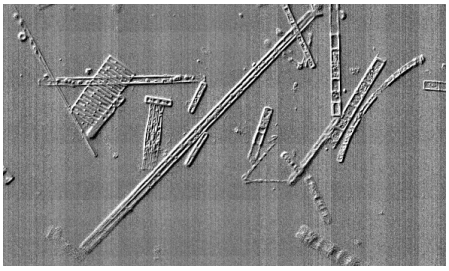
LTER Research in Microbial Ecology:

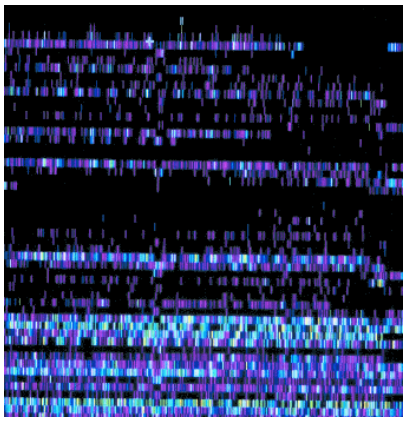
A synopsis of microbial studies at LTER sites

Introduction:



Research and interest in microbial studies is both extensive and broad within the Long Term Ecological Research (LTER) Network program of the National Science Foundation. The information here is compiled from data provided by LTER sites regarding microbial ecology research within LTER. A white paper on LTER Microbial Ecology Efforts was also written by and LTER ad-hoc committee.

Although not an explicit core research area of LTER, observations and investigations of microbial activity are well integrated into the general research conducted at each of the LTER sites. The microbial investigations range from intensive research like that of the Arctic Tundra LTER site in collaboration with research centers at the Woods Hole Marine Biological Laboratory, to individual graduate student investigations such as those of the Niwot Ridge LTER and of the Laboratory of Microbial Ecology at the University of Virginia and Virginia Coast Reserve LTER. The investigations range from identification and DNA sequencing work to biogeochemical measures of microbial processes. Studies range from microbial observations of undisturbed ecosystems such as the microbial mats in the polar Antarctic lakes of the McMurdo (MCM), Taylor Dry Valleys LTER, to perturbation experiments such as the soil warming experiments at the Konza LTER and elevated CO_2 studies at the Harvard Forest LTER. Identification of microbes from the field using sequencing techniques has been one current focus of microbial studies in general. Studies within the LTER program are in general extensive, including microbial function, survival, biogeography, succession throughout the season, rates of grazing on microbes, measurement of activities at low levels, use of probes to identify organisms from the field, active vs. inactive microbes, and gene expression in the field. The studies include bacterial numbers, bacterial productivity, species/sequencing, bacteria/flagellate food webs, protozoans, and phytoplankton.A new initiative in microbial studies could go beyond already developed methods, and use new methods and technologies to answer questions related to microbial ecology itself.



Microbial Observatory efforts within LTER could be enhanced by further collaborations with existing centers, such as the NSF Center for Microbial Ecology at Michigan State University. Collaborations with private industry such as use of identification/ sequencing kits provided by Perkin Elmer offer potential mutual benefits. LTER sites are ideal locations for studies in the spatial extent and distribution of microbes using DNA sequencing techniques and databases integrated with Geographic Information System (GIS) databases. This work is important not only with more conventional studies of the LTER program but also within the new framework and interests of the Phoenix (CAP) and Baltimore (BES) Urban Ecosystem LTER sites. The investigations could include use of information within databases of National Institutes of Health (NIH) and Center for Disease Control (CDC).

The National Science Foundation, Directorate for Biological Sciences (BIO), has an active program in Microbial Observatories, with a fourth call for proposals due in July, 2002. The long-term goal of this activity is to develop a network of sites or "microbial observatories" to discover novel microorganisms, microbial consortia, communities, activities and other novel properties, and to study their roles in diverse environments. Individual investigators or teams of investigators are encouraged to develop and conduct research at a site or habitat dedicated to discovery and study of these novel microbes over time and across environmental gradients. Development and application of new

experimental approaches to these studies, including the use of genomic and functional genomic methods, is strongly encouraged.

