Decomposition of dissolved organic carbon after soil drying and rewetting as an indicator of metal toxicity in soils

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Abstract

One of the drawbacks of respiration tests to identify metal toxicity on C mineralization in soil is that the result depends on the type of substrate added (Giller et al., 1998). Carbon mineralization in metal-contaminated soils was measured using the native soil organic matter as the substrate. The method is based on monitoring the decrease in the dissolved organic carbon (DOC) in soil solution after the DOC flush, following two drying and rewetting cycles. Four agricultural topsoils were spiked with ZnCl$_2$ at 50, 150 and 500 mg Zn kg$^{-1}$. The DOC concentration in soil solutions did not change during the 23 days of moist incubation following spiking. Metals slightly reduced the DOC in all soils but this effect was significant ($P < 0.05$) only in one soil. After the two air-drying and rewetting cycles, the DOC concentrations significantly ($P < 0.05$) increased by factors between 2.5 and 5.3. The flush in carbon after rewetting consistently decreased in the following 24 days of moist incubation in all uncontaminated soils, and this decrease was less pronounced in metal-contaminated soils. The first-order degradation constant varied between $34 \times 10^{-3}$ and $90 \times 10^{-3}$ day$^{-1}$ for the uncontaminated soils. The degradation constants at the highest Zn rate were significantly lower by between 2.4 and 12-fold compared to the control for all soils ($P < 0.05$). Inhibition of the DOC decomposition at 150 mg Zn kg$^{-1}$ was only significant ($P < 0.05$) in two soils. Since drying--rewetting events are natural processes that promote C-mineralization in the topsoil, we believe that the decomposition of the DOC flush may be a relevant indicator of the effects of contaminants on C-mineralization in the long term. © 2001 Elsevier Science Ltd. All rights reserved.

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1. Introduction

There are numerous reports that show toxicity of metals as indicated by the decomposition of organic matter. These reports are based on litter decomposition tests, respiration tests and, in extreme cases of metal pollution, observations of a build-up of organic matter in soils (see Tyler et al., 1989; Giller et al., 1998 for reviews).

The effects of metal toxicity on C-mineralization in soil can be measured in the laboratory using substrate-induced respiration tests. The initial respiratory response after substrate addition is found to be very sensitive to even low metal-contamination (Doelman and Haanstra, 1984; Nordgren et al., 1988; Reber, 1989; Hattori, 1991). A drawback in the substrate-induced respiration tests is that the test result depends on the type of substrate. Various substrates have been used, ranging from model C-compounds (glucose, amino acids) to plant materials. Witter et al. (in press) have used a range of substrates and found that the response was not the same for all substrates. Decomposition of $m$-hydroxybenzoic acid was even faster in a high metal soil than in a low metal soil.

Mineralization of native soil organic C has also been used in metal toxicity tests. The results of such tests are conflicting and metals have both stimulating and inhibiting effects on respiration (Walter and Stadelmann, 1979; Lighthart et al., 1983; Doelman and Haanstra, 1984; Leita et al., 1995). A potential confounding factor in the respiration responses may be the effect of the metal on substrate availability: the death of cells may increase substrate availability but metals can also affect complex soil organic matter and render it less available (Giller et al., 1998).

Here, we present an alternative way to detect metal toxicity on C-mineralization using native soil organic C as a substrate. Whereas changes in total C in soil are obviously undetectable in the short term, this may not be true for a labile native substrate. We used the dissolved organic carbon (DOC) in soil solution as this substrate. The choice for DOC was suggested by unexpected results obtained in soils that were contaminated with Zn$^{2+}$ and Cd$^{2+}$ salts (Brans, unpublished data). The pore water analyses of
these soils showed gradual increases of DOC with increased metal application rates, and DOC concentrations exceeded even 400 mg C l\(^{-1}\) in some soils at the highest metal application rates. The metals did not increase soil pH, rather they slightly decreased the pH. The soils had been subjected to a drying–rewetting cycle during the more than 10 months aging period prior to testing. We hypothesized that drying–rewetting the soils may have provoked a flush in DOC in all soils and that metal toxicity may have limited the decomposition of this flush during incubation. Drying–rewetting is a natural process that is accompanied by a flush in respiration and a release of soluble C in soil (Powlson and Jenkinson, 1976; Van Veen et al., 1985; Zsolnay, 1996).

