC [mmolc/kg]	102.0	31.0	133.8
Base saturation [%]	99.9	35.9	99.9
Cu _{tot} [mg/kg]	28	7.4	14
Zn _{tot} [mg/kg]	97	39	58
Cd _{tot} [mg/kg]	0.1	< 0.2	0.2
Pb _{tot} [mg/kg]	37	13	19

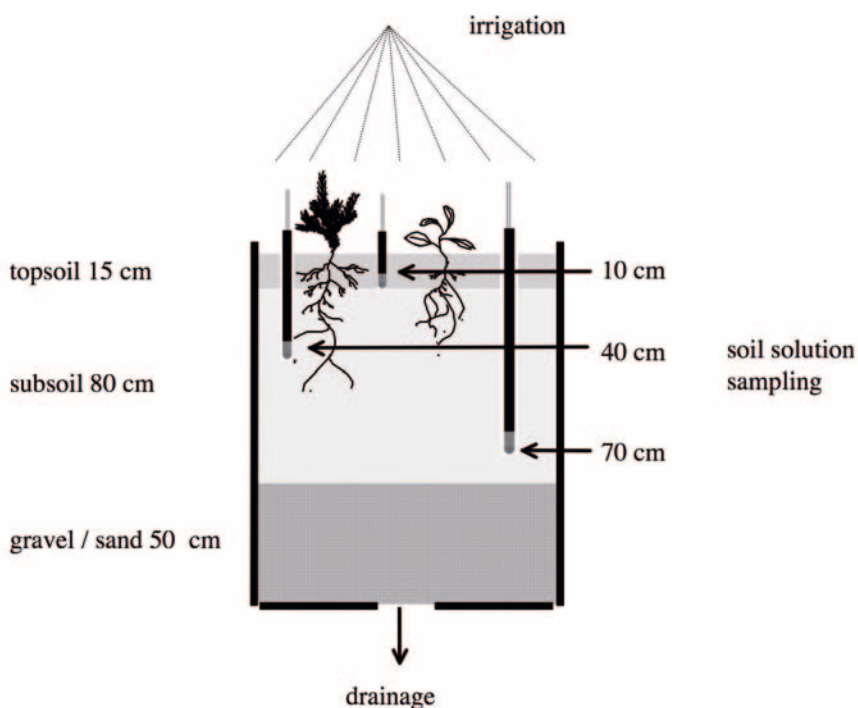


Fig. 1. Cross-section of one lysimeter with the three soil layers and the depth of soil solution sampling.

Table 2: Treatments of the open top chambers with the three variables pollution, rain, and subsoil.

	Ambient rain	Acid rain
	CO	AR
Topsoil unpolluted	– Acidic subsoil – Calcareous subsoil	– Acidic subsoil – Calcareous subsoil
	HM	HMAR
Topsoil polluted	– Acidic subsoil – Calcareous subsoil	– Acidic subsoil – Calcareous subsoil

In the metal treatments (HM), the topsoil was contaminated with filter dust (average particle size of 8 μm) containing the following element contents [mg kg^{-1}]: Zn 800 000, Cu 170 000, Pb 15 500, and Cd 270. Additionally, cadmium oxide was mixed into the dust to increase its Cd content ten times. The dust was mixed manually into the topsoil layer by means of a small harrow and final concentration of Zn, Cu, Pb and Cd were 3000, 640, 90, and 10 mg kg^{-1} , respectively.

Each model ecosystem was planted with 14 trees (6 *Picea abies* [L.] Karst., 4 *Populus tremula* L., 2 *Salix viminalis* L. and 2 *Betula pendula* Roth.) and understory plants. These plants were chosen because they are often used in afforestations due to their fast growth and high biomass production. Willow and poplar accumulate high amounts of metals while spruce excludes metals quite efficiently from transfer to the shoots (HERMLE *et al.* 2006).

