Molecular Characterization of Bacterial and Fungal Decomposer Communities in a Southeastern U.S. Salt Marsh

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Abstract:

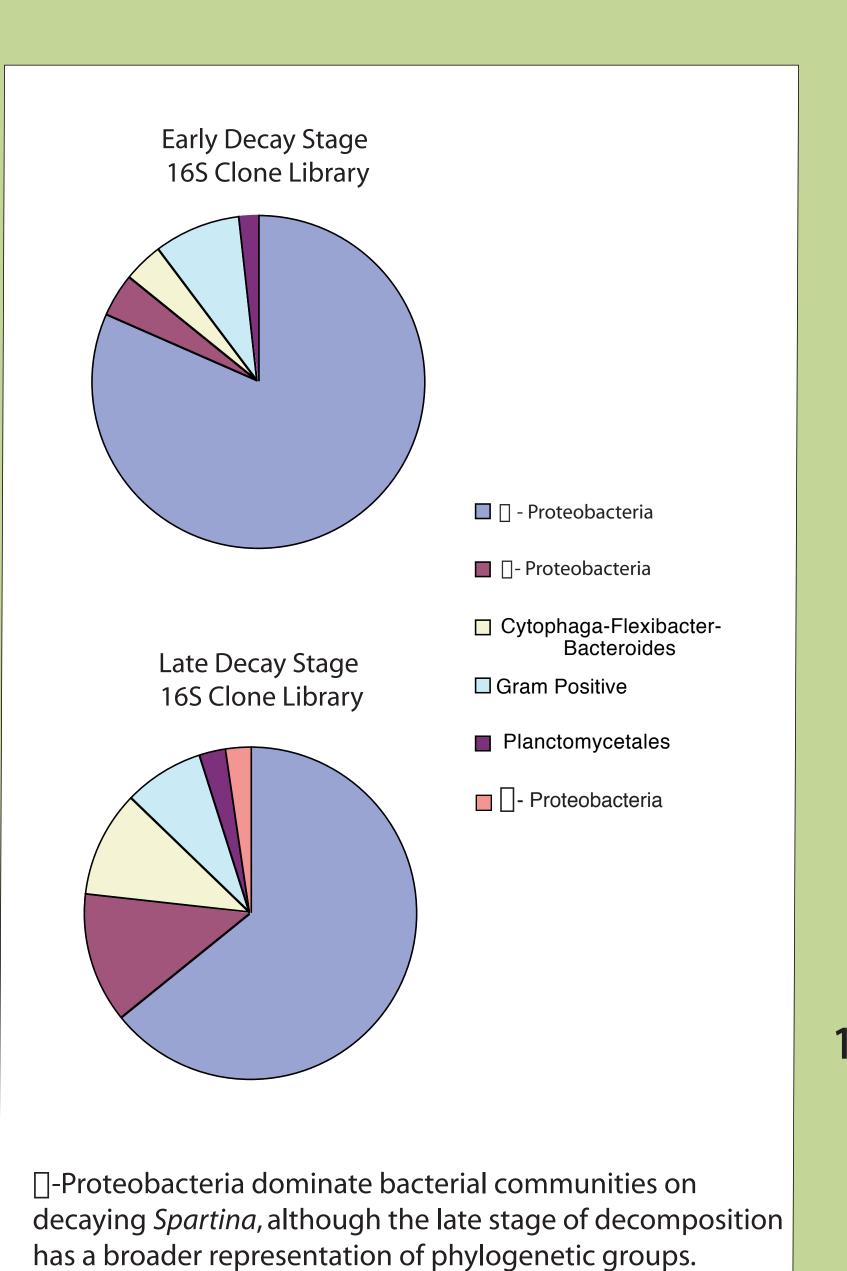
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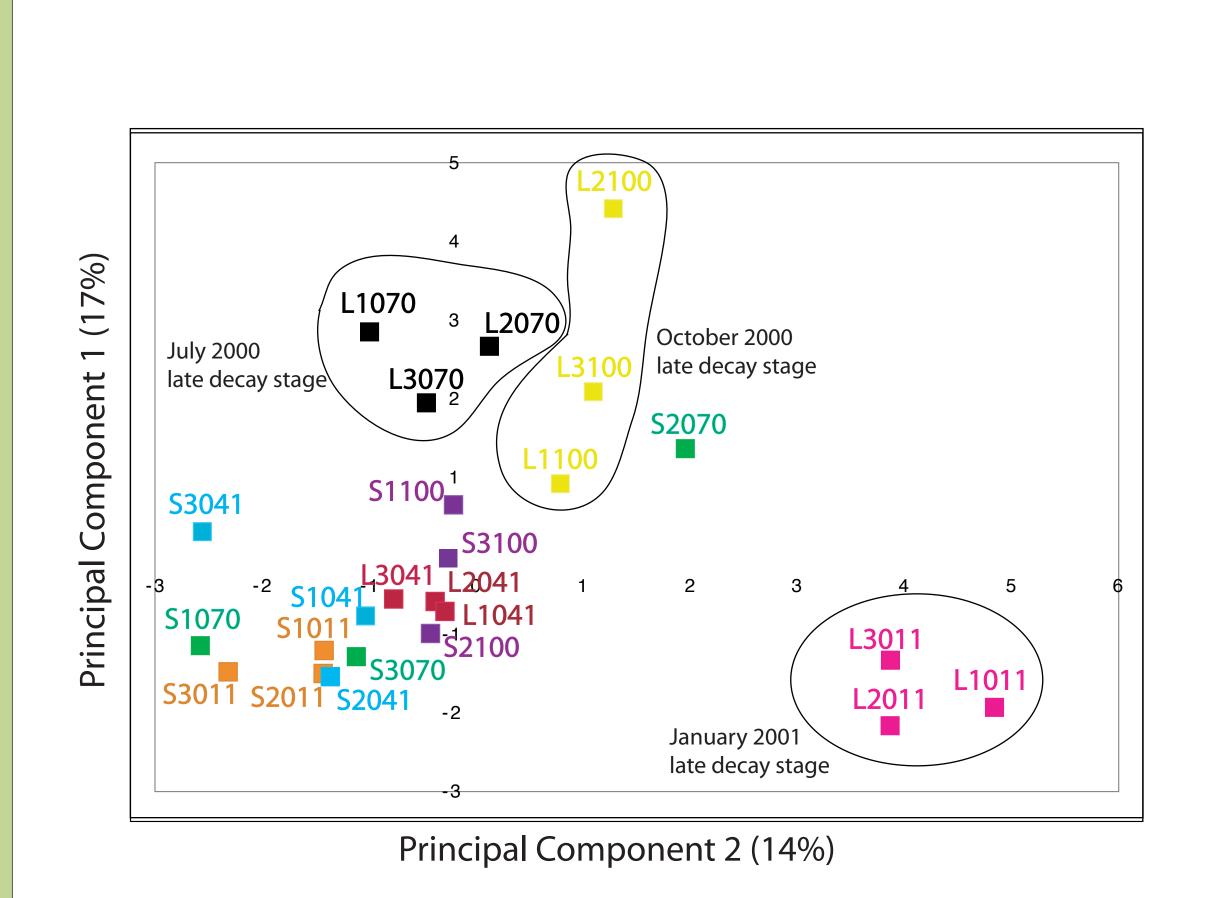
Both fungi and bacteria are recognized to play critical roles in decomposition processes in the salt marshes of the southeastern U.S. and elsewhere, but few studies have simultaneously examined both decomposer communities. We characterized bacterial communities using 16S rRNA genes and the ascomycete fungal community using 18S-28S internal transcribed spacer (ITS) regions, employing both clone library and T-RFLP fingerprinting approaches. Bacterial communities were dominated by []-Proteobacteria (up to 75% of the clone libraries) for two decomposition stages of Spartina alterniflora detritus over several seasons. The composition of the bacterial communities showed little spatial heterogeneity at a given sample date, but changes in the dominant groups were evident with season and decomposition stage. The ascomycete decomposer community was typically dominated by less than five species (or species clusters), with some shifts in the dominant species evident with season. Molecular characterization of fungal communities agreed very well with microscopic identification, indicating that many of the key fungal decomposers have already been successfully cultured. This is generally not the case for the bacterial decomposers, although some groups of []-Proteobacteria (e.g., Roseobacter and Erythrobacter /Porphyrobacter) have close relatives in culture.

