Soil microbial eco-physiology as affected by short-term variations in environmental conditions

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Abstract

Short-term variations in abiotic and biotic environmental conditions modify microbial metabolism. These effects were studied in an arable soil during the oat straw decomposition. Cumulative CO₂ evolution was significantly higher when soil was subjected weekly to drying–rewetting cycles (DR) or had been initially fertilised with nitrogen (+N, as NH₄NO₃) in comparison to samples incubated at constant conditions (CC). The microbial metabolic quotient (respiration rate per unit microbial C) of DR and +N exceeded CC by approximately 100–150%, indicating that variable water availability and improved N availability induced a higher activity level of the microbial biomass. In addition, the metabolic-responsive microbial communities, calculated by the ratio of microbial C estimated by substrate-induced respiration to fumigation–extraction technique, increased from 1.0 to approximately 1.3 after 7 weeks for DR and +N, but remained essentially at 1.0 for CC and control. A high abundance of soil nematodes significantly affected respiration rate, metabolic-responsive biomass and metabolic quotient during the early stages of decomposition. We concluded that short-term variation in environmental conditions promoted decomposition processes by influencing the physiology of soil microorganisms and increasing the metabolic-responsive biomass. Such a stimulated microbial metabolism seems to be unspecific due to stressing environmental conditions. The role of the interacting fauna such as nematodes varied during decomposition. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Microbial biomass; Decomposition; Drying–rewetting; Eco-physiology; Soil fauna; Nitrogen

1. Introduction

Decomposition is of crucial importance in biogeochemical cycling and ecosystem functioning. Many aspects dealing with this process such as the liberation of nutrients, mineralisation of pollutants and xenobiotics, and organic matter conservation are important for sustainable agriculture and environmental quality. The process rates are self-regulated by a set of biotic and abiotic factors in stable ecosystem. Climate and substrate composition including C/N ratio, lignin content and soluble compounds are important factors regulating the decomposition rate. In ecosystems under moderate climate the fauna is one of the key components modulating mineralisation in soil (Anderson et al., 1985). Environmental factors and their long- and short-term variations affect decomposition rate through microbial activity and growth. However, there are few studies dealing with the response of the soil microbiota to short-term impacts and disturbance (Degens et al., 2001).

Abiotic and biotic environmental factors regulate soil microbial communities by affecting microbial biomass, activity and growth. Soil microbial biomass represents the main driving force performing the decomposition process (Kjøller et al., 2000) and can be reduced by stressors such as heavy metal (Fritze et al., 1996). However, stressors may stimulate the biomass-specific activity (Fließbach et al., 1994; Anderson and Domsch, 1993). Such modifications are often attributable to ecosystem disturbance (Dilly et al., 1997) or changes in agricultural management practice (Lupwayi et al., 1999).

Environmental conditions are generally unfavourable for extensive microbial growth. The soil microbiota need, therefore, to adjust their physiology. Non-active organisms, including profoundly dormant forms, are suggested to dominate soil microbial communities in natural ecosystems (Heinemeyer et al., 1999) and the addition of limiting nutrients generally promotes the decomposition of organic matter as demonstrated for N (Schlesinger, 1997). However, the effect of N availability may vary between early, later and final stage of decomposition (Berg and Matzner, 1997). In addition, the soil fauna may have stimulating or suppressive
effects on microbial biomass and activity dependent on soil N status (Mamilov et al., 2001). Thus, substrate availability and its quality regulate microbial activity and it is unclear how microbial physiological state and organic matter transformation are interrelated. The aim of this study was, therefore, to investigate the effect of short-term changing abiotic and biotic environmental conditions on both activity rate and eco-physiology of soil microbiota.

2. Materials and methods

2.1. Soil

Soil samples were taken from the A horizon (0–20 cm soil depth) of the crop rotation field in Bornhöved Lake District in northern Germany (Dilly and Munch, 1998). According to ISSS/ISRIC/FAO (1998), the soil was classified as a Eutri-cambic Arenosol. The soil contained 12.1 mg organic C g\(^{-1}\) dry soil, 1.01 mg total N g\(^{-1}\) dry soil, and had a pH (CaCl\(_2\)) value of 5.4. The soil has been under oat (Avena sativa L.) before sampling. Three independent soil samples were taken in the field and treated separately throughout the whole experiment. Soil were sieved through 2 mm mesh and stored with natural moisture content at 4 °C for 7 days until the experiment commenced.

