Tentative objectives for grant proposal:

**Objective 1:** To determine whether natural soil microbial populations are altered by the application of biodegradable mulches

**Objective 2:** To determine whether biodegradable mulches alter populations of a key plant beneficial bacteria in the rhizosphere

**Objective 3:** To determine the effect of biodegradable mulches on germination of relevant plant pathogenic fungal propagules

**Figure 1.** Flowchart to clarify the research question and what types of analyses might be used to address it.
Role of Brodhagen in reaching these objectives:

1. I can oversee collection of soil samples from various habitats/management regimes/soil types for analysis. My laboratory could also perform simultaneous assays for microbial degradation of polymer emulsions if we decide that we would like to go that route.

2. My laboratory can extract samples for both lipids and fatty acid analysis.

3. I can analyze the results of the PLFA analysis, and decide whether to proceed with a molecular (i.e. nucleic acid) analysis (e.g. DGGE) to identify any microbial populations affected by mulches.

4. After appropriate training, I can oversee the molecular analysis in my laboratory.

5. I can interpret the results of the molecular analysis.

6. I can design and implement bioassays to test the effects of biodegradable polymers on germination of specific fungal pathogens.

**LIST OF USEFUL REFERENCES**

**General soil microbial diversity**


*This is a good beginning review for those who are unfamiliar with soil microbial diversity. It gives a short, up-to-date review of microbial ecology with specific reference to soil ecosystems.*


*Reviews current understanding of distribution and patterns of diversity for free-living bacteria.*

This review is dense in mathematical theory, but drives home our limitations in measuring or estimating microbial diversity.

**Microbes and biodegradable mulches**


*Use of biodegradable plastic reduced overall soil microbial community diversity. Pseudomonas spp. appeared to be correlated to crop productivity.*


*Tomato plants were protected from wilt caused by Rastonia solanacearum when the soil was treated with a copolymer comprising N-benzyl-4-vinylpyridinium chloride and styrene in equimolar parts (PBVP-co-ST). This copolymer captures microbial cells on the surface and is highly biodegradable when exposed to sludge. Perhaps in the case of disease problems, this reagent could be considered as a supplement or additive to biodegradable mulches.*

***the next few references have to do with microbial degradation of films, and Doug knows far more about this than I do… but some of the methods seemed notable.*****


*Explores the results of embedding a mulch-degrading *Bacillus* in polylactic acid films.*


*Bacteria capable of degrading polymers of PLA, PHA, PCL, and PTS were isolated by plating slurries from soil samples onto agar plates containing emulsions of these polymers as a carbon source. Colonies arising that produced clearing zones were further analyzed by isolating DNA and amplifying and sequencing SSU rDNA for phylogenetic analysis. Such a method might prove useful to us if we wish to amend soils with naturally-occurring strains that could enhance degradation of biofilms post-harvest.*

Using methods like those described by Suyama et al (1998) above, the authors identified a strain of Paenibacillus amylolyticus that degraded PLA to monomeric lactic acid. Moreover, strain also degraded other polyesters such as PBS, PBSA, PES and PCL. The degradation was preliminarily correlated with esterase and/or protease activity. This article, like the one above, outlines methods that may be useful in our studies.


This is a thorough review of the role of fungi in degradation of biodegradable polyesters. Table I neatly lists fungal species known to degrade polyesters, which class, and by whom it was reported.


SEM micrographs of microbial biofilms degrading poly(butylene adipate-co-terephthalate) (PBAT) biodegradable mulch films.

Reviews covering general methods for assessing soil microbial communities


Discusses current technologies (mostly DNA-based molecular methods) for measuring abundance, diversity, and phylogeny in microbial populations in the environment. Good brief introduction to FISH, RFLP, clone libraries, DGGE, microarrays, etc.


Discusses the use of reporter genes that are appropriate for monitoring a specific microbial species (OTU, ecotype…) within a complex matrix such as soil. Bioreporters would also be useful for monitoring expression of a key gene (e.g. one central to degradation of biodegradable mulches). Also discusses biosensors, which are probes in which a biological component (e.g. enzyme or nucleic acid) interacts with an analyte, which is then detected by an electronic component and translated into a signal.