Clone Designation	Closest Described Relative	Bacterial Division	% Similarit
SIB20/SIB27/SIB49/SIB50/LIB08/LIB26/ LIB29/LIB31/LIB32/LIB35/LIB41/LIB55/LIB67	Er ythrobacter litoralis	□-Proteobacteria	90-99
SIB01/SIB29/LIB05/LIB12/LIB30	Erythrobacter citreus	□-Proteobacteria	94-95
SIB37	Erythrobacter sp. AS-45	□-Proteobacteria	95
SIB46	Er ythrobacter sp. MED13	□-Proteobacteria	90
SIB54	Porphyrobacter neustonensis	□-Proteobacteria	97
SIB15/SIB17/SIB22/LIB24/LIB36	Agrobacterium san guineum	□-Proteobacteria	90-97
SIB02/SIB03/SIB04/SIB06/SIB40	Agrobacterium sp. SKA40	□-Proteobacteria	93-98
SIB34	Agrobacterium stellulatum	□-Proteobacteria	90
SIB13/SIB16/SIB21/			
SIB28/SIB33/SIB39/SIB41/SIB44	Roseivivax halodurans	<pre>[]-Proteobacteria</pre>	98
LIB64	Roseobacter litoralis	<pre>-Proteobacteria</pre>	95
SIB35	Roseobacter sp. KAT3	<pre>-Proteobacteria</pre>	94
SIB57	Roseobacter sp. QSSC9-8	<pre>[]-Proteobacteria</pre>	94
SIB24	Roseobacter sp. SCB34	-Proteobacteria	94
SIB30/SIB48	Roseobacter sp. KT1117	<pre>[]-Proteobacteria</pre>	95
LIB51	Keto gulonogenium robustum	<pre>[]-Proteobacteria</pre>	96
SIB07/SIB26	Ketogulonogenium vulgarum	□-Proteobacteria	95
SIB45	Citromicrobium bathoceanense	□-Proteobacteria	96
LIB09	Hyphomonas oceanitis	□-Proteobacteria	95
SIB55/LIB03/LIB59	Methyloarcula marina	□-Proteobacteria	94-96
LIB04	Methylocystis parvus	□-Proteobacteria	93
SIB36	Paracoccus sp.	□-Proteobacteria	89
SIB23	Paracoccus sp. MBIC4017	□-Proteobacteria	96
LIB20	Pedomicrobium manganicum	□-Proteobacteria	88
LIB58	Rhodobacter veldkam pii	□-Proteobacteria	94
LIB16	Rhodomicrobium vannielii	□-Proteobacteria	91
SIB32	Rhodo planes roseus	□-Proteobacteria	93
SIB18/LIB43	Rhodovulum adriaticum	□-Proteobacteria	93-95
SIB58	Rhodovulum iodosum	□-Proteobacteria	95
LIB10	S phingomonas subterraneae	□-Proteobacteria	93
SIB11	Flavobacterium sale gens	CFB	91
SIB56/LIB18	Flexibacter tractuosus	CFB	85-86
LIB27	Microscilla furvescens	CFB	86
LIB38	Microscilla sp. Nano 1	CFB	90
LIB61	Salinibacter ruber	CFB	87
LIB17	Achromatium oxaliferum clone (ACJRRDD)*	Proteobacteria	91
SIB59/SIB60	Alteromonas macleodii	- ∏Proteobacteria	88-97
LIB14/LIB19	Marinobacter hydrocarbonoclasticus	- ∏Proteobacteria	98
LIB15	Methylocaldum tepidum	- ∏Proteobacteria	89
LIB11	Pseudomonas elongata	- ∏Proteobacteria	92
LIB65	Aureobacterium kitamiense	Gram Positive	89
SIB31/SIB38	Bifidobacterium bifidum	Gram Positive	89
SIB53	Geobacillus subterraneus	Gram Positive	87
LIB33	Nocardia sp. R441	Gram Positive	90
LIB66	San guibcter inulinus	Gram Positive	86
SIB52	Thermus oshimai	Gram Positive	86
LIB62	clone (AF010040)*	∏Proteobacteria	82
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SIB42 LIB60	clone #0319-7F4 (AF23144)* clone (AF010081)*	Planctomycetales Verrucomicrobia	96 94

