NOTES

Changes in Cytoplasmic Carbon and Nitrogen Pools in a Soil Bacterium and a Fungus in Response to Salt Stress

JOSHUA P. SCHIMELO,† WARREN J. SCOTT, AND KENNETH KILLHAM

Department of Plant and Soil Science, University of Aberdeen, Aberdeen AB9 2UE, Scotland

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The effects of water potential on the cellular compositions of a soil bacterium and a fungus were examined by growing the organisms in media with various KCl concentrations. In media containing up to 1 M KCl, C/N ratios in Aspergillus flavus increased significantly, while those for Pseudomonas sp. did not. For both organisms, the proportions of cellular C and N in cytoplasm increased by a factor of 10 as salinity increased from 0 to 1 M KCl. Such compositional changes have implications for microbial biomass dynamics in soils of varying water potential and for biomass measurement by chloroform fumigation.

Microbial biomass is a critical nutrient pool in soil. Microbes act as both a source and a sink of nutrients, and processing through biomass is a key step driving nutrient cycles (3, 8, 14). Factors that can alter the size and cellular composition of the microbial biomass may therefore be important in controlling soil nutrient availability; one such factor is soil water potential.

To maintain internal water potentials at equilibrium with the soil, microbes accumulate internal solutes, generally amino acids in bacteria and polyhydric alcohols in fungi (2, 6, 11). A decrease in water potential would therefore be expected to increase fungal cytoplasmic C content and C/N ratios. Bacterial C and N contents would be likely to increase with water stress, although the C/N ratio should change little. Changes in water potential may thus affect microbial nutrient requirements. Increasing the proportion of material in soluble constituents relative to cell wall polymers could also affect the dynamics of microbial nutrient pools (5).

The implications of changing cellular constituents in response to soil water potential have received little consideration in the literature on nutrient cycling. A model of soil nutrient dynamics proposed by Van Veen et al. (14) incorporated changes in microbial dry matter with water potential; however, these authors reported no changes in the C/N ratio with water potential. A model of fungal growth (9, 10) suggests that decreasing the water potential may increase cell wall growth relative to cytoplasmic growth, thus increasing the fungal C/N ratio. Kief et al. (5) examined the effects of changes in soil water potential on microbial biomass and found that up to 60% of the microbial C was released and metabolized when a dry soil was moistened.

The changes in microbial cytoplasmic content in response to water potential may also have implications for the measurement of soil microbial biomass C and N. Current techniques for measuring biomass C and N use fumigation with CHCl₃ vapor to release microbial C and N, which are either extracted (1, 13) or determined by an incubation bioassay (4). With both techniques, only a portion of the total microbial C or N is recovered; this is related to the total by an empirical constant (Kᵣ or Kᵣᵠ). Most of the material recovered presumably is cytoplasmic, and if the proportion of cytoplasmic C or N varies, the value of Kᵣ or Kᵣᵠ should vary as well. Effects of water potential on Kᵣ and Kᵣᵠ may therefore be a source of error in microbial biomass measurements, particularly direct-extraction methods, which are considered applicable in a wider range of soil conditions than is fumigation-incubation (1).

In this study we examined the relationship of microbial cell composition and water potential. We grew a soil fungus and a bacterium in media with various KCl concentrations (i.e., over a range of water potentials) and measured both their total and chloroform-labile (cytoplasmic) C and N contents.

Two microorganisms, a Pseudomonas sp. and Aspergillus flavus, were studied. Both microorganisms were recent soil isolates and had been maintained on agar slants. The microorganisms were grown for 10 days in shake cultures (100 rpm) at 20°C and harvested in the late log phase. Batch cultures consisted of 2.5-liter Erlenmeyer flasks containing 1 liter of the appropriate growth medium. The fungal inoculum consisted of two disks (1-cm diameter) of mycelia from the leading edge of colonies grown on Czapek Dox agar. The bacterial inoculum consisted of a starter culture (10 ml) of cells in the late log phase grown in the appropriate basal medium.

The basal media (i.e., no additional salt stress) for liquid batch culture were as follows (all amounts are per liter): (i) for A. flavus, sucrose, 10 g; NaNO₃, 2.0 g; K₂HPO₄, 0.1 g; KCl, 0.5 g; MgSO₄ · 7H₂O, 0.5 g; and FeSO₄, 5 mg; and (ii) for the Pseudomonas sp., glucose, 10.0 g; peptone, 1.0 g; yeast extract, 1.0 g; K₂HPO₄, 0.4 g; (NH₄)₂HPO₄, 0.5 g; MgSO₄ · 7H₂O, 0.05 g; MgCl₂, 0.1 g; FeCl₃, 0.01 g; and CaCl₂, 0.1 g. Added to these basal media were various concentrations of KCl (0, 0.5, 1.0, 1.5, and 2.0 M) to create a range of salt stresses. Three replicate flasks were used for each microorganism and each salt concentration.