There are currently 8 LTER research project funded by NSF's program on Microbial Ecology:

(MCB-9977933) Diversity of Nitrogen-Cycling Microorganisms at the H.J. Andrews LTER PI: David Myrold

(MCB-9977882) Observing Patterns of Prokaryotic Diversity along Land use Gradients of the CAP LTER PI: Fred Rainey

(MCB-9977907) Spatial Scales of Genetic and Phenotypic Diversity Among Streptomycetes in Native Soils (takes place at Cedar Creek LTER) PI: Linda Kinkel

(MCB-0084223) Microbial Biogeochemistry and Functional Diversity across the Forest-Tundra Ecotone in the Rocky Mountains (Niwot Ridge LTER) PI: Steve Schmidt

(MCB-9977903) A Microbial Observatory for the Northern Temperate Lakes Long Term Ecological Research Site PI: Eric Triplett

(MCB-9977897) Microbial Observatories: Salt Marsh Microbes and Microbial Processes: Sulfur and Nitrogen PI John Hobbie

(MCB-0084164) Prokaryotic Diversity of a Salt Marsh/Estuarine Complex at the University of Georgia Marine Institute, Sapleo Island (Georgia Coastal Ecosystems LTER) PI: Mary Ann Moran

(MCB-0132085) Microbial Observatories: Collaborative Research: A Cold Microbial Observatory: Collaborative Research in an Alaskan Boreal Forest Soil PI: Jo Handelsman

John R. Vande Castle, LTER Network Office, Editor - Last edited Thursday, July 25, 2002

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AND – H.J. Andrews Experimental Forest**,** *Blue River, Oregon*

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There is a wide range of microbial observations conducted at the HJA/LTER site in the Central Oregon Cascade Mountains. They can be generally characterized as: (1) studies of microbial activities and microbially mediated processes or (2) studies on fungal ecology. These studies have included the following:

The effects of riparian stand management on the microbial characteristics of stream sediment fine particulate matter:

This work has shown a potential link between forest management practices and stream biological productivity. These seasonal studies have shown that headwater stand harvest can alter the microbiology and chemistry of stream sediments passing through these stands. In the future, we would like to continue these studies documenting the influence of riparian vegetation on seasonal patterns of organic particulate matter quality in stream sediments. This will eventually allow us to make direct connections between riparian management and stream productivity as mediated through microbial activity.

Studies on ectomycorrhizal fungi:

Since the mid 1980s, there have been a number of studies centering around ectomycorrhizal fungi. Fungi that form ectomycorrhizae establish an essential interface between soil and plants by extending the nutrient-absorbing surface area of the roots, producing extracellular enzymes that increase phosphorous and nitrogen availability, and protecting against pathogens. One set of studies have focused on a specialized group of ectomycorrhizae that form fungal mats. One of these studies addressed the role these mats play in forest soil biogeochemistry and forest productivity as well as factors influencing their distribution. We would like to expand this work to study factors influencing ectomycorrhizal mat inoculation potential and the extent to which these mats are capable of interconnecting different types of plants as well as their possible role in seedling reestablishment. Studies to date suggest that these fungi are capable of transferring both organic and inorganic nutrients from overstory trees to seedlings; increasing the probably of seedling establishment on disturbed sites.

Two major studies have examined the community structure of ectomycorrhizal fungi in and near the HJA/LTER site. Despite the importance of ectomycorrhizal fungi to these ecosystem processes, little is known about their community structure and dynamics. The first of the two studies focused on hypogeous truffle species. While ecologically important, the hypogeous fungi comprise a small fraction of ectomycorrhizal species. In a second study, both epigeous mushroom as well as hypogeous species were included. Permanent plots were established in multiple stands of various age classes of Douglas-fir. This comprehensive four-year study yielded an extensive database providing the backbone for addressing new and critical research topics including: 1) conservation biology of rare fungal species, 2) sustainability of edible, commercially harvested fungi, 3) fungi as important indicators of old-growth legacy and forest community dynamics, 4) development of habitat models for fungi, and 5) implementation of studies employing molecular techniques to more effectively answer questions about belowground community dynamics.

