Microscale distribution and function of soil microorganisms in the interface between rhizosphere and detritusphere

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Abstract
We used a three-compartment pot design to study microbial community structure and enzyme activity in the interface between rhizosphere and detritusphere. All three compartments were filled with soil from a long term field trial. The two outer compartments were planted with maize (root compartment) or amended with mature wheat shoot residues from a FACE experiment (residue compartment). Soil, residues and maize differed in ^{13}C signature ($\delta^{13}C$ wheat residues -44.1‰, soil -26.5‰ and maize roots -14.1‰) which allowed following the transfer of residue and root-derived C into microbial phospholipid fatty acids. In the interface between rhizosphere and detritusphere, activities of β-glucosidase, xylosidase and phosphatase and the abundance of bacterial and fungal PLFAs were higher in the first 1-2 mm of the root and residue compartment, with generally higher activities in the vicinity of the residue compartment. The $\delta^{13}C$ of the PLFAs suggests that incorporation was limited to the first 1 mm from the residue or root compartments with residue-derived C being incorporated by the soil microorganisms to a greater extent than root-derived C.

Key Words
^{13}C, C flow, enzymes, maize, microbial community structure, roots, wheat

Introduction
The soil influenced by the roots (rhizosphere) and the soil surrounding plant residues (detritusphere) are characterised by high concentrations of easily available compounds and therefore are hot-spots of microbial activity. Close to the roots or residues, microbial density and activity and enzyme activity are high and decrease with increasing distance; forming distinct gradients in mm scale. Moreover, microbial community structure changes with distance from roots or residues (e.g. Kandeler *et al*. 2001, Poll *et al*. 2006). Properties of the rhizosphere and detritusphere have been studied extensively, but separately. However, in the field, roots usually grow in the vicinity of decomposing plant residues. Therefore, it is important to study the interface between rhizosphere and detritusphere on a mm scale. We used a three-compartment pot system with maize on one side and soil with wheat residues on the other, each separated from the 5 mm middle compartment by a 50 µm mesh. Soil, plant and residues differed in ^{13}C signature. The middle compartment was sliced into 1 mm sections. In each section, enzyme activity and microbial community composition by PLFA were measured. Using the differential ^{13}C signatures, we tracked residue or root-derived C in the PLFAs. We hypothesised that: (i) the gradients in enzyme activity and microbial community structure of rhizosphere and detritusphere overlap leading to intermediate values in mid-distance from roots and residues, or (ii) the gradient from either roots or residues dominates, diminishing the gradient from residues or roots, respectively.

Methods
Experimental set up
The experiment was conducted in a three compartment pot system with two outer compartments, each separated by a 50 µm mesh from a 5 mm wide middle compartment. The outer compartments were filled with 664 g and the middle compartment with 99 g dry soil equivalent.
The soil was an agricultural top-soil (Chernozem; C_{org} 1.4%; pH ($CaCl_2$) 7.0; P_{CAL} 93 mg kg^{-1}; N_{total} 0.06%; K_{CAL} 137 mg kg^{-1}; soil texture: sand 12%, silt 66%, clay 22%) from a long-term field trial in Bad Lauchstädt (Germany) with rotations including only C3 plants. Soil was sieved to 2 mm and filled in all three compartments. The mature wheat residues were obtained from a Mini-FACE experiment conducted at the University of Hohenheim (Erbs and Fangmeier 2006). The shoot residues were cut into 2 cm length and mixed thoroughly into the soil of one outer compartments at a rate of 7.5 g kg^{-1} dry soil. Pre-germinated maize (*Zea mays*, cv Amadeo) was planted in the other outer compartment. The $\delta^{13}C$ values were: wheat -44.1‰, soil -26.5‰ and maize roots -14.1‰. There were four treatments which differed in configuration of the outer compartments: unamended soil-un-amended soil (Soil-Soil), maize-un-amended soil (Maize-Soil),

maize-soil with wheat residues (Maize-Residue), soil with wheat residues-un-amended soil (Residue-Soil). The pots were sealed and placed in a water bath in order to maintain the soil temperature at 20°C. The water bath was situated in a glasshouse with ambient light and temperature (summer). Soil moisture was maintained by weight daily.