In summary, the model ecosystem experiment was set up with treatments varying the following parameters (see Table 2): contaminated vs. uncontaminated topsoil, uncontaminated acidic subsoil vs. calcareous subsoil and acidic (pH 3.5) vs. ambient rain (pH 5.5). Treatment levels ($2 \times 2 \times 2$) were replicated 4 times (16 OTC arranged in a Latin square; each OTC hosting one lysimeter with acidic subsoil and one lysimeter with calcareous subsoil). We abbreviate the treatments as: CO (control), HM (metal contaminated topsoil), AR (acidic rain) and HMAR (combination treatment).

2.2 Sampling and analytical methods

Detailed sampling procedures and analytical methods are described elsewhere. The sequential extractions have been carried out according to the method of ZEIN and BRÜMMER (1989). The use of DGT to assess the resupply kinetics of Cu is described in NOWACK *et al.* (2004). The measurement of the soil water household and the root density is reported in MENON *et al.* (2005). The methods to measure the microbial activity are described in FREY *et al.* (2006). Soil solution sampling and analysis are reported in RAIS (2005) and RAIS *et al.* (2006).

3 Results and discussion

3.1 Solubility and availability of metals

Stable conditions with respect to solid phase speciation were reached after half a year for Zn, while Cu transformation was completed only 1.5 years after establishing the contamination. Sequential extractions at the end of the experiment (Fig. 2) showed that for Cu about 40 % were present in the first two fractions and were therefore only weakly bound. About 70 % of the Zn was found in these two fractions and 85 % of Cd. Only 10 % of Pb was present in these two fractions. Significant fractions of Cu and Pb were extracted in the so-called “organic” fraction while the more refractory iron oxide and residual fractions only played a significant role for Pb with about 30 %. EXAFS analyses showed that Zn was present as (in decreasing importance) Zn-layered double hydroxide, Zn-phytate, adsorbed Zn, Zn-phylosilicate, and Zn-birnessite (VOEGELIN *et al.* 2005). The results show that a large fraction of Cu, Zn, and Cd was present in highly plant available fractions during the whole time of the experiment.

Measurements by DGT revealed that the kinetics of Cu and Zn resupply from the solid phase are characterized by fast initial mobilization indicating a pool of rapidly available metal (NOWACK *et al.* 2004). With time this pool was exhausted and the availability of Zn was very low while that of Cu remained relatively high and constant. This indicated that part of the Zn was present as labile, easily mobilizable phase and a larger fraction of a refractory phase. Cu on the other hand was more available on the long-term. This is also in agreement with the results from the sequential extractions and the EXAFS measurements.

The metal concentrations in soil solution of the topsoil varied little over time (RAIS 2005). They were significantly larger in the contaminated topsoils than in the control. Table 3 shows the range of observed metal concentrations in the topsoil and the two subsoils. There was a strong correlation between Cd and Zn concentrations. In the acidic subsoil, Zn concentrations were somewhat larger below contaminated topsoil than below uncontaminated topsoil, with maximum concentrations in winter and spring. In the calcareous subsoil, none of the metals was increased below the contaminated topsoil.

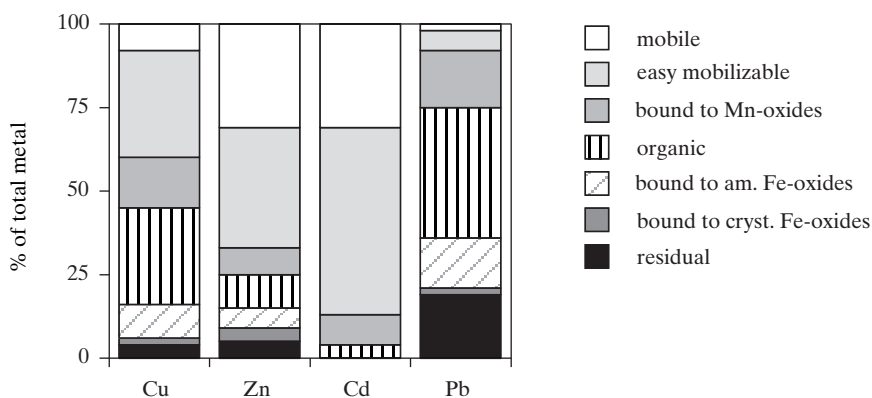


Fig. 2. Sequential extraction of the metal polluted topsoil at the end of the experiment.