2.2. Experimental design

Glass flasks were filled with 160 g of fresh soil that corresponded to approximately 140 g oven dry soil and amended with oat straw. The straw was harvested in August 1999, air-dried and milled. Soil was amended with 0.2% (w/w\(^{-1}\)) of oat straw and thoroughly mixed. This amount was equivalent to the annual in situ C input (Kutsch and Kappen, 1997). Two vials containing 4 ml of 1 M NaOH to trap CO\(_2\) and water to maintain humidity were put in each flask. All flasks were closed and incubated at 22 °C in the dark.

The following treatments were considered: (1) soil incubated at constant conditions (Control), (2) soil plus oat straw incubated at constant conditions (CC); (3) soil plus oat straw weekly subjected to drying and rewetting (DR) as follows: material was transferred to a plastic surface, spread in a thin layer and uniformly air-dried for approximately 3 h at 22 °C. Lost water was gravimetrically determined and samples were then re-moistened with deionised water; (4) soil plus oat straw and fertilised with N [+N] as NH\(_4\)NO\(_3\) equivalent to 131 µg per g soil (oven-dry weight) that corresponded to the amount of fertiliser applied in field conditions; (5) soil plus oat straw and nematodes added at day 14 to increase the natural abundance fourfold; (6) soil plus oat straw and nematodes added at day 28 to increase the abundance four times. Nematodes were extracted with Baermann funnels from equal amounts of native soil which had previously been pre-incubated for 10 days. The extracted volume was reduced to 20 ml by settling and decanting; 2.5 ml was applied on the surface of each sample. The addition of 2.5 ml soil extract without nematodes did not affect the soil respiration rate at constant conditions (data not shown).

Three replicate flasks were set up for all treatment. The samples for analyses were taken out from these independent flasks of each treatment after 7, 14, 21, 28 and 35 days of incubation.

2.3. Respiration rate and microbial biomass measurements

Respiration rate was estimated by trapping CO\(_2\) in NaOH solution during the incubation period. The remaining alkali was titrated with 0.1 M HCl after adding BaCl\(_2\) solution correspondent to the NaOH equivalents for the precipitation of CO\(_2\).

Soil microbial C was estimated by the substrate-induced respiration (SIR) method (Anderson and Domsch, 1978) on the base of the maximum initial respiratory response obtained after addition 5 mg glucose (mixed with talcum in ratio 3:5) g\(^{-1}\) dry soil. The amount of CO\(_2\) emitted was measured using gas-chromatography with a thermal conductivity detector. In addition, microbial biomass C was also measured by fumigation–extraction (FE) technique with \(k_{FC} = 2.64\) (Vance et al., 1987). Organic C in 0.5 M K\(_2\)SO\(_4\) extracts was determined using an automatic TOC/TNb-analyser (Fa. DIMATEC, Essen, Germany) with a catalysed dry-digestion procedure for organic C and chemo-luminescence procedure for total N determinations. The metabolic quotient, \(q_{CO2}\), was calculated as respiration rate per unit of microbial biomass estimated by FE.

The fungal and bacterial biomass were estimated on the base of its substrate-induced respiration following Anderson and Domsch (1973, 1978). Fresh soil corresponding to 2 g of oven-dry material received (A) 5 mg glucose and no inhibitors, (B) glucose +8 mg streptomycin (Merck), (C) glucose +25 mg cycloheximide (Sigma), (D) glucose +streptomycin +cycloheximide. The applied concentrations of antibiotics were tested for the selectivity of the inhibition effect and were preliminary adjusted to meet the prerequisite \(A - ((A - B) + (A - C)) = D \pm 7\%\). The ratio of fungal-to-bacterial biomass was calculated from the equation \((A - C)/(A - B)^{-1}\).

2.4. Animals measurement

Nematodes were extracted at room temperature with Baermann funnels containing 10 g of fresh soil. In the extracts total numbers of nematodes were counted under a light microscope without identifying species (Cairns, 1960).

2.5. Statistics

All measurements were carried out in triplicate. Standard deviations of the difference of the mean were calculated for
all data. Statistical analyses were performed using STATISTICA 5.0 (StatSoft, Inc., USA). Analysis of variance (ANOVA) and Tukey honest significant difference (HSD) test were additionally applied to evaluate differences between the treatments. Differences obtained at a level of $P < 0.05$ were considered significant.

3. Results

Oat straw amendment increased soil respiration rate (Fig. 1). Microbial biomass was also increased but only after 14 days in CC and +N and after 21 days in DR at a significant level ($P < 0.05$, Fig. 2). The respiration rates continuously decreased after 7 days when soil was incubated at CC. Respiration rate of DR was higher than the other treatments ($P < 0.01$) and exceeded the CC values by approximately 40%. Simultaneously, the cumulative amount of CO$_2$–C emitted from DR and +N was also higher in comparison to CC (Fig. 1). Noteworthy, microbial biomass C estimated by FE of DR was similar or lower than that of CC (Fig. 2).