Sampling
Four replicates of each treatment were harvested on day 14 and 23 after planting (DAP) of the germinated maize seeds. The middle compartment was frozen at -20°C and sliced into 1 mm vertical slices using a kitchen knife. The soil from each 1 mm slice was analysed separately. The soil from the slices and the outer compartments was stored at -20°C until analyses.

Analyses
The activities of β-glucosidase, N-acetyl-ß-glucosaminidase, xylosidase, phosphatase and leucin aminopeptidase were measured using 4-methyl-umbelliferone- or 7-amino-4-methylscoumarin-labeled substrates as described in Marx *et al*. (2005). PLFAs from 4 g of soil (four replicates per treatment, slice and harvest) were extracted using the procedure described by Frostegård *et al*. (1993). ^{13}C in the PLFAs was measured by GC-C-IRMS.

Results
Plant shoot and root dry matter increased 2.5 fold from 14 DAP to 23 DAP and did not differ significantly between the treatments. On 14 DAP, maize had formed a few roots close to the mesh of the middle compartment; by the second harvest, 23 DAP, a dense root mat had formed. Moreover, root density was very high in the lower part of the pots, resulting in relatively dry soil, suggesting that although the water content of the pots was maintained by weight, the water did not penetrate sufficiently into the lower part of the pots to compensate for water uptake by the roots.
Particularly on 14 DAP, activities of β-glucosidase, xylosidase and phosphatase were higher in 1-2 mm distance from roots and residue-amended soil, with generally higher activities in the vicinity of the residue-amended soil than of the roots. Figure 1 shows the β-glucosidase activity as an example, with black bars indicating 14 DAP. The gradients did not overlap in the Maize-Residue treatment resulting in a U-shaped pattern of activities in the rhizosphere-detritusphere interface.

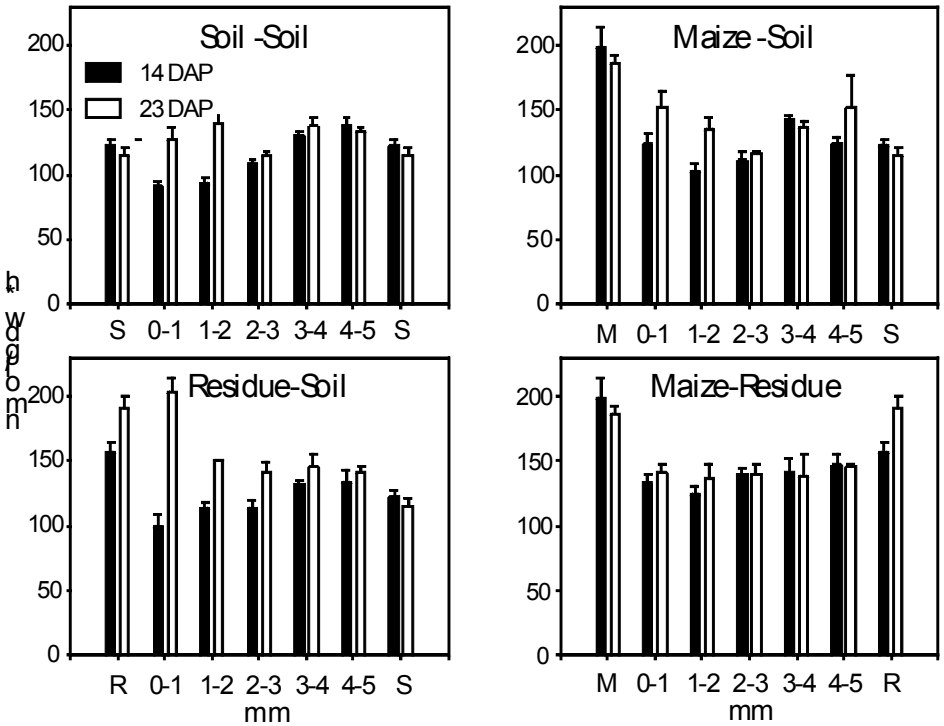

Figure 1. β-glucosidase activity in 1 mm slices of the middle compartment and the outer compartments in Soil-Soil, Maize-Soil, Residue-Soil and Maize-Residue treatments, with S: soil, M:root compartment, R: residue compartment.

