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Microbial Biomass and ATP in Smelter-Polluted Forest Humus

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Many aspects of microbial activity in soil have been studied in connection with heavy metal pollution (Babich and Stotzky 1985; Duxbury 1985; Doelman 1986; Bååth 1989), but few investigations have included microbial biomass. Brookes and McGrath (1984) and Brookes et al. (1986), however, showed that amendments with heavy-metal rich sewage sludge to agricultural soils decreased microbial biomass as measured by the fumigation-incubation method. A decrease in ATP content of the soil was also found, but the biomass-C/ATP ratio was unaffected by the soil concentration of heavy metals. Soil heavy metal content, however, was only increased to 3-5 times the background values.

To study how biomass-C and ATP were affected over a wide range of metal concentrations, these variables have been measured around the Gusum brass mill in south Sweden. Near the smelter more than 20,000 ppm Cu + Zn g^{-1} dry soil have been found (Tyler 1984). This area has been extensively studied from microbiological, zoological and botanical points of view (Tyler 1984).

MATERIAL AND METHODS

Samples were taken from the organic layer (A_{01}/A_{02}) of a spruce forest in a transect (0.2 to 8 km) from the Gusum smelter during two sampling periods (June and December). The samples from the first collection were adjusted to 200% soil water g^{-1} dry weight and stored at 4°C for one month before processing. Humus from the second period was used after one week at 4°C and without adjustment of the ambient moisture levels found in the field (appr. 175% water g^{-1} dw).

The samples were incubated at 22°C for 1 day before respiration measurements. Basal respiration rate was measured gas-chromatographically using 10 g wet weight of humus incubated for 20 h at 22°C in 300 ml flasks (Nordgren et al. 1983). The flasks were then flushed with air. Glucose

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+ talcum (4:1, 30 mg per flask) were added and the flasks were vigorously shaken. After 30 min the flasks were again flushed with air, closed, and the respiration rate measured for 2-3 h at 22°C. This substrate-induced respiration rate (SIR) was converted to microbial biomass assuming that 100 μg CO_2 h^{-1} were equivalent to 2 mg biomass-C (Anderson and Domsch 1978).

ATP content of 0.5 g samples was determined according to Eiland (1983). The samples were not transferred to 22°C before the measurements. ATP was only measured on samples from the second sampling period.

Cu content of the humus was determined with a Cu-sensitive electrode after the samples had been ignited at 650°C and the ash moistened with concentrated HNO_3 and then diluted with distilled H_2O (see Nordgren et al. 1988). Approximately equal amounts of Zn and Cu are present in the polluted soil (Nordgren et al. 1983), but Cu content was used as an index of pollution. Loss on ignition at 600°C for 4h was used to estimate organic matter content. Total soil C was calculated assuming that 45% of organic matter was carbon (Persson et al. 1980).

RESULTS AND DISCUSSION

Data from the second sampling period are presented in Fig. 1. Microbial biomass-C (SIR) decreased with increasing heavy metal pollution level. Non-polluted samples had 9-14 mg biomass-C g^{-1} organic matter, which decreased to 3-5 mg biomass-C g^{-1} organic matter in the most polluted samples near the mill. This is equivalent to 2.0-3.1% biomass-C of total soil-C in the non-polluted samples and 0.7-1.1% in the most polluted samples. The first sampling had lower values in the non-polluted sites, between 6 and 9 mg biomass-C g^{-1} organic matter, which decreased to less than 3 mg C in polluted soils. Basal respiration rate decreased with increasing Cu content of the soil (data not shown). Respiration rate was well correlated with biomass-C (r= 0.86 and 0.98 for the first and second sampling period, respectively).

ATP content of the soil was also well correlated to biomass-C (r=0.94). ATP, however, decreased more then biomass-C along the heavy metal gradient (Fig. 1). Thus, the ATP/biomass-C ratio (ATP measured at 4°C) decreased from about 3.3 μg ATP mg^{-1} biomass-C in non-polluted soil to about 1.7 μg mg^{-1} in the most polluted ones. Often soil ATP is measured after incubation at 25°C for 5 days (Tate and Jenkinson 1982). ATP measurements are temperature dependent and storage of humus samples for 5 days at 25°C gave ATP values 1.5 times higher than samples stored at 4°C (K. Arnebrant, unpublished). Using this conversion factor, the ATP/biomass-C ratio in non-polluted soils was around 5 μg mg^{-1}, which is comparable to previously published figures (Oades and Jenkinson 1979; Tate and Jenkinson 1982; Vance et al. 1987).