At harvest, cells were collected by filtration onto a polycarbonate membrane filter (0.2 μm). The cells were then washed three times with isotonic solutions (25 ml each). A
subsample of the cells (25 mg) was oven dried (80°C) to determine moisture content. The remaining cells were divided into thirds. One-third was freeze-dried, ground, and analyzed for total cellular C and N as described below. One-third was fumigated with chloroform vapor for 24 h to lyse the cells, which were then extracted with 0.5 M KC1 to determine soluble cytoplasmic C and N (1). The final third was extracted with KCl to determine extractable noncytoplasmic C and N; this was the control for the fumigation treatment. C and N were not detectable in any of these control extracts, so the amounts of C and N in the fumigated samples were equal to the amounts of soluble cytoplasmic C and N. All cell extracts were filtered through nylon membranes (0.1 μm) and then freeze-dried. Samples of the freeze-dried cells and cell extracts were analyzed for C and N contents by using a Carlo Erba dry combustion C-N analyzer, which was calibrated with glutamic acid and adjusted for ash content. A C-N analyzer was used for measuring organic C because Cl− can interfere with Cr2O3 wet digestion (7).

For each organism, the effect of salt concentration on various characteristics was tested by one-way analysis of variance and Duncan’s multiple-range test. Effects were considered significant at P = 0.05.

While the two organisms tested grew differently in response to salt stress, they generally showed a pattern of relatively stable growth over a range of salt concentrations, followed by a rapid decline in growth at some critical concentration. This may correspond to the point at which the organisms could no longer accumulate organic solutes and started accumulating the stress solute KC1 (6).

A. flavus grew well with salt concentrations up to 1.5 M KC1, at which the biomass produced was 75% of that in the control sample (Table 1). Only at 2 M KC1 did biomass production drop off substantially. The Pseudomonas sp. was less tolerant of salinity; growth decreased sharply at 1.0 M KC1 and there was no growth with salt concentrations above 1 M KC1.

Both the total cellular and the cytoplasmic C/N ratios in the Pseudomonas sp. were low and were unaffected by salt stress (Fig. 1). These results were as expected; bacteria accumulate amino acids as compatible solutes (2, 6) and their normal C/N ratio is low, so a change in response to salt stress would be unlikely.

In the Pseudomonas sp., the amounts of cytoplasmic C and N increased significantly with salt concentration (Fig. 2), to up to 28% of total cell C and 57% of cell N. This increase was certainly due, in part, to amino acid accumulation. The increases in cytoplasmic C and N were, however, larger than the increase in the free amino acid pool of two streptomycetes (6), each of which accumulated approximately 15% of
crobes, then this should be considered in studies and models of soil nutrient dynamics. In soils with changing water potential, microbes would need to periodically take up and then release large amounts of C and N, producing major fluctuations in the microbial cellular composition (5, 12). Some of the material accumulated by the *Pseudomonas* sp. and *A. flavus* was probably not compatible solutes and would therefore not have to be flushed from the cell immediately after an increase in water potential. It is likely, however, that this material would shift from an osmoregulatory function to a growth substrate. While microbial biomass is frequently thought of as being stable or protected (14), these results suggest that there could be two functionally distinct components to the soil biomass, a stable structural component and a labile cytoplasmic component, the sizes of which are determined by soil water potential.

Soil organisms are frequently limited by either C (8) or N (12a), and the need to accumulate C and N may cause nutrient limitation to be a major component of the microbial response to water stress. This problem would be exacerbated in soils with low matric potentials, as thin water films can limit nutrient diffusion (2, 9, 10).

Our data also indicate the hazards of using CHCl₃ fumigation biomass assays in dry or saline soils. If soil is moistened before fumigation, biomass C and N may be released (5, 12); if soil is not moistened, the correction factors (Kₑ and Kₐ) may vary. If only cytoplasmic C and N are recovered after fumigation, then Kₑ and Kₐ could vary by a factor of 10 over a range of water potentials common in soil (Fig. 2). We therefore recommend careful consideration of soil water potential before use of CHCl₃ fumigation microbial biomass assays, particularly those using direct extraction of cytoplasmic constituents (1, 13).

In conclusion, water potential is an important control of microbial cell composition and may thereby have major effects both on the dynamics of the microbial biomass and on its measurement.

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**LITERATURE CITED**