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Assessing the effect of management practices on soil microbial communities in a Vertisol using enzymes and ^{15}N-DNA stable isotopic probing techniques

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Abstract
The effect of different management practices, on soil enzymes activities and organic carbon content were evaluated in a Vertisol from a long-term field experiment conduced in Venezuela. The most important results from 5 years of evaluation showed a contrasting behavior in enzyme activities either with tillage systems or residue management. To identify the active bacterial community involved in decomposition of crop residues of different quality an incubation experiment was conducted with highly ^{15}N-enriched residues using the stable isotopic probing technique. The quality of plant residues determined enzyme activities and changed the composition of microbial communities. The stable isotopic probing technique allowed the separation of the active microbial community.

Introduction
Management practices have been shown to affect soil microbial activity and population structure. Molecular biological techniques allow for analysis of the overall structure and species composition of a microbial community (Dilly et al., 2004). The labeling of microbial DNA with stable isotopes (^{15}N,^{13}C) (stable isotope DNA probing) opens new opportunities to trace *active* microbial populations to achieve a better understanding of the processes that drive C and N cycling in soil (Cadisch et al., 2005). The objectives of this study were, to evaluate the effect of managament practices on soil enzyme activities and organic carbon content in a Vertisol from a long-term field experiment and to identify the active bacterial community involved in decomposition of crop residues of different quality.

Materials & Methods

- *Long-term field experiment*

Soil samples were collected during five years from a long-term experiment conduced at Aragua state in Venezuela since 1997 where the effect of different management practices (tillage, residues and cropping systems) were evaluated. The soil was a Vertisol (Typic Haplusterts) with a clay loam texture, organic carbon content of 15.2 g kg^{-1} and pH(H_2O) of 6.7. The mean annual precipitation and temperature of the study area were approximately 870 mm and 26.9 ºC respectively. Dehydrogenase and acid phosphatase activities were measured according to Casida et al. (1964) and Tabatabai & Bremner (1969), respectively.

- *Incubation experiment*

Highly ^{15}N-enriched plant residues of different quality i.e. maize (C:N 32) and soybean (C:N 15) were mixed (1%) with soil samples from the same field experiment and incubated for 30 days (25 ^{o}C) at 40 % WHC. A control without residue was also used. Soil samples were analyzed for invertase and xylanase activities after 3, 7 and 15 days according to Schinner and von Mersi (1990). Soil DNA was extracted after 7 and 15 days and centrifuged in a CsCl gradient (69h at 140K g) (Cadisch et al., 2005). The DNA samples were fractioned and analyzed by DGGE after PCR amplification using general bacterial primers (F984GC / R1378) (Heuer et al., 1997).

Results & Discussion

Long-term field experiment

During a period of five years dehydrogenase, as a measure of total microbial activity of soil microorganisms was higher in conventional tillage (CT) than in no tillage treatment (NT) (Fig.1). In a previous study, enzymatic activities related to N mineralization (urease, protease) showed the same trend (España et al., 2002). The amount of N released from crop residues was higher in CT than in NT, indicating a faster decomposition rate of residues (España et al., 2002). This was supported by a study on chemical-structural properties of soil organic matter, where the mineralization index was higher in CT compared to NT (Rodriguez et al., 2004). Soil organic carbon (SOC) decreased in the top soil under conventional tillage compared to no tillage (Fig. 2). This could be due to a physical disruption of aggregates reducing the protection of organic matter due to tillage practices. Additionally a redistribution of soil organic carbon with depth under conventional tillage was observed after 6 years (Fig. 2). This indicated that conventional tillage diluted organic matter through mixing the organic carbon-rich surface horizons with horizons low in SOC. Therefore, over the whole soil depth (0-20 cm) SOC was not different between tillage systems.