The effect of the heavy metal contamination on the specific respiration rate

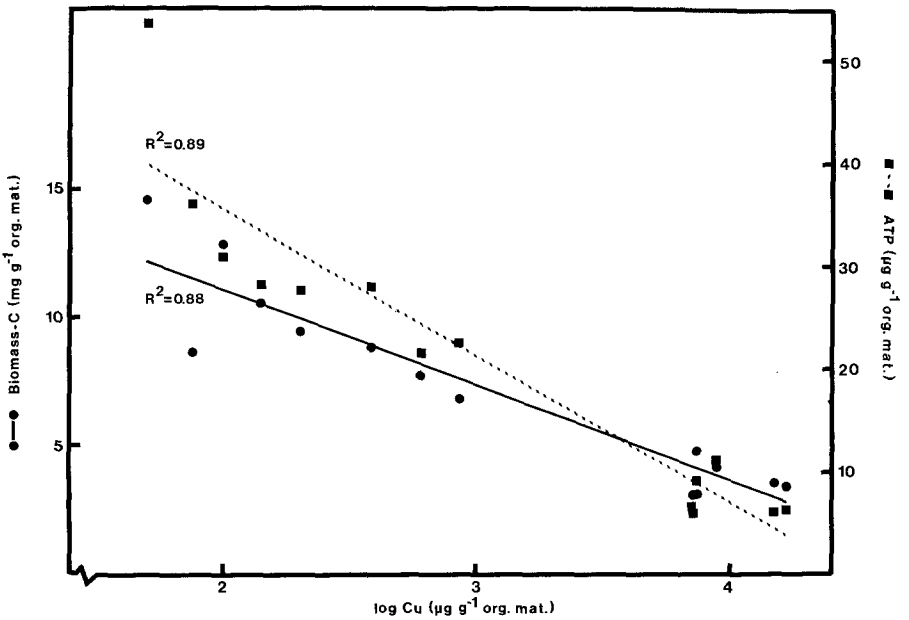

Figure 1. Microbial biomass-C, estimated with the substrate-induced respiration method, and ATP in the humus horizon of polluted coniferous forest around the Gusum smelter in Sweden. Lines were fitted by linear regression.

($q\text{-}CO_2$) was unclear. If expressed as respired CO_2-C per biomass-C unit measured with SIR, $q\text{-}CO_2$ decreased slightly with pollution level. At the second sampling occasion, slightly polluted plots (<400 ppm Cu) had a mean $q\text{-}CO_2$ value of $3.1 \pm 0.15 \times 10^{-3}$ mg CO_2-C mg^{-1} biomass-C (n = 4), while heavily polluted samples (around 10,000 ppm Cu) had a value of $2.7 \pm 0.17 \times 10^{-3}$ mg mg^{-1} (n = 6). The corresponding values from the first sampling occasion were $4.0 \pm 0.26 \times 10^{-3}$ and $3.7 \pm 0.19 \times 10^{-3}$ mg CO_2-C mg^{-1} biomass-C. On the other hand, when $q\text{-}CO_2$ was expressed as respired CO_2-C per unit ATP, an increase was found with increasing pollution levels, from 1.0 ± 0.16 µg CO_2-C μg^{-1} ATP in slightly polluted samples to 1.4 ± 0.08 µg CO_2-C μg^{-1} ATP in the most polluted samples.

The effect of the heavy metal pollution on microbial biomass was similar to that found earlier for soil respiration rate, enzyme activities and fungal biomass (Tyler 1974, 1984; Nordgren et al. 1983) The EcD_{50}, which is the metal concentration, where values from non-polluted soils are halved (Babich et al. 1983), was about 2,500 ppm Cu for biomass-C and ATP in the present study. This is similar to that calculated for the other measurements cited above.

Biomass-C as a proportion of total soil-C is usually very constant and varies little between different soils (Jenkinson and Ladd 1981). Values around 2%

found in non-polluted forest humus are within this range for natural soils. The decrease to below 1% in polluted soils thus emphasizes the disturbed biological function at these sites.

The decreased ATP/biomass-C ratio in polluted soils can be a further indication of a disturbed microbiological situation in these soils. Brookes and McGrath (1984) did not find any changes in the ATP/biomass-C ratio because of metal pollution. However, differences exist between our two studies. Brookes and McGrath (1984) studied pollution levels that were much less extreme (e.g., no effect on basal respiration rate was found). They also used the fumigation-incubation method to measure the microbial biomass-C content of the soil. Although biomass-C measured with the fumigation-incubation and the substrate-induced respiration technique are often well correlated (Anderson and Domsch 1978; West et al. 1986; Martens 1987), this might not be the case under the extreme conditions found near the Gusum mill. Further, there might exist a threshold value above which the ATP/biomass-C ratio indicate microbiological disturbances of the soil. Below this level of contamination, the ratio might be less important as an indicator of pollution.

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