Although this methods paper was written with the marine microbiologist in mind, we landlubbers will benefit from reading a straightforward how-to on environmental sampling. Topics include
amplification of SSU rDNA, making clone libraries, SSU rDNA sequencing, rarefaction analysis, and phylogenetic analysis.

**Specific methods: phospholipid fatty acid (PLFA) analysis of soil microbial communities**


*PLFA provides an unbiased analysis of the gross structure of complex soil communities. This review highlights the utility and limitations of PLFA for community analysis.*


*Data from both PLFA and DGGE were concurrent in revealing that application of Bt is associated with changes in the microbial community structure of the pepper phyllosphere.*


*PLFA was used in concert with soil ergosterol content to determine whether the application of three broad spectrum fungicides caused short-term changes in microbial community structure. (It did.)*


*PLFA analysis was used to assess changes in microbial community structure during composting. Notably, the depletion of the starch fraction coincided with the beginning of a microbial biomass decrease, leading the authors to conclude that starch is an important substrate for thermophilic microorganisms during composting.*


*PLFA was compared with two other methods for analysis of microbial community structure, the microbial identification (MIDI) method and the ester-linked (EL) procedure, to assess community*
structure in compost. PFLA gave the most detailed information about growth and overall succession of the microbial community.


Length heterogeneity PCR is a SSU rRNA based method for microbial community analysis that is similar to the more commonly used T-RFLP method. In this study, FAME and LH-PCR methods were used to compare microbial community composition at four sites differing in soil type or crop management. The methods were complementary and correlations were found between LH-PCR and FAME.


A combination of PLFA and DGGE was used to assess changes in microbial community structure during composting, with particular attention to Actinobacteria.


PLFA was used to assess richness of rhizosphere sub-communities able to utilize specific rhizosphere exudates. This study may provide a useful model for us to test degradation products of biodegradable mulches.


Mycorrhizal fungi are significant contributors to plant productivity in most soil ecosystems. The signature fatty acid, 16:1[9\]5, can be used to estimate biomass of arbuscular mycorrhizal fungi (AM). Ectomycorrhizal fungi can be estimated with the signature fatty acid 18:2[9\]6,9.

Specific methods: DNA reassociation for estimating species richness in soil


Specific methods: terminal-restriction fragment length polymorphism (T-RFLP) analysis of soil microbial communities


Analyzes a number of restriction enzymes for their utility in revealing differences in microbial communities. Shows that a single enzyme never detected more than 70% of the actual OTUs in a sample.


See PLFA section above.

Specific methods: denaturing gradient gel electrophoresis (DGGE) analysis of soil microbial communities


Isolation of abundant bands from the Chrysanthemum rhizosphere vs. bulk soil revealed that Pseudomonas are among the bacteria most enriched in the rhizosphere.


See PLFA section above.


See PLFA section above.

Effect of C-source (or sugar derivatives) on microbial community structure

*Perhaps the best understood relationship between microbial community structure and C-source is that between plants (root exudates, decomposing biomass) and soil microbes. Although this review does not address biodegradable mulches as a nutrient source, it discusses some ideas that are good to bear in mind, e.g. priming effects.*


*Carbon flow to the soil microbial population can drive changes in community structure. A strain of lux-marked P. fluorescens could be used to assess the degree of impact of specific carbon sources. Emitted light reflects population density, which changes in response to the quantity and quality of C-sources.*


*Nod factors, exopolysaccharides, lipopolysaccharides, and cyclic glucans all play key roles in physiology and communication by soil microbes. We may want to consider whether biodegradable mulch breakdown products might mimic any of these polymers.*


*Discusses the mechanisms of fungal spore germination, including the effect of small molecules (e.g. sugars, amino acids, salts) which may be germane to our study if breakdown products inadvertently lift dormancy for pathogenic fungal spores.*