COMPETITION FOR NITROGEN DURING MINERALIZATION OF PLANT RESIDUES IN SOIL: MICROBIAL RESPONSE TO C AND N AVAILABILITY

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Summary—In an experiment where N-poor (barley straw) and N-rich (clover leaves) plant residues were placed into soil as separate layers, negative effect of plant roots on microbial growth was observed. The depression effect occurred predominantly in N-poor plant residue layers, which indicated that microbial growth can be limited by N availability in such microsites in soil. More energy substrates were available in the planted straw layer, as indicated by a significantly higher N immobilization potential in the planted soil compared with that in the unplanted soil. However, in the N-rich sites, plant roots stimulated N mineralization during 1 wk incubation of soil slurries. Microbial response to initial growth conditions, including C and N availability, are also discussed. © 1997 Elsevier Science Ltd

INTRODUCTION

Soil microbial biomass as an N sink has been stressed (Paul and Juma, 1981), since microbial cells and metabolites are preferred substrates by active microorganisms due to their low C-to-N ratio of the chemical compositions (Jawson *et al.*, 1989). Dead cells of microorganisms are easily decomposable, and based on this a biochemical method to estimate soil microbial biomass has been developed (Jenkinson, 1966; Jenkinson and Powlson, 1976a,b). The predation of microbes by protozoa and soil animals also stimulate mineral N release (Coleman *et al.*, 1978; Elliott *et al.*, 1979; Clarholm, 1985). Some of the evidence has shown that, during the early stage of plant residue decomposition, when straw mixed with soil was incubated, most of the mineral N (labelled as ^{15}N) was immobilized into the fractions which are associated with microbial biomass (Cheng and Kurtz, 1963; Stewart *et al.*, 1963; Jawson and Elliott, 1986). Under field conditions, Jackson *et al.* (1989) demonstrated that more than one-third of injected mineral N could be recovered in the microbial biomass fraction in a California grassland soil, within 24 h of incubation *in situ*.

Soil microorganisms are very active, provided that substrates are available. However, soil bacteria in general are oligotrophs, and the small cells ("dwarf cells") dominate due to limitation of available C and energy sources. Yet some investigations have also indicated that microbial growth in soil is limited by the availability of mineral nutrients rather than energy substrates in the rhizosphere (Helal and Sauerbeck, 1986; Merckx *et al.*, 1987). In a growth experiment with groundwater bacteria, it was found that bacterial growth could be limited by phosphorus rather than by C (Bengtsson, 1989). In an investigation with plants in pots (Wang and Bakken, 1989) we found that microbial growth was depressed as a result of the competition for N between plant roots and the microflora.

Under a plant cover, the continuous flux of organic C materials from active plant roots represents a significant input of energy sources to the immediate soil, and sustains a larger community of rhizosphere microflora compared to that in bulk soil (Rovira and Davey, 1974). The flush of organic C, occurring with an amendment of plant residues, is another important C source for soil microorganisms. In both cases there must be some microsites where microbial growth may not be limited temporarily by energy substrate, rather but by mineral nutrients, especially N availability.

In the present study, with further refinement under the control conditions compared to a previous study (Wang and Bakken, 1989), soil microbial biomass and potential N mineralization or immobilization were determined, attempting to take

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the experiment one further step by looking at the microbial response to N-depletion, resulting from the competitiveness of plant roots for mineralized N.

MATERIALS AND METHODS

In a phytotron experiment (experiment 1 described in detail by Wang and Bakken, 1997) studying competition between plant roots and heterotrophic microflora for N during decomposition of plant residues in soil, bacterial numbers, hyphal lengths and potential N mineralization or immobilization in various soil samples were determined. Five treatments in the experiment were prepared with varying distance between N-rich and N-poor (C-rich) plant residues (clover leaves and barley straw). Then, barley plants were transplanted into the pots and the experiment was conducted under controlled conditions for 6 wk. Two weeks after planting, samples were taken every week. These soil samples were either composite samples from whole pots (200 g soil dry wt pot⁻¹), or samples from the separate layers of straw or clover material plus adjacent soil (about 100 g of each). Later in the text, "clover layer" and "straw layer" refer to corresponding plant residue together with nearby soil (see Wang and Bakken, 1997 for more detailed information).