Factors influencing respiration and nitrogen fixation rates in decomposing logs:

This ongoing research was established in 1985 and continues today through the efforts of two graduate students. A key finding, determined by remeasuring sporocarp production for 8 years, was that fungal sporocarps were exporting many nutrients (including N and P) during the early stages of log decomposition. This result was total unanticipated, and has caused us to rethink the mechanisms controlling nutrient loss from woody detritus. This work will continue in the future as we collect data to parameterize and validate log decomposition models.

The effects of climate on forest soil carbon and nitrogen dynamics:

We have conducted studies of soil processes at the HJA employing the same techniques and experimental design used in similar

studies of Oregon Coastal Ranges and southern Oregon forests. These studies have permitted us to compare the effects of regional climate differences on carbon and nitrogen cycling as mediated by microbial transformations and direct effects on vegetation. These studies have shown that moisture is apparently the major driver of soil C and N cycling at this scale. Studies conducted at the HJA have shown other factors being primary drivers of C and N cycling at watershed and stand levels.

At the HJA, two sets of well documented permanent forest floor study sites have been established which have been used by ourselves and others to study the effects of local climate and forest disturbance on forest soil biogeochemistry. One set of 24 permanent plots have been used in monthly measurements of soil respiration at a few selected sites and another set of 184 sites have been established to obtain maximum spatial coverage of the HJA. This ongoing study has allowed us to determine which components of carbon and nitrogen cycling are most influenced by climate by comparing climatic gradients across the HJA with various elements of biogeochemical cycling. These data will be used to parameterize predictive models of landscape-scale climate change scenarios on soil biogeochemistry. The large quantities of soil biogeochemical data have been collected at both sets of permanent sites will also be an invaluable resource to future researchers as they assess long-term changes in forest soils in response to global climate change.

The effects of forest disturbance on soil carbon and nitrogen dynamics:

The effects of stand harvest on soil carbon and nitrogen cycling has been studied at both the stand and landscape levels. Since C and N cycling is primarily controlled by microbial processes, these should be considered microbial observations. These studies are being continued under LTER 4 with the assessment of microbial factors influencing successional trajectories of sites after harvest as well as the influence of slow vs. fast regeneration on forest soils. These ongoing studies will be used to parameterize predictive models which will eventually allow us to assess the effects of forest management practices on carbon and nitrogen pools over the landscape.

In the future, we would like to set up manipulated plots at high elevations to evaluate the conditions that lead to the degradation of forest soils after a disturbance. There are a number of sites at high elevations in both the mountains of southern Oregon and the Central Cascades in which repeated efforts to reestablish conifers seedlings after harvest has failed; even 45 years after harvest. These studies will permit us to assess the factor/s responsible for this phenomenon; with this information, management practices could be devised to reduce the generation of degraded forest soils in the future. We are going to need considerable additional resources to maintain and monitor those plots over the coming years.

The effects of vegetation on soil biology and chemistry:

This is actually an extension of the forest disturbance studies since disturbances invariably result in the changes in vegetation. There are conditions under which there is a fundamental shift in vegetation that results in much different plant succession that is normally found at the HJA. In these cases, the normal bootstrapping that occurs between vegetation and soils appears to have failed. To better understand this feedback phenomenon, we have been studying and would like to continue studying the basic differences that occur in soil microbiology in response to the establishment of different vegetation types.

There has also been another study recently initiated at the HJA to assess the impact of different organic inputs on forest soil chemical and microbial characteristics. This NSF sponsored study will continue over the next few years as the impact of these manipulations are assessed.

As has traditionally been the case in most microbial studies, most of the studies described above have been of relatively short duration. We see, however, major benefits in continuing many of these measurement programs. Although the function of individual microorganisms is very limited in time and space, their collected effects over large temporal and spatial scales is considerable. In fact, much of our understanding of biogeochemistry on which forest productivity and litter/log decomposition models are based is incomplete. Long-term studies of microbial processes within the context of stand regeneration under different climatic regimes are critical to obtaining this understanding. In addition, long-term studies of fungal distribution patterns are needed to manage for this increasingly valuable commodity for maintenance of both biodiversity and productivity. In summary, the microbial studies currently and recently conducted at the HJA provide an excellent framework on which future long-term studies can be built.

