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# Spatial pattern of enzyme activities depends on root exudate composition

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Roots increase microbial activities, depending on exudate composition, especially on the ratios of sugars, carboxylic and amino acids, and thus structure enzyme activities in the rhizosphere. We introduce a new approach combining soil zymography and simulated exudates released from Rhizon® samplers to avoid the direct release of enzymes by living roots. This enabled visualizing, localizing and analyzing the effects of simulated root exudates on activity of five enzymes involved in carbon (C) ($\beta$-glucosidase, cellobiohydrolase), nitrogen (N) (leucine aminopeptidase), phosphorus (P) (phosphatase) and sulfur (S) (sulfatase) cycles. We tested the hypotheses that 1) artificial exudates stimulate microorganisms for enzyme production and form spatial gradients around roots, and 2) the extent of microbial enzyme activities in the rhizosphere is component-specific. In line with these hypotheses, the activities of P-, N- and S-related enzymes were higher near the artificial root and gradually decreased as a function of distance to the root, whereas the pattern for C-cycle enzymes was uniform and independent of the exudate composition. Among all components, alanine increased the rhizosphere extent much stronger than other substances, while methionine had no effect on the spatial distribution of enzymatic activities. Vmax of all enzymes increased with glucose and alanine addition, but decreased after adding citrate. The ratios of enzyme activities demonstrated that rhizosphere microorganisms release more leucine aminopeptidase than other enzymes to meet their N demand. Glucose increased the Km of cellobiohydrolase and $\beta$-glucosidase, while alanine had the greatest effect on the Km of protease and sulfatase. Phosphatase had the most sensitive enzyme system to the composition of artificial root exudates, which means that any factor influencing root exudate composition can strongly affect the P cycle. We conclude that the rhizosphere extent of microbial-derived enzyme activities is component- and enzyme-specific and that this extent depends on the substrate stoichiometry and soil microbial nutrient demand.