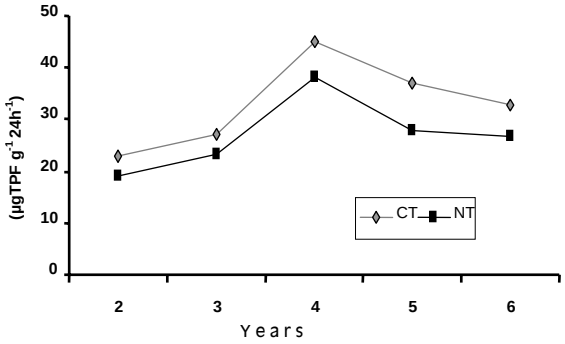

Fig.1. Dehydrogenase activity in soil (0-10 cm) with different tillage systems during 5 years of evaluation.

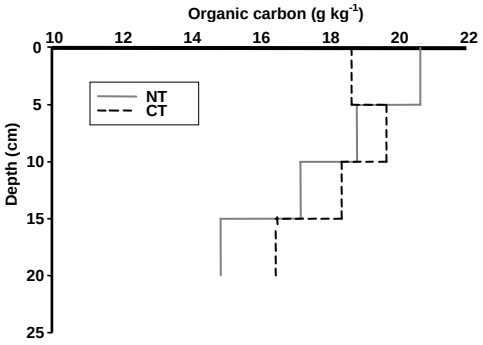

Fig.2. Redistribution of soil organic carbon in different tillage systems after 6 years

Dehydrogenase and acid phosphatase activities in the top soil (0-10 cm) decreased after removing residues (maize, soybean) from the field showing that crop residues left in the field after harvest represent an important source of nutrients to soil microorganisms (Data not shown).

Incubation experiment

The enzyme activities increased during incubation time with addition of residues (Fig. 3). Xylanase and invertase activities were directly affected by residue quality. Both enzyme activities were higher in soybean treatment (high quality residue) compared to maize treatment (low quality) and in the case of xylanase those were between two and three times higher than in the maize treatment. Luxhøi et al. (2002) interpreted this difference also as a function of the physical properties of the plant materials, where the low quality residues retain its rigid structure at sub-cellular level over a long time, reducing the access of microorganisms to the substrate.

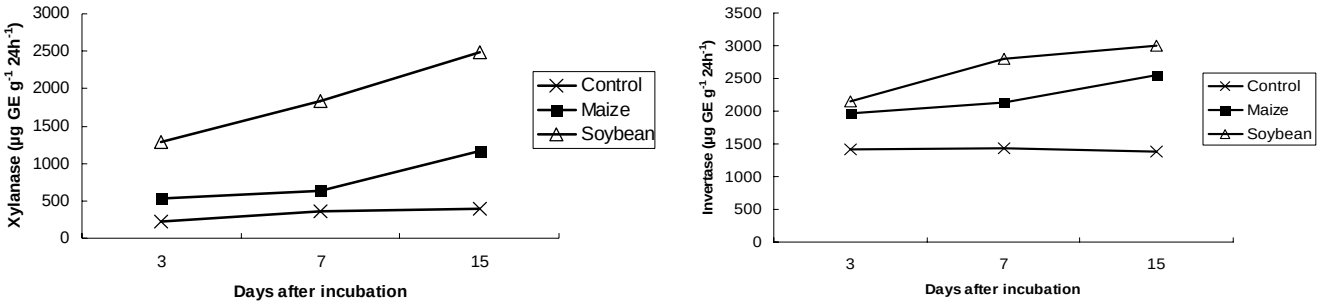

Fig. 3. Xylanase and invertase activities in soil after residues application of different quality

DGGE obtained from amplified 16S rRNA gene sequence indicated that the number of bands increased with application of residues. New bands that were not present in the control treatment indicate the activation of bacterial communities involved in the decomposition process (Table 1). The number of the new bands was higher in the maize compared to the soybean treatment. Additionally the pattern of bands changed with residue quality suggesting that different groups of microorganism are involved in the decomposition of residues of different quality. Using the DNA profile as a semi-quantitative measure of bacterial diversity, it can be suggested that low quality residues (maize) developed microbial communities with higher bacterial diversity compared to the high quality residues (soybean) at 15 days after application. Other authors have also shown that quality of residues affect the community structure, and have suggested that apparently, more species (or genotypes) are required for decomposition, or are able to grow, when the litter quality is low (Marschner et al., 2003, Dilly et al., 2004).

