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Introduction

Bacteria drive biogeochemical cycles of the biosphere and are key to the decomposition of organic materials. Molecular techniques reveal an overwhelming number and diversity of bacterial species in the soil which, parenthetically, has revolutionized the concept of soil microbial richness and diversity. Yet, despite the obvious gains in understanding of the link between decomposition and bacterial function, their have been only a few detailed look at the bacterial community members that colonize and grow during the early stages of residue decomposition, We thus investigated the composition, turnover ,and diversity of bacterial communities and the associated bulk soil during the early stages of rice straw decomposition.

Methodology

Experimental Set-up

- Two-cm rice straw mixed into soil at the rate of 0.1 % g straw/g soil the mixture was placed in 500 mLcanning jar and moisture was adjusted to -33KPa
- temperature were maintained at ~21-23 ^{o}C for 2 incubation periods: one week and two weeks

The Treatments

- Nonincubated rice straw
- Detritusphere at 1 week incubation
- Detritusphere at 2 weeks incubation
- Bulk soil at 1 week incubation
- Bulk soil at 2 weeks incubation

Sample Preparation

- At termination, rice straw were vigorously shaken to remove adhering soil particles and placed in clean tubes (detritusphere)
- The bulk soil and detritusphere were kept in separate tube and stored at -80^{o}C until analyses

The 16S rRNA Clone Library

DNA was extracted from 5-10 g of soil (0.25 g for detritusphere) using the PowerMax Soil DNA isolation kit (MOBIO Labs). Fifteen cycle-PCR reaction were used to amplify the rRNA genes. The standard conditions include 10 ng of template DNA, 0.15 mM of the 27f and 1492r primers and Taq polymerase. The PCR products were cloned immediately after the amplification and clones were produced using the TOPO TA cloning kit from Invitrogen. Well isolated colonies were then picked into 96-well blocks, where each well contained 2 ml of medium plus 10 % glycerol. After one day of growth, a 0.1 ml portion is transferred to a second 96-well block, and both blocks are frozen at -80 C. The second block was sent to the Sequencing and Synthesis Facility at University of Georgia for sequencing. Replicate libraries were made for each treatment. LIBSHUFF analysis revealed that the compositions of the replicate libraries were not significantly different. Thus, the replicate libraries for each treatment were pooled for subsequent analyses.

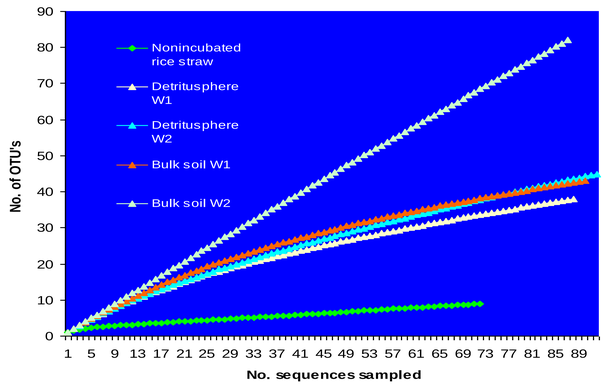

Fig. 1. Rarefaction curves of the clone libraries

Table 1. Diversity indeces for the 16S rRNA libraries of the different treatments

Index	Nonincubated rice straw	Detritusphere W1	Detritusphere W2	Bulk soil W1	Bulk Soil W2
No. of clones	72	88	92	90	87
No. of OTU's	9	38	45	43	82
Shannon (H)	1.08	3.28	3.38	3.55	4.38
Evenness (E)	0.49	0.90	0.88	0.94	0.99
Simpson (1/D)	2.55	24.54	23.87	43.06	623.44
Chao 1 estimator	30.0	80.17	115.86	68.67	832.75
95% COI	14.15-94.67	52.52-160.49	72.37-228.45	52.03-115.93	384.08-1947.82

Table 3. Phylogenetic affiliation and distribution of the most abundant operational taxonomic unit (OUT's) at D=0.03, n≥5

RDP Phylogenetic Affiliation	Sequence Similarity (%)	Non incubated rice straw	Detritus-phere W1	Detritus-phere W2	Bulk soil W1	Bulk Soil W2
Uncultured bacterium	99.1	43	-	-	-	-
Pantoea ananatis	99.9	15	-	-	-	-
Erwinia sp.	99.9	7	-	-	-	-
Uncultured Eubacterium	94.5	5	-	-	-	-
Pantoea sp.	99.9	22	13	8	-	-
Uncultured Planctomycetes	100	-	-	-	6	-
Stenophomonas maltophilia	99.9	-	-	-	5	-
Uncultured Acidobacterium	96.5	-	-	-	5	-
Pantoea aglomerans	99.3	-	5	-	-	-
Pseudomonas flourescens	99.8	-	6	-	-	-
Chitinophaga sancti	98.5	-	4	1	-	-
Uncultured Chloroflexi bacterium	98.2	-	-	5	-	-
Rhodopila globiformis	98.6	-	-	11	-	-

Note: Libshuff comparison reveals that bacterial communities from each treatment differed among each other

Results

- Except for the unincubated rice straw, rarefaction curves failed to reach saturation indicating high bacterial diversity in these treatments (Fig 1). This is supported by the Chao 1 estimator which predicted about 2- to 10-fold more bacterial OTU's (D =0.03) than were actually sampled (Table 1).
- Compared to the nonincubated rice straw number of OTU's in the detritusphere increased by five times after one and two week of incubation while more OTU's were detected in the soil at week 2 (Table 1). This finding is supported by the fact that bacterial communities developing in the detritusphere had no common taxonomic units with those in the bulk soils. The communities in the detritusphere overlapped considerably between week one and two, however, there was no overlap between the communities sampled from the bulk soil at either time (Table2).
- The communities in the detritusphere and the bulk soil during week 1 were dominated by a relatively few members (34 and 45 OTU's, respectively). In contrast, the community in the bulk soil during week 2 showed no dominance and was described by an even distribution (78 singletons)

Discussion

We hypothesize that during the early stages of decomposition that the high concentration of available soluble carbon favored a few community members in both the detritusphere and bulk soil. During the second week, the production and diffusion of these water soluble compounds probably slowed, resulting in substantial turnover of the bacterial community, particularly in the bulk soil. The fact that similar taxonomic units were not detected in the bulk soil in weeks 1 and 2 suggests that these members are rare in soil and grow opportunistically during high substrate availability.

The apparent surge in bacterial richness in the bulk soil in the second week is probably an artifact of the high degree of dominance of a few members during the early stages of decomposition, and probably more closely reflects the real bacterial richness of the soil.

The survival of Pantoea sp. In the detritusphere throughout the study suggests that they may have a tight association with Rice during its life cycle. These results provide a novel and resolute look at bacterial dynamics in soils.