The sum of PLFAs, as a measure of active microbial biomass was increased in 1-2 mm distance from the roots and the residue-amended soil with approximately 30% greater values in the detritusphere than the rhizosphere. Compared to the un-amended soil, the concentration of bacterial, Gram-negative and Gram-positive bacterial fatty acids were not increased in the rhizosphere, but were nearly two-fold increased in 1-2 mm distance from the residue-amended soil. The most distinct gradient was found for fungal fatty acids, with 5-7 fold greater concentrations in the vicinity of roots and residue-amended soil than in the un-amended soil (Figure 2). Both roots and residue-amended soil strongly increased the fungal/bacteria ratio up to 4 mm distance. Gradients were generally more distinct at 14 DAP than at 23 DAP. In the Maize-Residue treatment, there was no overlap of gradients for bacterial and fungal fatty acids; however the gradients overlapped for the fungi/bacteria ratio, resulting in increased ratios compared to the un-amended bulk soil throughout the interface.

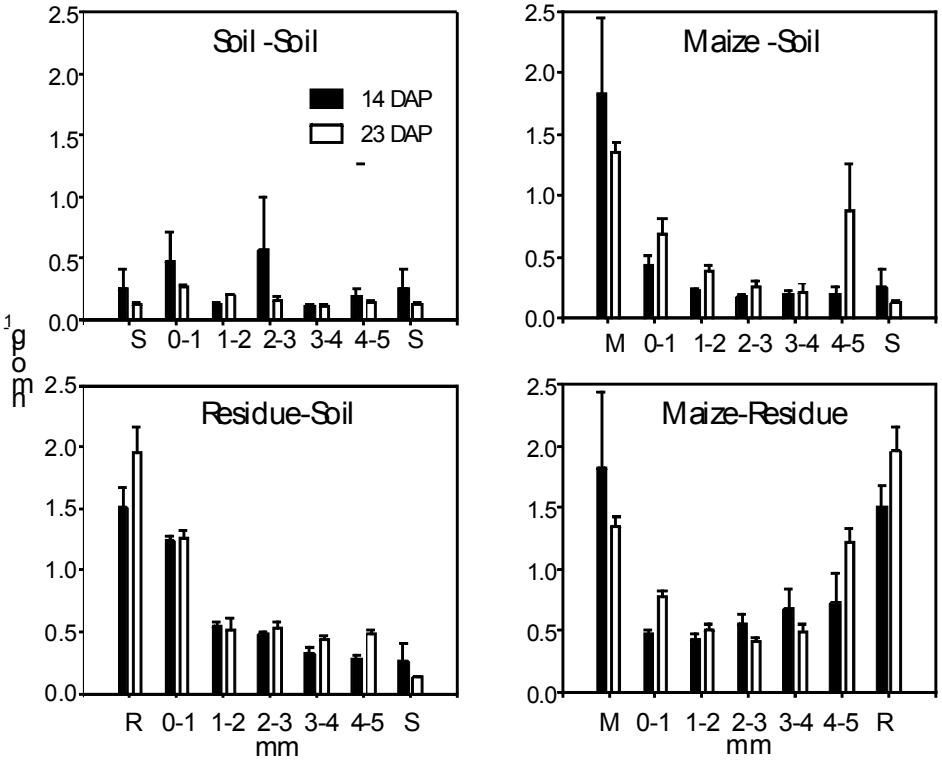

Figure 2. Abundance of fungal PLFA (nmol g^{-1}) in 1 mm slices of the middle compartment and the outer compartments in Soil-Soil, Maize-Soil, Residue-Soil and Maize-Residue treatments, with S: soil, M:root compartment, R: residue compartment.