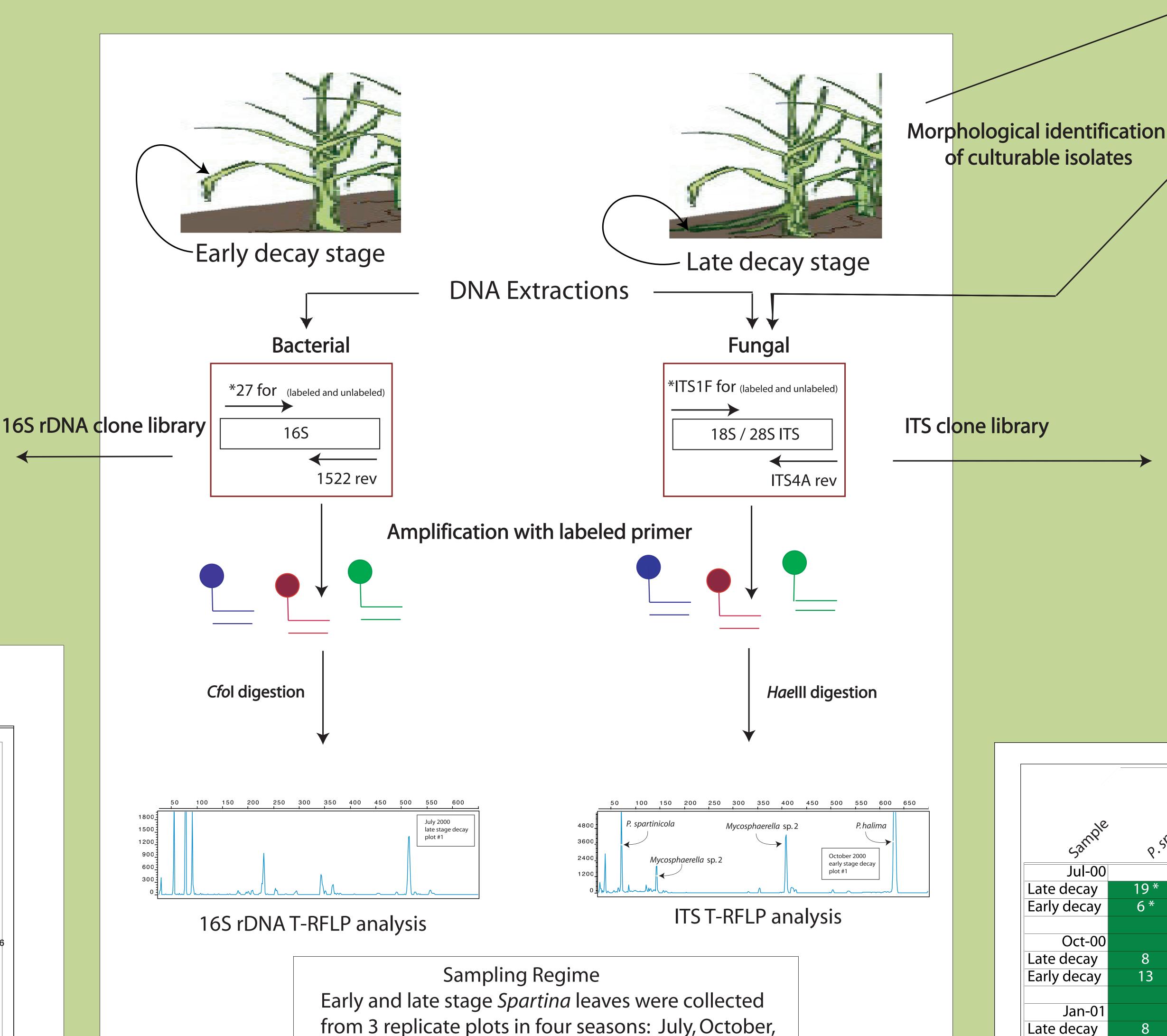
The majority of the 16S rDNA clones retrieved from both stages of decomposition during the July 2000 sampling were affiliated with the

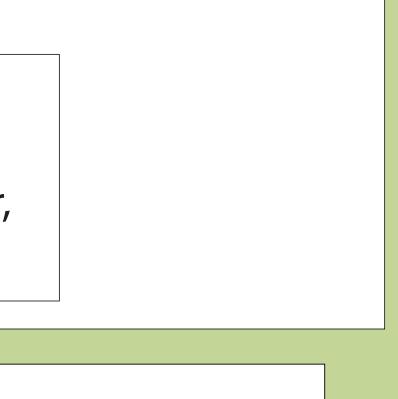
-Proteobacterial Erythrobacter/Porphyrobacter, Agrobacterium and Roseobacter groups. SIB = early decay stage, LIB = late decay stage.