Here, we report on an experiment to test the aforementioned hypothesis. The DOC concentrations were monitored in four soils at four Zn application rates during a sequence of metal application, drying–rewetting and moist incubation.

### 2. Materials and Methods

Four soils (K, A, N and T) were sampled in September 1998. They were in production as grassland (K, A) or arable land (N, T), at the time of sampling. Soil K is an Anthrosol with a fimmic A-horizon. Soils A, N and T are Luvisols. All soils were air-dried and sieved (2 mm) and were stored at 4°C. Selected characteristics are given in Table 1. The test was started (day 0) by rewetting about 3.25 kg of each soil to 40% of its water holding capacity (WHC, see Table 1). The soils were incubated in darkness at 20°C in closed jars. The first sampling of pore water was made after 4 days. Soils were contaminated at day 4, after pore water sampling, using stock solutions of ZnCl\(_2\) to obtain soil Zn concentrations of 0, 50, 150 and 500 mg Zn per kg oven-dry soil. The soil moisture content was increased through spiking to between 50 and 70% of the WHC of each soil. Soil moisture content was the same for all Zn treatments and was 24% (K), 26% (A), 19% (N) and 27% (T). The soils were mixed and the samples were incubated until day 28, at 20°C. Pore water was sampled at day 7 and day 28. The soils were subsequently divided in two treatments: one was continuously incubated moist at 20°C until day 44 and the other was subjected to two drying–rewetting cycles. This second treatment was dried in a layer of about 2 cm at 25°C under continuous illumination (650 μmol photons m\(^{-2}\) s\(^{-1}\)) for 2 days. The soils were rewetted with deionised water to the soil moisture content before drying, mixed and dried a second time for 2 days at similar conditions as given above. After rewetting, the soil samples were transferred to closed glass jars (about 500 g in a 2.5 l jar) and incubated at 20°C in darkness until day 59. The soil samples that had been incubated moist continuously were similarly transferred to closed glass jars. Pore water of the soils, subjected to the drying and rewetting cycles, was sampled at days 35 (3 days after the last rewetting), 37, 44, and 59. Pore water of the first treatment (continuous moist incubation) was sampled at days 35, 37 and 44.

Pore water was isolated by a direct centrifugation method. A disposable 30 ml syringe without a plunger was filled with quartz wool and 50–60 g of soil sample. The syringe was transferred to a centrifuge tube and centrifuged for 1 h at 1100 relative centrifugation force. At each occasion, two replicate samples were taken of each soil. The DOC of each solution was measured by dry catalytic combustion at 950°C followed by infrared CO\(_2\) detection at 4200 nm (Skalar Formacs TOC analyzer). In preliminary trials using all four soils, we found that neither membrane filtration (0.45 μm) nor removal of inorganic C by an acid purge in the TOC analyzer affected the concentration of soluble C in solution. Therefore, none of the solutions was membrane filtered in this experiment. The soluble C comprised both inorganic and organic C, but since inorganic C was marginally small, we denote soluble C as dissolved organic carbon (DOC) in this study.

Soil moisture content was determined at the end of the experiment by drying at 105°C. Oven-dried subsamples were homogenized in a mortar and extracted in boiling aqua regia (2 h). The Zn concentrations in the extracts were measured by atomic absorption spectrophotometry.

### 3. Results

The Zn addition caused a small decrease in DOC at day 7, i.e. three days after spiking. This decrease was significant (P < 0.05) only in soil A where the DOC was halved at the highest Zn rate compared to control (Fig. 1). These effects on DOC may be due to Zn\(^{2+}\) decreasing the solubility of humic substances, or to small decreases in pH (not measured).

The two drying and rewetting cycles provoked a significant increase in the DOC, whereas there were no marked changes in the DOC during moist incubation. A typical example is given for soil K in Fig. 2. Similar trends were
found for the other soils at all metal treatments (not shown). The increases in DOC upon drying and rewetting varied between 60 and 250 mg C l$^{-1}$ between soils and treatments. These DOC flushes were larger in the coarse-textured K and A soils than in the N and T soils with larger clay content (Fig. 1). The DOC decreased after the last rewetting in all the uncontaminated soils. This decrease is most likely due to the decomposition of the C flush released by the soil drying and rewetting. The first observation of DOC was made three days after the last rewetting. Some C-mineralization may already have occurred in these three days, therefore it is likely that the DOC increases between day 28 and 35 underestimate the total DOC increases, which occurred upon drying–rewetting steps.