Table 3. Concentration range of protons and metals in the soil solution for the CO/HM treatments. Data from RAIS (2005).

	Topsoil		acidic subsoil		calcareous subsoil	
	CO	HM	CO	HM	CO	HM
pH	7.4–8.0	7.5–8.0	5.8–6.8	6.0–7.0	8.4–8.7	8.4–8.8
Cu (μM)	0.19–0.62	1.0–1.6	0.02–0.04	0.02–0.05	0.15–0.24	0.12–0.16
Zn (μM)	0.02–1.0	13.1–24.9	0.02–0.09	0.02–0.24	0.03–0.04	0.02–0.03
Cd (nM)	0.10–0.51	20.0–44.3	0.28–1.1	0.37–0.90	0.11–0.51	0.10–0.32

While Cu was governed by complexation with DOC, Zn and Cd concentrations were negatively correlated with phosphate in the contaminated topsoils. The concentrations of Cu and Zn could be described with the following equations (RAIS 2005):

$$\log [\text{Cu}] = -4.90 + 0.29 \cdot \log [\text{DOC}] \quad (n = 36, R^2 = 0.37, p < 0.001)$$

$$\log [\text{Zn}] = -8.63 - 0.93 \cdot \log [\text{PO}_4] - 0.06 \cdot \text{pH} \quad (n = 36, R^2 = 0.63, p < 0.001)$$

These equations include the most important soil solution parameters needed to explain the temporal changes in Cu and Zn in the polluted topsoils.

3.2 Water household

The metal contamination produced small but significant effects on the soil water household (MENON *et al.* 2005). The evapotranspiration determined from the water balance was reduced under metal stress. The effects became more pronounced from year to year with the

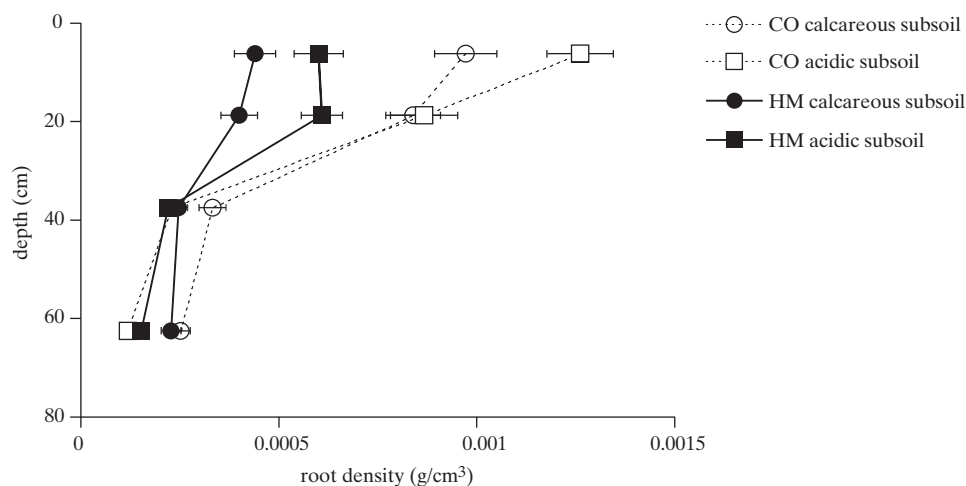


Fig. 3. Vertical profiles of the fine root density distribution at the end of the third growing season in the lysimeters. Data from MENON *et al.* (2005).

development of the forest ecosystem. Root growth in the topsoil was much stronger above acidic subsoil and metal pollution affected the root density strongly in the topsoil but not in the subsoil (Fig. 3). The relative proportion of the root system in the subsoil was therefore larger in the HM than in the CO treatment.