T-RFLP profiles of 16S rDNA amplicon pools indicate that bacterial communities associated with early stage decomposition of *Spartina* are quite similar spatially and seasonally. In contrast, those communities associated with the late stage decomposition show seasonal variability. Each stage of decay (S = early, L = late) was sampled in 3 replicate plots (e.g S1, S2, S3) during July 2000 (070), October 2000 (100), January 2001 (011), and April 2001 (041). For example, \$1070 indicates the early decay stage sample collected from replicate plot #1 during July 2000.



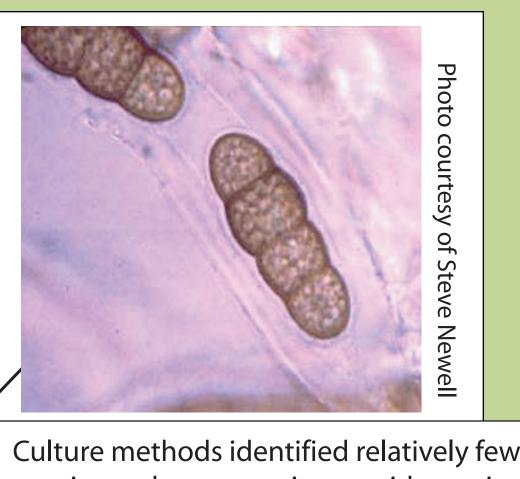


Collaborative Studies

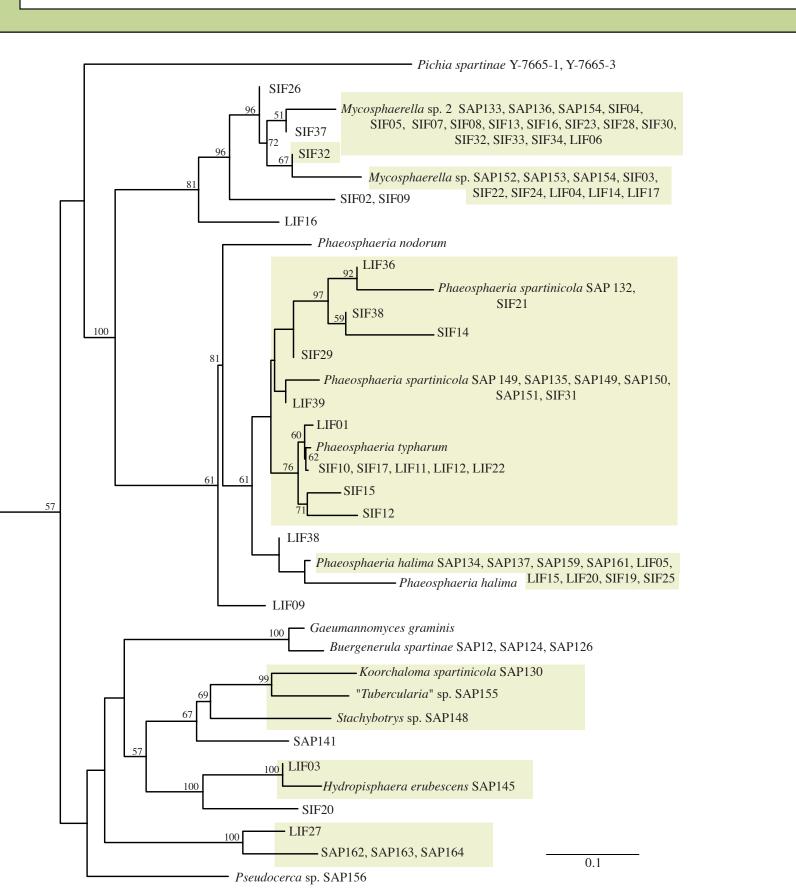
January, and April.