There was no significant change in DOC in any Zn treatment during continuous moist incubation (results not shown). After drying and rewetting, however, the DOC decreased faster in uncontaminated soils than in soils at high Zn rates. As a result, DOC concentrations increased significantly ($P < 0.001$) with increasing Zn rate at the end of the incubation period in all soils. The soil analyses after the incubations showed that measured soil Zn concentrations were within 25% of the concentration expected, based on the added concentration and soil background Zn concentration (details not shown).

The decrease of the DOC flush after the drying–rewetting (days 35–59) was fitted to a first-order degradation model, i.e.

$$\ln \text{DOC}_t = A + kt$$  \hspace{1cm} (1)
where DOC$_t$ is the flush in DOC (the difference in DOC before and after rewetting), $k$ the first-order degradation constant, $A$ the intercept of the regression line and $t$ the time (days). The DOC$_t$ was calculated as the difference in DOC concentration between the rewetted soils and the average DOC concentration in the soil solution at day 28 (the average for each Zn treatment). We preferred to describe the decomposition of the flush in DOC rather than the DOC concentration itself, because it is assumed that the DOC will degrade only to the steady state concentration observed in soils kept moist continuously. The regressions were all significant ($P < 0.05$) except for soil K at the highest Zn rate. The first-order degradation constants steadily decrease with increasing Zn rate in all soils (Fig. 3). This degradation constant was significantly lower at the highest Zn rate as compared with the controls of all soils ($P < 0.05$, based on $t$-tests). Inhibition of the DOC composition at 150 mg Zn kg$^{-1}$ was only significant in soils K and A ($P < 0.05$, based on $t$-tests).

4. Discussion

4.1. The ecological relevance of the flush in DOC upon soil rewetting

In the topsoil, drying and rewetting events are natural processes known to stimulate the overall C-mineralization (Sørensen, 1974; Amato et al., 1984; Van Gestel et al., 1993). Drying and rewetting is associated with a flush in soluble C and respiration (Powlson and Jenkinson, 1976; Van Veen et al., 1985), confirmed by this study. The C flush can be sensitively detected in the DOC of the soil solution (Figs. 1 and 2). The C flushes for the four soils were equivalent to between 14 and 62 mg C kg$^{-1}$ dry soil (mean: 35 mg C kg$^{-1}$) when converted using the soil moisture content. Assuming that the upper 7 cm of the soil is affected by drying and rewetting and that the soil has a density of 1.4 kg l$^{-1}$, amounts between 14 and 62 kg C ha$^{-1}$ would be liberated during a drying and rewetting cycle. This DOC flush upon rewetting a soil is marginal compared to the annual C turnover in agricultural soils (typically about 5 ton C ha$^{-1}$). However, the flush in DOC is only a fraction of the total C released upon soil rewetting, because some of the C that is released is sorbed in the soil. Powlson and Jenkinson (1976) used 1 N K$_2$SO$_4$ extraction to quantify flushes in C after soil rewetting. The extractable C flushes of six soils were higher (mean 130 mg C kg$^{-1}$) than the soil solution DOC flush in our study. The extra CO$_2$-C release (10 days) in a rewetted soil, compared to a soil kept moist, was generally even greater than the extractable C flush (Powlson and Jenkinson, 1976). We have previously compared 1 N K$_2$SO$_4$ extractable C with soil solution DOC in the four control soils used in this study (Brans, unpublished data). The salt solution extracted between 2.2 and 4.5 times the amount of C present in the soil solution (both expressed on a unit soil weight basis). It is assumed therefore that the DOC flushing in soil solution mirror the flush in labile C in soil, but that this flush is only a fraction of the total labile C released after soil rewetting.

Monitoring the C flushes through soil solution DOC has some technical advantages over monitoring extractable C or CO$_2$ evolution. The changes in DOC are pronounced due to the low background, i.e. the soil solution DOC is small in soils kept moist continuously. In addition, DOC concentrations are usually larger in soil solution than in water or salt extracts.