Table 1. Number of bands obtained in DGGE from amplified 16S rRNA gene sequence with the application of residues of different quality after 15 days

	Treatment	
	Maize	Soybean
New bands	16	11
Common bands	7	7
Different bands	9	4

New bands, bands not present in the control treatment. Common bands, bands present in both residues treatment. Different bands, bands present only in the residue treatment indicated.

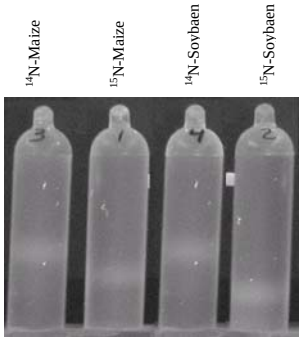

Fig.4. CsCl gradient centrifugation of DNA samples 7 days after application of ^{15}N-enriched residues of different quality

The CsCl gradient centrifugation indicated that 7 days was enough time to get DNA samples highly enriched in ^{15}N (Fig. 4). In a previews work Cadisch et al. (2005) found that the DNA sample at the bottom of the visible band in the CsCl gradient was heavily ^{15}N-enriched, e.g. up to 90 atom% after 7 days of application of *Lolium perenne* residue. The DGGE patterns of DNA samples extracted from a CsCl gradient 15 days after ^{15}N-maize residue application (Fig. 5) showed that more than 75 % of the active bacterial diversity involved in the decomposition process were present in the heavier, highly ^{15}N-enriched fraction.

Conclusions

Conventional tillage (CT), presented not only higher enzyme activities, but also reduced surface organic carbon accumulation. No tillage increased organic carbon accumulation in the topsoil (0-5 cm). However, there was no difference in SOC over 20 cm between tillage treatments.

Residue additions stimulated soil microbial activities, and the quality of plant residues determined enzyme activities and changed the composition of microbial communities.

Our results showed that the stable isotope probing technique allowed the separation of the active microbial community and thereby provides new opportunities in soil biology research.

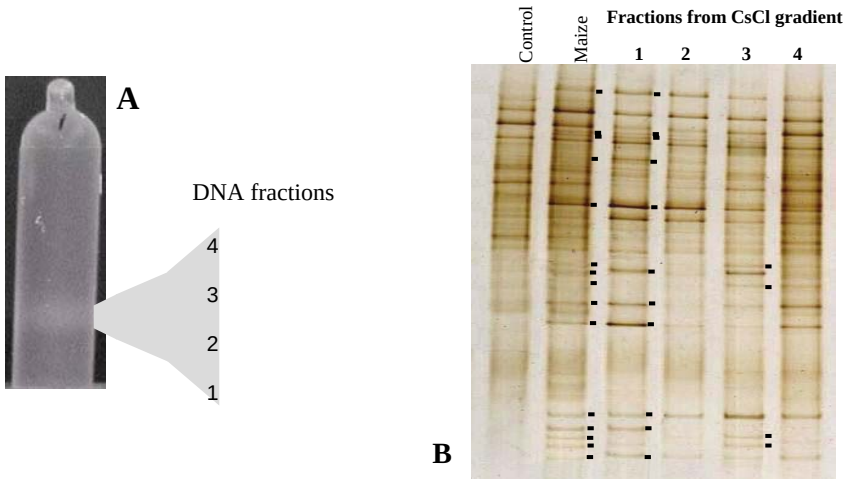

Fig. 5. A. Separation of ^{15}N-DNA from a CsCl gradient 15 days after ^{15}N-maize application. B. DGGE patterns of the DNA samples extracted from the CsCl gradient. Control= unamended soil, Maize= unfractioned soil DNA from maize residue treatment, the numbers correspond with the position of the fractions from the bottom, (➥) indicate the active bacterial diversity (by difference to the control).

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