Under sufficient availability of water, the roots extracted water mainly from the topsoil. Under water stress conditions, root water uptake was shifted into the subsoil, especially with calcareous subsoil which was much more fertile than the acidic subsoil (MENON *et al.* 2005). The results support the hypothesis that the uncontaminated subsoil offered a possibility to compensate the reduction in root water uptake under metal stress. This was more pronounced with the calcareous subsoil, which was more favorable for root growth than the acidic subsoil.

3.3 Microbial activity

The exposure to the metals negatively affected soil microbial activity and changed the microbial community structure in the topsoil (FREY *et al.* 2006). The HM treatment reduced the microbial biomass to 55 % of the control topsoil, the combined HMAR to 45 %. The phospholipid fatty acid analysis (PLFA) revealed a similar picture with a significant decrease induced by the HM and HMAR treatment (Fig. 4). Both Gram-positive as well as Gram-negative bacteria were affected equally and fungal biomass was also depressed by the HM treatment. Parameters describing microbial activity such as basal respiration, phosphatase, β -glucosidase, β -glucuronidase, and nitrification potential were significantly reduced in the presence of metals (Fig. 4) (RAIS 2005; FREY *et al.* 2006).

The PLFA analysis together with nucleic acid-based molecular techniques for fingerprinting 16S ribosomal DNA were able to discriminate metal effects (FREY *et al.* 2006). The total number of taxonomic units was not reduced in the HM treatment compared to CO topsoils. However, the results showed that the bacterial communities differed in species composition and in the proportions of the different species. Time-dependent shifts of the microbial communities were also detected and patterns in microbial communities were clearly separated between the second and the fourth year of the experiment.

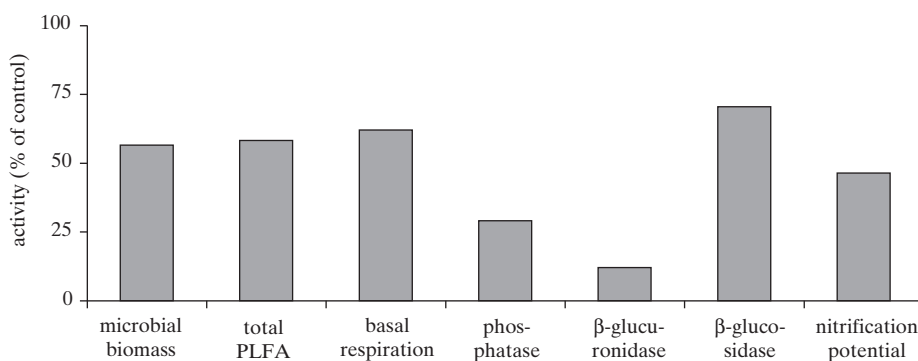


Fig. 4. Microbial parameters in the metal-polluted topsoil (HM) in % of the activity the control soil (CO) (both for ambient irrigation). Data from FREY *et al.* (2006) and RAIS (2005).