The origin of the DOC flush upon soil rewetting is unclear. There is evidence that the drying process kills organisms, i.e. that biomass C is released upon drying a soil (Stevenson, 1956). The C-flush observed here was smaller than the total biomass C of the soils. We have used a fumigation-soil solution centrifugation method for the biomass C analysis of the four control soils, according to the method of van Ginkel et al. (1994). The flushes in soil solution DOC upon fumigation were 1.1-4.7 fold greater than the corresponding flushes after soil rewetting (data not shown). Similarly, Powlson and Jenkinson (1976) found that a 24 h drying event released, on an average, 2.4-fold less extractable C than soil fumigation by chloroform. Other processes than killing of biomass may also operate during drying andrewetting. Powlson and Jenkinson (1976) suggested that drying may release organic matter trapped in small pores, and that this material is not readsorbed upon rewetting because of the hysteresis effects accompanying the drying–rewetting events. Perhaps it is even the rewetting (through slaking) that releases the organic matter trapped in small pores.

The degradation of the DOC after soil rewetting (Figs. 1 and 2) suggests that the material released upon soil rewetting is not recalcitrant. Zsolnay (1996) derived first-order degradation constants $k$ from different studies, where DOC (or water extractable organic C) was monitored over time. The $k$ values were in the range of $-31 \times 10^{-3}$ and $-61 \times 10^{-3}$ day$^{-1}$, in studies where $^{14}$C labeled plant
material (roots or root exudates) was added to the soil. In studies where cellulose was added as a substrate, the $k$ values can be as small as $-1.8 \times 10^{-3}$ day$^{-1}$. The $k$ values of the four uncontaminated soils ($-34 \times 10^{-3}$ to $-90 \times 10^{-3}$ day$^{-1}$) are of a similar magnitude as those found in studies where plant material was added to the soil, suggesting that the DOC flush after soil rewetting represents labile C. Powell and Jenkinson (1976) found that the flush in extractable C after soil drying and rewetting was almost completely degraded in 10 days.

4.2. DOC decomposition indicating metal toxicity

Metals in soil significantly influence the decomposition of C, released after drying and rewetting. The dose-response curve in Fig. 3 most probably indicates that the decomposition process is inhibited by metals, rather than that metals have an effect on the type of material that is released. Reduced C-mineralization in Zn contaminated soils has frequently been found in laboratory tests (Chaney et al., 1978; Chang and Broadbent, 1981; Lighthart et al., 1983; Haanstra and Doelman, 1984; Doelman and Haanstra, 1984; Ohya et al., 1985; Saviozzi et al., 1997). One of the drawbacks of the substrate-induced respiration tests is the dependency of the toxic response on the choice of the substrate (Giller et al., 1998). The C released during drying and rewetting of soils is a native substrate. Testing toxicity in soils based on the degradation of this C flush therefore, overcomes substrate-dependency problems that occur in substrate-induced respiration tests (Witter et al., in press).

This DOC degradation test has a similar metal sensitivity than the substrate-induced respiration tests. The inhibition of the degradation constant at 500 mg Zn kg$^{-1}$ was significant in all soils and ranged from 58% (A) to 92% (K). The inhibition at 150 mg Zn kg$^{-1}$ was also significant in two soils with coarser texture, where it was 56% (K) and 27% (A). The onset of Zn toxicity in metal salt contaminated soils is generally found at far greater concentrations (e.g. Chaney et al., 1978; Lighthart et al., 1983; Haanstra and Doelman, 1984; Doelman and Haanstra, 1984; Saviozzi et al., 1997), although some tests report effects on respiration starting around 50 mg added Zn kg$^{-1}$ soil (e.g. Chang and Broadbent, 1981). Obviously, threshold concentrations can hardly be compared between different tests because of differences in, e.g., soil characteristics, substrates or endpoints. We have previously used the four soils of this study in a substrate-induced respiration test, with 1 mg glucose g$^{-1}$ soil as substrate (Bierkens et al., in preparation). Soils were similarly contaminated with ZnCl$_2$ at 300 and 600 mg Zn kg$^{-1}$ but, in contrast with the current study, they were equilibrated moist at 4°C for over 1 year prior to testing. We failed to find significant toxicity in the substrate-induced respiration test at 300 mg Zn kg$^{-1}$ in any of the soils. At 600 mg Zn kg$^{-1}$ there was a significant reduction of the CO$_2$-C flush after substrate addition, and the inhibition was in the range of 46–70% for the four soils.

5. Conclusion

Monitoring the soluble C flush in soil solution after a drying and rewetting event is a sensitive way of monitoring C-mineralization in soil. The DOC in soil solution is a native, labile C substrate, and elevated Zn sensitively affects its decomposition rate. Since drying–rewetting events are natural processes that promote C-mineralization in the topsoil, we believe that the decomposition of the DOC flush may be a relevant indicator of the effects of contaminants on C-mineralization in the long term.

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