The dynamics of soil N extracted with 0.5 M K$_2$SO$_4$ solution during oat straw decomposition did not reveal any difference between DR and CC treatments, whereas the amount of extractable N increased continuously in control and was significantly higher in +N treatment (Fig. 3).

The $q$CO$_2$ values of CC decreased during oat straw decomposition suggesting the decline of actively mineralising microorganisms and the increase in C-use efficiency as it will be discussed later (Fig. 4). On the contrary, metabolic quotients of DR did not decreased continuously and exceeded those under CC throughout the experiment by approximately 100–150%.

Fig. 1. Soil respiration during oat straw decomposition as rate (graph above) and cumulative CO$_2$ evolution (graph below); bars indicate standard deviations.

Fig. 2. Microbial C estimated by fumigation–extraction technique during oat straw decomposition related to soil mass (graph above) and related to the control (graph below); bars indicate standard deviations.

Fig. 3. Soil N content extracted with 0.5 M K$_2$SO$_4$ during oat straw decomposition; bars indicate standard deviations.
At the beginning of oat straw decomposition the ratio between the microbial biomass estimates (SIR and FE) varied between 0.82 and 1.00, and remained close to 1.00 during the course of the experiment in CC and Control (Fig. 5). However, DR, +N and high nematode abundance substantially modified SIR/FE ratio throughout the experiment.

The elevated abundance of soil nematodes significantly reduced microbial biomass and metabolic responsive component ($P < 0.01$) after 2 weeks of oat straw decomposition (Table 1). Such an effect was not observed after 4 weeks incubation. However, nematodes concurrently enhanced soil respiration rate, metabolic quotient after 2 and 4 weeks.

The ratio of fungal-to-bacterial biomass in soil continuously decreased during the course of decomposition from 3.02–3.25 to 1.66–2.33 in control and DR, respectively. At the end of the experiment the ratio also decreased in soil stored at constant conditions (Fig. 6) indicating an increasing bacterial portion in the total microbial biomass.

Additional N increased respiration rates by approximately 150% at day 10 in comparison to CC and exceeded significantly the values during following period. Fig. 1 showed furthermore that values of cumulative respiration with +N exceeded those of CC.

4. Discussion

4.1. Microbial biomass and mineralisation

Decreasing respiration rates in soil under constant conditions is attributed most likely to nutrient depletion in available straw fractions and the increasing dominance of recalcitrant compounds such as cellulose, hemicellulose and lignin (Neely et al., 1991). Concurrently, decreasing
microbial activity with proceeding incubation was earlier reported by e.g. Ocio et al. (1991), Dilly and Munch (1996) and Dilly (1999). However, soil subjected to drying–rewetting cycles demonstrated elevated CO₂–C emission values. These results showed similarities to those of Birch (1958), Sparling et al. (1986) and Clein and Schimel (1994) who demonstrated increasing mineralisation rates in rewetted soil and litter immediately after dry periods. Thus, short-term changes in environmental conditions promote the decomposition process. Comparable or even lower biomass values of DR suggest that constant conditions favoured microbial biosynthesis and reduced mineralisation in comparison to short-term changing environments.

Fig. 2 shows that straw addition sustained microbial growth for 14 days. It suggests further that substrate use efficiency increased with proceeding decomposition, which may be related to the predominance of K-selected organisms (Atlas and Bartha, 1998).

Mary and Recous (1994) supposed that drying–rewetting cycles caused an N flush. According to Sparling and Ross (1988) net C and N mineralisation after air-drying gave the CO₂–C/NMin ratio of 12.1 for the air-drying treatment and, thus, organic substrates with low C/N ratio such as microbial cells seems to be preliminary mineralised. In addition, Sparling et al. (1985) found that phosphorus released from killed microbial cells may explain extractable phosphorus following air-drying. However, Sparling and Ross (1988) showed that the N flush following the drying increased with increasing total C and N content, which implies decomposition of non-microbial compounds. Seneviratne and Wild (1985) concluded that N mineralised after mild drying derived from both killed microorganisms and organic matter of wide C/N ratio. According to Marumoto et al. (1982) microbial biomass N to total N-mineralisation flush after soil rewetting varied between 35 and 75%. Thus, increased mineralisation rate of soil subjected to drying–rewetting cycles may not completely be explained by elevated nutrients turnover from dead microbial biomass. Since extractable N was similar in DR and CC over 35 days (Fig. 3), the two treatments seem to have similar nutritional conditions for N. Moreover, significantly lower values of available N in DR than in +N treatment suggests that stimulated metabolism of microbial communities in soil subjected to drying–rewetting seems to be related to modified microbial eco-physiology rather than N availability. The following results indicate that short-term changes in environmental conditions modified the microbial eco-physiology by promoting organic matter decomposition.