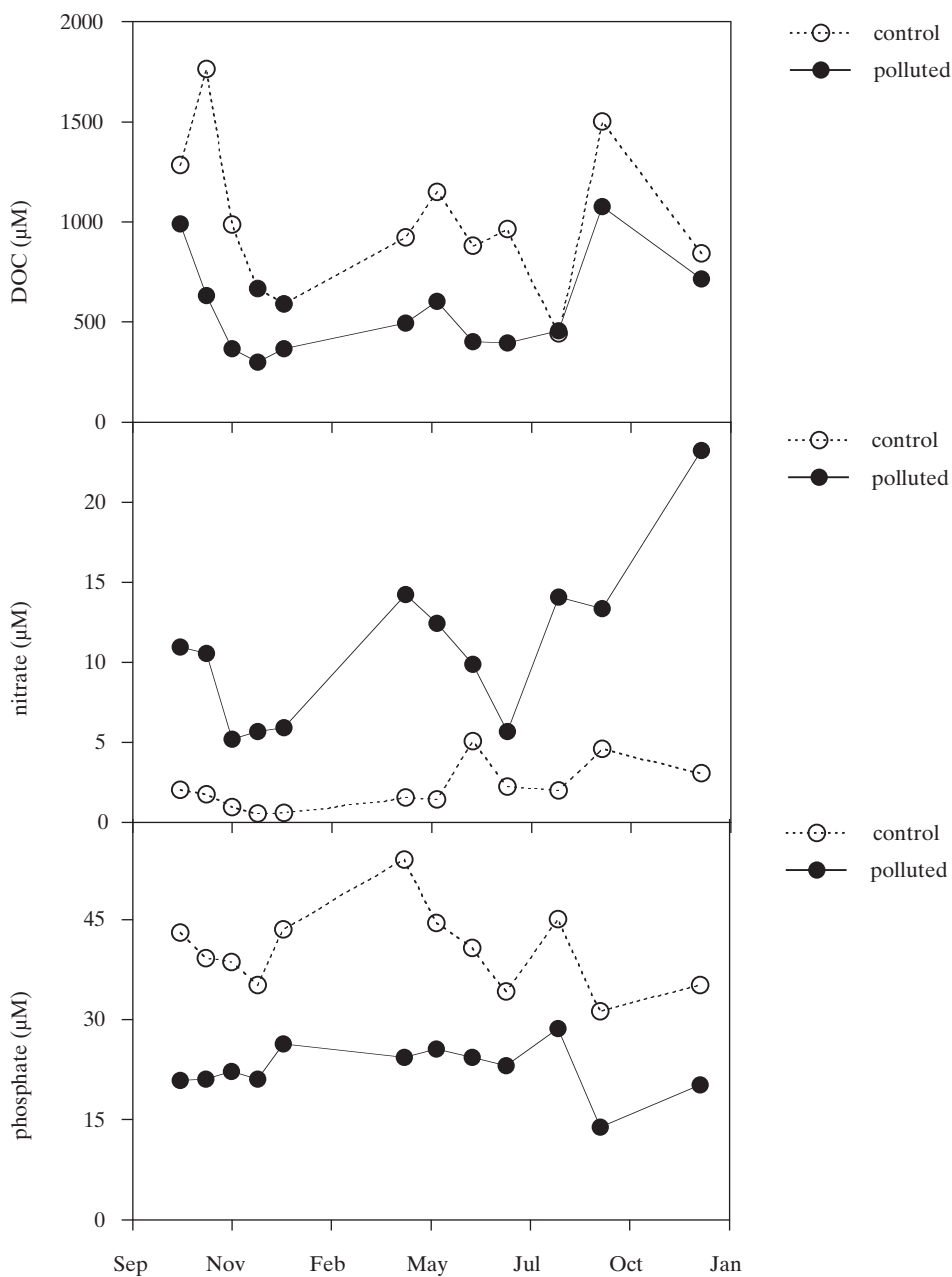


Fig. 5. DOC, nitrate, and phosphate concentrations in the topsoil in the CO and the HM treatments over 14 months. Data from RAIS (2005).

3.4 Inorganic anions and dissolved organic carbon in the soil solution

Dissolved organic carbon (DOC) and sulfate concentrations were smaller in the contaminated topsoil than in the uncontaminated topsoil (Fig. 5) (RAIS 2005). Considering the low DOC and sulfate concentrations without metal effects in a plant free reference system, as well as the metal effects on root density in the topsoil (Fig. 3), led RAIS (2005) to the conclusion that the stimulation of the microbial activity by root exudates seemed to be reduced under metal stress.

Also phosphate concentrations were smaller in the contaminated than in the control topsoil (Fig. 5). This can be explained on one hand by the reduced microbial phosphatase activity in the HM treatment, on the other hand by oversaturation of the contaminated topsoil solution with respect to Zn phosphate (RAIS 2005).