Determination of microbial biomass

Preparation of soil samples Roots were first separated from the soil by dry sieving (2 mm), and soil adhering to the roots was washed with two portions of 100 ml distilled water (or 50 ml distilled water for the separate layers), which were combined with the sieved soil into a slurry. Subsamples were taken from this slurry by a syringe during rapid stirring. The subsamples of soil slurries for biological analysis were homogenized further with a Warring Blender (three 1 min steps, cooled in ice periodically) and sampled for determination of bacterial numbers and fungal hyphal length.

Bacterial counts The numbers of bacterial cells were counted by fluorescence microscopy after staining with Acridine Orange (Acridine Orange Direct-Count, AODC; Hobbie *et al.* (1977). Separate counting within six different size groups was conducted (Bakken, 1985). The calculated mean cell volume (μm^3) in each size group were: I, 0.034; II, 0.11; III, 0.32; IV, 0.90; V, 2.35; VI, 6. The biovolume was converted to biomass dry weight and N by assuming 0.33 g dry wt ml⁻¹ biovolume, and 12% N in biomass (Bakken, 1985).

Fungal counts Fungal hyphal length was measured by fluorescence microscopy, stained with fluorescein isothiocyanate I (FITC) and cross-points in a grid in the ocular were counted. The hyphae were divided into three diameter groups: 0–3, 3–6

and >6 μm . Fungal biovolume was calculated separately for each group and combined. The fungal dry weight and N content was calculated assuming 220 mg dry wt ml⁻¹ (Bakken and Olsen, 1983) and 4.5% N in fungal dry weight.

N mineralization or immobilization potential

Soil N mineralization or immobilization potential was determined by measuring the increase or decrease in mineral N during a 1 wk incubation of soil slurries, with or without the addition of nitrate prior to the incubation. Subsamples were taken as described above, and for each soil sample (slurry), four replicates of 150 ml Erlenmeyer flasks containing 5 ml slurry (3 g soil dry wt) were prepared. Control samples for determination of initial concentrations of mineral N were frozen immediately.

The flasks was either given 1 ml $\text{Ca}(\text{NO}_3)_2$ solution (0.3 mg $\text{NO}_3^- \text{-N ml}^{-1}$) or 1 ml distilled water prior to incubation. Nitrate was only given to those samples where net N-immobilization was expected (straw layer and mixture of clover and straw layers).

All flasks were covered with aluminum foil and placed on a reciprocal shaker at 80 rev min⁻¹ at 21°C. A week later, the incubated and frozen slurries were extracted with 1 M KCl solution (final concentration), and NH_4^+ - and $\text{NO}_3^- \text{-N}$ were determined using a flow injection analysis technique (FIA).

Statistical analysis

Standard statistical methods (analysis of variance, Stagraph) were used to test the effects of plant roots, distance between two plant residues and the interaction between these factors (samplings treated as blocks), and the effects were tested against a residual mean square.

RESULTS

Microbial counts and biomass

Bacterial counts and dry weight (average across treatments C–E) are shown in Fig. 1. As in a previous experiment (Wang and Bakken, 1989), plant roots had a negative effect on bacterial growth. Although the bacterial numbers were not significantly different between the planted and unplanted treatments [Fig. 1(a)], bacterial dry weight (DW) in the planted soil was significantly lower ($P = 0.01$) compared with that in the unplanted soil [Fig. 1(b)].

Bacterial counts and dry weight in the clover and straw layers (average across treatments A and B), are shown in Fig. 2. As expected, the negative effect of plant roots on bacterial growth occurred predominantly in the straw layers, and the difference in bacterial DW between the planted and the unplanted treatment was statistically significant ($P = 0.01$).

Fig. 1. The effect of plant roots on bacterial growth in the mixed layer samples, averaged across treatments C–E. (a) Bacterial counts; (b) biomass. Standard error shown as vertical bars ($n = 3$).

Bacterial biomass in each cell size group, averaged across treatments and samplings, is shown in Fig. 3. The difference in microbial biomass between the planted and the unplanted soil was attributed to the increase in the numbers of the large-celled bacteria (size groups III–V) in the mixed layer samples (treatments C–E, Fig. 3, upper part), and those large cells were major contributors to bacterial biomass in the soil. The bacterial numbers in cell size group VI were very low, and were of no significance to the total bacterial biomass. When the layers were separated, it was observed that the negative effect of plant roots on the growth of the large-celled bacteria occurred mainly in the straw layer (Fig. 3, lower part), whereas in the clover layer no such significant effect ($P = 0.05$) was observed (data not shown).

The fungal length and dry weight fungal biomass was a minor part of the total microbial biomass, and there was no significant difference at the $P = 0.05$ level in fungal biomass between the planted and unplanted soil (data not shown).