4.2. Metabolic quotients

Several microbial quotients were proposed to characterise the soil microbiota. The quotients have not been widely used, as doubts exist as to their ecological significance. Papers from Anderson and Domsch (1990), Sakamoto and Oba (1994), Wardle and Ghani (1995) and Dilly and Munch (1998) have tried to fill this gap. Metabolic quotient such as respiration rate per unit microbial C was suggested to evaluate the effect of environmental conditions (Fließbach et al., 1994; Anderson and Joergensen, 1997; Alon and Steinberger, 1999).

Continuously decreasing metabolic quotient was also observed during leaf litter decomposition by Wardle (1993) and Dilly and Munch (1996) and was explained as the depletion of available nutrients and the development of K-selected organisms during successional changes. Breland and Eltun, 1999 hypothesised that low qCO₂ values were caused by a smaller proportion of active biomass. The metabolic quotients of DR did not decrease with stages of incubation indicating both more active microorganisms and lower microbial C use efficiency. The two facts explain higher dry matter losses via cumulative CO₂–C emission in DR (Fig. 1).

Odum (1985) and Anderson and Domsch (1993) mentioned that high qCO₂ values are attributed to stress. Therefore, we conclude that microbial communities of DR

### Table 1

<table>
<thead>
<tr>
<th>Day</th>
<th>CC Nematodes</th>
<th>CC Nematodes</th>
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<tbody>
<tr>
<td>14–21</td>
<td>Microbial biomass (µg C g⁻¹ soil)⁶</td>
<td>270⁶</td>
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<tr>
<td></td>
<td>Microbial biomass (µg C g⁻¹ soil)⁷</td>
<td>383⁵</td>
</tr>
<tr>
<td></td>
<td>Respiration rate (µg CO₂–C g⁻¹ soil h⁻¹)</td>
<td>0.63⁶</td>
</tr>
<tr>
<td></td>
<td>qCO₂ (mg CO₂–C g⁻¹ Cmic h⁻¹)</td>
<td>1.64⁵</td>
</tr>
<tr>
<td></td>
<td>SIR/FE ratio (g SIR-Cmic g⁻¹ FE–Cmic)</td>
<td>0.70⁵</td>
</tr>
<tr>
<td></td>
<td>Fungal/bacterial ratio (w/w)</td>
<td>2.82⁵</td>
</tr>
<tr>
<td>28–35</td>
<td>293⁵</td>
<td>298⁶</td>
</tr>
</tbody>
</table>

⁶ Microbial C estimated by SIR.
⁷ Microbial C estimated by FE.
were under stress and modified metabolism by accelerating nutrient turnover. Pratt and Barreiro (1998) observed increased turnover of nutrients in algal communities stressed by herbicide application. Fließbach et al. (1994) and Moreno et al. (1999) detected increased CO₂ in soils polluted with heavy metals. Thus, enhanced metabolism rate seems to be the microbial unspecific response to changing environmental conditions and corresponds to the stress response (Selye, 1950).

4.3. SIR/FE ratio

Significant correlations between biochemical and physiological microbial biomass estimates were frequently recorded (Lin and Brookes, 1996; Anderson and Joergensen, 1997). However, ratios between microbial biomass and activity estimates were shown to be an informative tool in evaluating microbial eco-physiology and environmental conditions (Dilly and Munch, 1998).

Good correlations between SIR and direct counts were determined during ryegrass decomposition by Lin and Brookes (1999). In our oat straw experiment, FE and SIR were correlated in Control and CC with \( r = 0.86 \) and 0.87 \( (P < 0.02) \), respectively. In contrast, the SIR/FE ratio in DR increased to up to 1.33–1.44. Considering that Anderson and Domsch (1978) described SIR-method for measuring microbial C in non-resting soil biomass, where 40 ml CO₂ corresponded to 1 mg biomass C, some difficulties might occur when the proportion of deeply dormant microorganisms is modified. Therefore, the SIR/FE ratio was used here to quantify the metabolic-responsive biomass, i.e. the component responsive to addition of available substrate. The quotient reflects the amount of microorganisms that might be potentially activated. The dynamics of metabolic-responsive component showed that the portion of microorganisms involved in decomposition process increased under changing environmental conditions.