The $\delta^{13}C$ values of the PLFAs were only determined for the plant-residue amended soil and the residue-unamended soil treatments. Of all PLFAs, only i15:0, 16:1, cy17:0/17:1/i17:0, C18:0 and C18:2ω6/C18:1ω9 showed gradients in $\delta^{13}C$ values (Figure 3 shows C18:2ω6/C18:1ω9). The $\delta^{13}C$ values in 1 mm distance from the residue-amended soil were depleted strongly compared to those in the soil in greater distance whereas the $\delta^{13}C$ values in the vicinity of the root compartment were only slightly enriched, suggesting that in the 14-23 days studied here, microorganisms incorporated residue-derived C to a greater extent than root-derived C.

Conclusion

The results of this study show, for the first time, microscale gradients in enzyme activity and abundance of bacterial and fungal fatty acids in the interface between rhizosphere and detritusphere as well as carbon flow from residues into soil microorganisms. Interestingly, the effect of the residue compartment was greater than that of the root compartment. This was surprising, given the relatively low rate of residue addition and the large residue particle size. In fact, at harvest, many residue particles were still intact suggesting relatively low decomposition rates. We had expected a greater effect of the maize roots because of the release of easily available compounds in root exudates. High rates of exudation can be assumed because of the high growth rate of the maize (dry matter increased more than 2.5 fold between the two harvests). It is possible that the

gradient in the rhizosphere is much steeper than in the detritusphere. Our earlier study (Kandeler *et al*. 2002) suggested that enzyme activities were strongly enhanced in up to 0.5 mm distance from the roots. Since in the present experiment the slices were 1 mm thick, this rhizosphere soil may have been diluted with bulk soil.

The results suggest that the influence zones of root and residue compartment overlapped very little which may be due to the thickness of the middle compartment. To investigate the interface more closely, residue and root compartment may need to be separated by 2-3 mm or less.

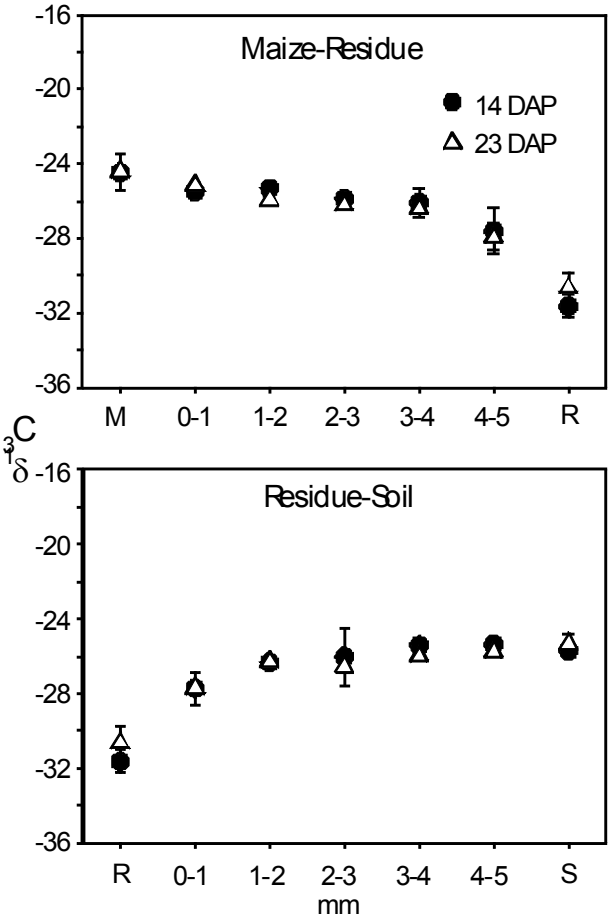

Figure 3. $\delta^{13}C$ in the fungal fatty acids C18:2ω6/C18:1ω9 in 1 mm slices of the middle and the outer compartments in Residue-Soil and Maize-Residue treatments, with S: soil, M:root compartment, R: residue compartment.

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