For the separate layers (data not shown), it was observed that in the first four samplings fungal dry weight was only slightly lower in the planted straw layer compared to the unplanted straw layer.

N mineralization or immobilization potential

Observed N-mineralization or immobilization in the slurries during a week of incubation has been transformed to a "per pot basis", i.e. mg N pot⁻¹. The data presented are based on the sum of ammonium and nitrate N. Most of the mineral N released during the incubation had been nitrified (data not shown).

Net N-mineralization or immobilization in incubated slurries of treatments C, D (layers not separated) and E (mixture) have been averaged, and are shown in Fig. 4. In the slurries with mineral N added, there was a high potential for immobilization throughout the whole experiment; significantly higher ($P = 0.01$) for the planted than for the unplanted soil. The slurries without nitrate N addition gave very low values for the planted soil (N-immobilization potential exceeded the amounts of mineral N present); in the unplanted soil there was a gradual increase in net N-mineralization.

Net N mineralization (in the clover layer) and immobilization (in the straw layer) during slurry incubation are shown in Fig. 5. As expected, straw and clover layers had considerable potentials for N immobilization and mineralization, respectively. Net N mineralization in the planted clover layer was sig-

Fig. 2. The effect of plant roots on bacterial growth in the separate clover and straw layers, averaged across treatments A and B. Bacterial counts (upper part) and biomass (lower part), with standard error bars ($n = 2$).

nificantly higher ($P = 0.01$) than that in the unplanted clover layer, except at the first sampling (statistically significant at $P = 0.01$ for each sampling). In the straw layer, there was a significantly higher ($P = 0.05$) N immobilization potential for the planted than for the unplanted soil.

When the net N mineralization or immobilization is plotted against both bacterial counts and bacterial dry weight, there was only a slight positive effect of microbial biomass on N mineralization potential in the clover layer (data not shown). No other correlations between microbial biomass and N mineralization and immobilization were observed.

Microbial N turnover rate

Microbial (bacterial) N turnover rate during a week of incubation in the separate layers has been calculated based on the following formula:

Turnover time (days) =

$$\frac{\text{bacterial N/net N mineralization}}{\text{or immobilization d}^{-1}}$$

and the results are shown in Fig. 6. Initially, there

was high microbial N turnover rate, in both the clover and the straw layers. As the plant residue decomposed, the turnover rates gradually decreased, especially for the unplanted clover layer. Towards the end of the experiment, the microbial N turnover rate also declined in the planted treatment.

DISCUSSION

Compared to the results of a previous greenhouse experiment (Wang and Bakken, 1989), fungal biomass was much lower (one-seventh to one-tenth of the previous estimate). Because we had to use low soil dilutions there were more soil particles on the membrane filters. This made it difficult for us to measure the fungal length accordingly.

Normally, microbial growth in soil has been thought to be limited by energy substrates rather than mineral nutrients. However, the evidence, provided in Fig. 1, suggested that N availability can also be a limiting factor for soil microbial growth. The significantly lower microbial biomass in the planted soil compared with that in the unplanted soil clearly demonstrated that the microbial growth was limited by plant roots, and the limiting factor was the N-depletion created by plant root uptake of

Fig. 3. Bacterial cell size distributions, as affected by plant roots. The data has been averaged across treatments C–E and samplings for the mixed layer samples (above, $n = 15$), and averaged across treatments A and B, and samplings for straw layer (below, $n = 10$).

Fig. 4. N Mineralization and immobilization during 1 wk incubation of soil slurries with both straw and clover treatments C–E (the data averaged across these treatments), as affected by plant roots and nitrate addition, with standard error bars ($n = 3$).

Fig. 5. N mineralization or immobilization (average across treatments A and B) during 1 wk incubation of soil slurries, as affected by types of plant residues and plant roots. Nitrate was only added to the slurries sampled from straw layer. Standard error bars are shown ($n = 2$).

Fig. 6. Estimated turnover time of microbial N in the slurries: bacterial N divided by the net N-mineralization (clover layers) or N-immobilization (straw layers) per day.

mineralized N (Wang and Bakken, 1997). As shown in Fig. 2, the depressing effects of plant roots principally occurred in the straw layer. A significantly higher N immobilization potential in the planted straw layer was observed (Fig. 5). Therefore the competitiveness of plant roots for mineralized N had induced a more severe N stress condition for the microorganisms at the N-poor sites. Our study agrees well with the previous investigation carried out in a greenhouse (Wang and Bakken, 1989). With the further refinement in the present study, it has been confirmed that N availability is actually more important than C availability in the promotion of microbial biomass production at some microsites in soil. Our results thus verified the hypothesis that microbial growth in soil can be limited by nutrient supply, as suggested by Sparling *et al.* (1982), Helal and Sauerbeck (1986) and Merckx *et al.* (1987).