Bacterial ring cleavage dioxygenases and fungal laccases, two enzymes involved in the breakdown of aromatic components of vascular plant material are being retrieved from cultured organisms and from communities associated with decaying Spartina.

Direct interactions between bacterial and fungal decomposer groups will be investigated using manipulative studies.



Culture methods identified relatively few dominant fungal species and were consistent with previous studies of this ecosystem.



The majority of ITS clones obtained from early and late decay stage samples during July 2000 matched cultured fungal species. SIF = early decay stage, LIF = late decay stage.

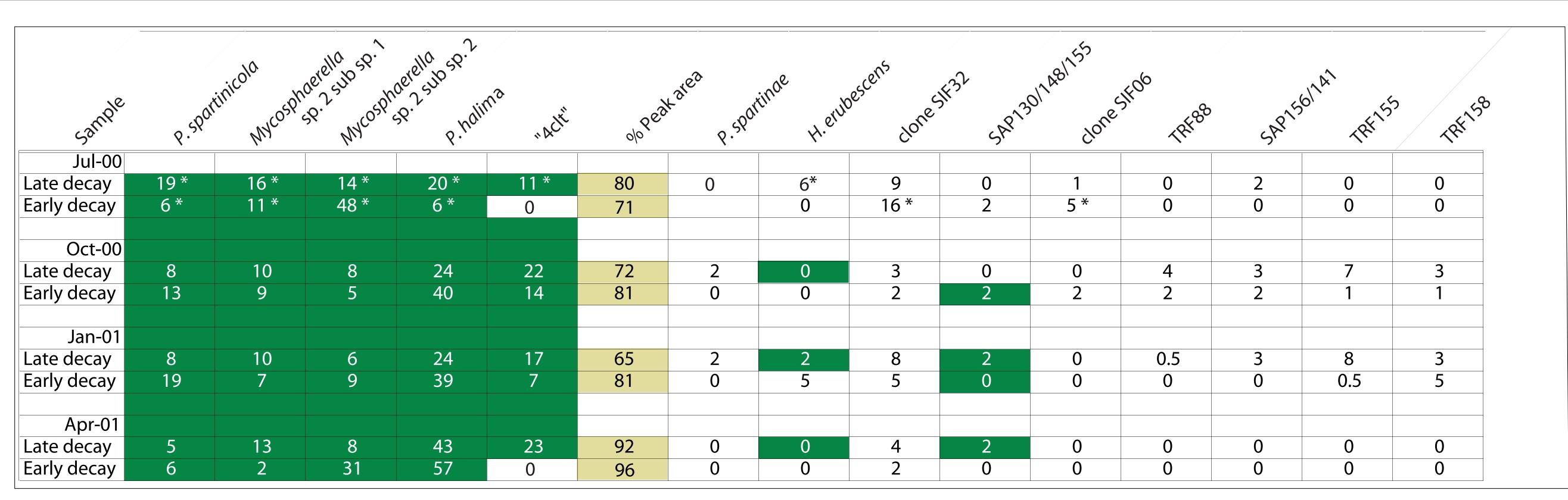
Conclusions

Fungal communities are dominated by relatively few species clusters, most of which are already known from culturing studies.

The presence of these fungal species in both stages of decomposition and all seasons suggests they would be good model organisms for future studies.

In contrast to the fungal players in *Spartina* decomposition, the key bacterial species are not yet known.

Because of the high diversity of the bacterial communities and the difficulties in culturing, the role of specific bacteria will be much more difficult to elucidate.



Most of the Terminal Restriction Fragments (TRFs) from decaying Spartina could be matched to ITS sequences retrieved from clones or fungal isolates. Of the four major ascomycete decomposers identified using culture independent methods (T-RFLPs and clone libraires) three have been previously demonstrated to be important colonizers of decaying Spartina using culture based methods. The molecular methods were instrumental in identification of the fourth major decomposer, "4clt", an organism that may play a more important role in the late stage of decomposition. Minor members of the fungal community were found primarily in the non-growing season. Almost half of these were identified by culturing, but the remaining half were identified only from the molecular methods.

Numbers are % relative area of the peak in ITS T-RFLP profiles, = identified using culture techniques, * = identified in ITS clone library