4.4. The effect of the fauna

The biotic impact was different during the stages of decomposition (Table 1), which may be attributed to the fact that newly formed mycelium was consumed more readily by higher trophic levels in the soil ecosystem due to mycelium palatability and nutritive value (Kaneko et al., 1995). However, the increased nematode abundance enhanced soil respiration rate, metabolic quotient and, furthermore, the amount of active microorganisms after 2 and 4 weeks. Thus, grazing controlled the microbial eco-physiology irrespective of the stages of decomposition. In contrast, the effect of soil microfauna on dynamics of metabolic quotient was found to depend on substrate quality and in particular the C/N ratio (Mamilov et al., 2001) and will, therefore, be discussed later.

4.5. Fungal/bacterial biomass ratio

Bacterial contribution to the total microbial biomass increased during plant residues decomposition. This observation concurs with those of Neely et al. (1991) who recorded decreasing fungal/bacterial ratios during litter decomposition. The comparison of the dynamics of total microbial biomass and fungal/bacterial ratio in CC led to the conclusion that straw amendment promoted mainly fungal growth, while drying–rewetting cycles suppressed fungal development.

Soil nematodes enhanced the fungal/bacterial ratio after 2 and 4 weeks similarly (Table 1). The decrease in fungal/bacterial ratio after 2 weeks coincided with reduction of microbial biomass measured both using FE and SIR-techniques. This indicates that changes in microbial community composition at the initial decomposition period are attributed to selective grazing on fungal biomass. However, significant changes in total microbial biomass were not detected after 4 weeks when fungal/bacterial ratio also slightly decreased. Earlier studies demonstrated that soil nematodes and micro-arthropods stimulated bacterial growth (Mamilov et al., 2001). Therefore, increased abundance of soil nematodes modified fungal/bacterial ratio through gut passage, selective grazing and/or affecting nutrients turnover (Couteaux et al., 1994).

In comparison to the other treatments, the fungal/bacterial ratio increased in N-amended soil to the day 28 indicating increasing dominance of fungi. Soil fungi seem to produce more biomass C per unit of available N due to better competition in comparison to bacteria favoured by their higher biomass C/N ratio. Lundquist et al. (1999) measured short-term variability of chemical and biological conditions after rye residue addition to intact soil cores and mentioned that microbial C changed rapidly following rye incorporation and microbial N remained essentially constant. The effect was probably a consequence of variations in fungal biomass. Considering that +N enhanced fungal/bacterial ratio and DR reduced it, we propose that increased soil metabolism of DR is more related to the changes in microbial eco-physiology than nutritional conditions.

4.6. The role of nitrogen

Oat straw has a C/N-ratio of approximately 80 and represents an N-poor substrate. Nitrogen addition improved nutritional conditions for soil microbiota (Fig. 3) and regulate with available C compounds the decomposition process (Groffman, 1999). Fig. 1 suggests that microbial communities were limited after the first week by available N. This result agrees with findings of Cochran et al. (1988) for wheat straw decomposition in soil amended with KNO₃. Fig. 1 shows further that cumulative respiration of +N exceeded that of CC. Concurrently, Skene et al. (1996) demonstrated that N addition favoured C mineralisation for the first 5 weeks.
Additional N affects the physiological state of soil microorganisms. The enhanced $q_{CO_2}$ values reflect the higher portion of actively mineralising microorganisms due to higher N availability that had limited the metabolism previously. Lovell et al. (1995) detected that the number of culturable bacteria were increased by four times due to the N amendment. The dynamics of metabolic responsive biomass indicates that N amendment de-repressed the metabolism of microbial communities and the portion of biomass contributing the SIR increased during the course of decomposition. The opposite dynamics of fungal/bacterial ratio in DR and +N soil indicates that microbial communities different in C use efficiency have been developed. The effect of N addition on microbial metabolic quotient and respiration rate corresponds to grazing when N-poor substrates are decomposed (Mamilov et al., 2001).

5. Conclusions

Short-term changing environmental conditions such as drying–rewetting cycles and mineral N fertilisation promoted oat straw decomposition by increasing metabolic–responsive component of the total microbial biomass most likely due to de-repression of soil microbial metabolism. Both, portion of physiological active microorganisms in total microbial biomass and the microbial C-use efficiency were higher under short-term changing environmental conditions, indicated by the increased values for SIR/FE ratio and metabolic quotient, $q_{CO_2}$. The biotic ‘nematodes abundance’ impact stimulated decomposition most apparently during the initial stage of decomposition. Additional nitrogen affects decomposition by modifying both microbial eco-physiology and nutritional conditions.

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