The depressing effect of plant roots on bacterial growth in the straw layer and the mixed soil samples occurred predominantly for the large-celled bacteria (Fig. 3), as was also shown by Wang and Bakken (1989). This seems contradictory to the general view that C starvation induces a reduction of the size of bacterial cells. As suggested by

Kjelleberg *et al.* (1983) and Moyer and Morita (1989), the size reduction of the C-starved cells was common for Gram-negative marine bacteria. For soil bacterial isolates, Luscombe and Gray (1974) demonstrated that the rod-type cells of four arthrobacter strains divided and produced cocci during C starvation. The dwarf cells are normally thought of as survivors or survival stage during energy limitation (Shnürer *et al.*, 1985; Bakken and Olsen, 1986).

However, changes in the relative concentrations of large bacterial cells might also reflect the nutritional status in the environment. The effect of nutritional status, for instance N stress in our case, on bacterial growth may not be direct, but rather indirect. The significantly higher potential N immobilization induced by plant roots indicated that C substrates were more abundant in the planted soil than that in the unplanted soil. However, those C substrates probably consisted of polymers and were not readily available to the bacteria. Therefore it would be necessary for the microorganisms to produce extracellular enzymes for utilization of the polymers. It was more likely, in our present study, that the synthesis of the exoenzymes was stressed more severely by N availability in the planted soil

Fig. 7. Scheme of the relation among possible N pools derived from clover.

compared to the unplanted soil, and consequently an inefficient use of energy substrate.

Regarding N mineralization potential in the clover layer, somewhat unexpected results occurred. To facilitate the discussion, a scheme is prepared and shown in Fig. 7. The observation that the soil organic N was practically identical (Wang and Bakken, 1997) in both the planted and unplanted clover layer shows that plant roots had a negligible effect on the overall net N-mineralization (2) in the layer. However, the potential N-mineralization in slurries of clover layers was substantially higher (except in the first sampling) for the planted than for the unplanted soil, indicating that more N was present in a labile form in these pots. These two observations taken together indicate that the plant roots had either stimulated the decomposition rate (1) or reduced the stabilization of N (3). One mechanism of N-stabilization which might be affected by plant root uptake of mineral N is the reaction between nitrite and organic material (Azhar *et al.*, 1986). Further investigation is required to clarify the phenomena.

Microbial biomass could hardly be taken as an indicator of the extra mineralizable N in the planted clover layers. Firstly, there was no clear link between microbial biomass and N mineralization or immobilization potential in treatments where straw and clover layers were mixed (treatments C–E), and a slight positive correlation between microbial biomass and N mineralization potential only occurred in the clover layer (data not shown). Secondly, the variation of size of microbial biomass was not clearly coincident with the variation of amount of N released during the incubation of soil slurries. Thirdly, the microbial N turnover rate changes with type of plant residues and the time they take to decompose.

As stated earlier, soil bacteria are living in starvation conditions with occasional flushing of energy substrates from plant roots and amendment of plant residues. When ample C materials are readily available, bacteria take up organic C for growth, and some of them even have unbalanced growth, including production of some storage materials, such as poly- β -hydroxybutyric acid (PHB), uncoupling respiration (Dawes, 1989) and bacterial cell

size enlargement (Morita, 1988), not necessarily with storage materials present. In our present study it could have occurred immediately after amendment with barley straw. In a situation like this, microbial biomass could hardly be related to any net N mineralization; rather a net N immobilization (in straw layer) if N was available. This is in contrast to the clover layer, where there was a slight correlation between microbial biomass and total N mineralization. It seems that the initial growth conditions, including C and N availability, rather than microbial biomass were decisive, since in time, N mineralization or immobilization potential decreased (Figs 4 and 5).

Obviously, the microbial N turnover rate was affected by plant roots in the present experiment as shown in Fig. 6, especially in the clover layer, where no difference in microbial biomass was found (Fig. 2). This demonstrates clearly that the part of active microorganisms, rather than total microbial community, determined potential N mineralization or immobilization. Also, stimulation of microbial activity by plant release of organic C materials might have occurred in the planted pots.

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