Larger nitrate concentrations in the HM treatment than in the control topsoil (Fig. 5) were inconsistent with the metal effects on nitrification potential (Fig. 4). The opposite effect on nitrate concentrations can be explained by a larger plant uptake of nitrate in the control topsoil than in the contaminated topsoil as suggested by root density measurements (Fig. 3). Due to the large nitrate demand by plants and its high mobility, concentrations of nitrate are mainly controlled by plant uptake (JUNGK 2002). Effects of metals on nitrification potential were therefore masked effectively.

Soil solution constituents, which consider the soil compartment as a whole and at natural conditions, are therefore complementary to microbial assays and place the results of the biological tests into an ecosystem perspective.

4 Conclusions

The contamination of the soil by filter dust resulted in high bioavailable metal fractions that persisted over the whole time of the experiment. Metal concentrations in the soil solution were considerably larger in the contaminated topsoil but showed rather small seasonal fluctuations. While Cu was governed by complexation with DOC, Zn and Cd were negatively correlated with phosphate. Metal concentrations in the subsoil were not affected by the metal pollution in the calcareous subsoil and only Zn was somewhat larger in the acidic subsoil. Overall little or no leaching of metals below the rooted zone was observed during four years.

Biological parameters such as root distribution as well as size and activity of microbial communities were reduced by metals in the topsoil. In accordance, DOC and sulfate concentrations were reduced in the topsoil solution due to the smaller microbial activity. Other parameters such as nitrate increased in the topsoil although the inhibitory effect on nitrification potential would have proposed a decrease. However, this was masked by a shift in water and nutrient uptake by plants from topsoil to the subsoil. This indicated that the response of a whole ecosystem might not be described sufficiently well by using single microbial parameters. However, combining such parameters with analyzing the soil solution composition can reflect also indirect metals effects on microbial activity induced by changed rooting patterns.

Figure 6 shows a conceptual model of the metal contaminated topsoil, which is based on the results presented above. It depicts the most important processes and interactions between metals in the soil solution and soil solid phase, plant roots, microorganisms, as well as carbon and nutrients in the soil solid phase. According to this model, elevated metal concentrations in the soil solution mainly affect the community structure of microorganisms

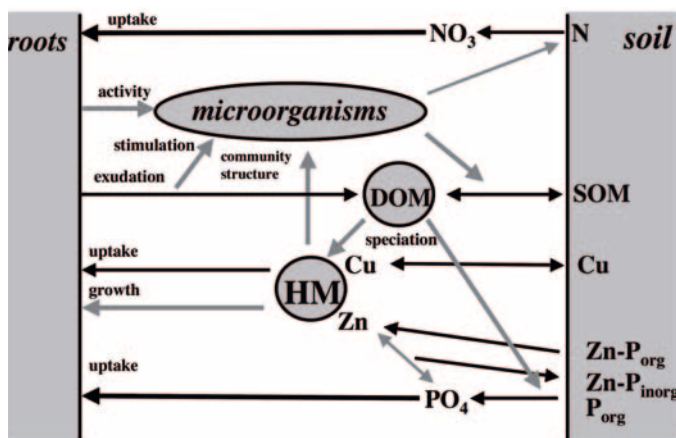


Fig. 6. Conceptual model of the metal contaminated topsoil. It depicts the most important processes and interactions between metals in soil solution and the soil solid phase, plant roots, microorganisms, as well as carbon and nutrients in the soil solid phase. Black lines denote mass fluxes, grey lines represent an influence.

and lead to a reduction in root growth. The latter means also smaller amounts of root exudates and lower microbial activity. Nitrate concentrations in the soil solution are governed on one hand by microbial activity and, on the other hand, by plant uptake. Microbial activity and community structure not only determine the degradation of soil organic matter (SOM) and dissolved organic matter (DOM) as well as the transformation between these two carbon pools, but, as a consequence, also the speciation and solubility of metals with a high affinity to organic ligands like Cu. The action of phytase, that is excreted by microorganisms and plant roots, leads to the release of both phosphate and Zn from Zn phytate. The solution concentrations of released Zn and phosphate are then controlled by plant uptake and solubility of Zn phosphate.

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