ARC - Arctic Tundra, Toolik Lake, *Brooks Range, Alaska*

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MICROBIAL ECOLOGY AT THE ARC LTER SITE

The information on microbial systems in Toolik Lake has developed over the years along the same lines as the developing field of aquatic microbial ecology. The first information came from direct counts (AO, DAPI); bacteria numbers were unexpectedly high in the summer (1-2 x 10^6/ml, maximum of 3.1) and as low as 0.1 x 10^6/ml during the winter (Hobbie et al. 1983, Johnston and Kipphut 1988). Radioisotope studies (thymidine, leucine methods) gave an estimate of 3-8 gC/m2 for the annual bacterial production (O'Brien et al. 1997). In this ultraoligotrophic lake, with an annual primary productivity of 15 gC/m^2, the DOC from land equals algal production as a source of carbon and energy for bacterial production. Several mesocosm experiments with lake water and DOC leached from tundra vegetation confirm the availability of this DOC to microbial degradation (Hobbie and Kling in prep.). In fact, the input of DOC to Toolik Lake was measured by Whalen and Cornwell (1985) as 98 g DOC/m^2/yr so only a few percent are likely used by the bacteria.

The benthos of Toolik Lake is found in the rocky littoral zone and in the soft sediment profundal zone. The sedge Carex aquatilis is found in stands in very shallow water and contributes about 70 g of litter for each square meter of the stands. Microbial decomposition of this litter is rapid and over 60% of the Carex leaf material is lost within the first month after the litter reaches the water (Federle and Vestal 1980). Large fungal-like forms grow rapidly on the surface of the leaves for the first two weeks after which bacteria and pennate diatoms become dominant (Federle and Vestal 1982). Rates of microbial decomposition are adversely affected by reduced pH (McKinley and Vestal 1982) or high turbulence (Federle et al. 1982). Rates of microbial decomposition are stimulated by increased temperature (Federle et al. 1982b) and nutrient enrichment (Federle et al. 1982a). This series of studies showed the importance of physical factors in determining the rate at which plant litter is colonized and degraded in aquatic environments.

The benthos of the Kuparuk River, another ARC LTER site, has been studied throughout a fifteen-year fertilization experiment. The addition of phosphorous has resulted in an overall metabolic shift from heterotrophy to autotrophy (Peterson et al. 1985). Some microbial processes were also stimulated by the increased photosynthesis caused by fertilization. Total respiration of the epilithon, acetate uptake (Hullar and Vestal 1989) and decomposition of lignin monomers were all stimulated but only in light-grown epilithon (Lock et al. 1990, Hershey et al. 1997). When epilithon was grown in the dark in the fertilized region of the river, there was no increased respiration. Also, phosphorous did not stimulate the decomposition of Carex litter (Peterson et al. 1993). Analysis of the micro-algal community structure of epilithon and moss epiphyton is an ongoing Kuparuk River project (Slavik and Peterson in prep.).

With the advent of molecular techniques, it became possible to assess the phylogenetic affinity of cultured and uncultured bacteria from Toolik Lake (Bahr et al. 1996). Ribosomal RNA genes were amplified and sequenced from cultures or DNA extracted from concentrates of bacteria from 40 liters of lake water. All of the genes were closely related to phyla represented in the ribosomal RNA database. Some were related to terrestrial forms or to forms typical of nutrient-rich habitats; these are likely from particles or from land. One group is a relative of the SAR11 cluster previously detected only in marine environments. This initial molecular survey of Toolik Lake microorganisms was not designed to detect Archaea or Eukarya.

In recent years, we have shifted our attention to the heterotrophic nanoflagellates (HNAN, 2-20 μm). An earlier Toolik study (Peterson et al. 1978) was the first to show that bacteria were not grazed to any extent by the larger zooplankton. We found that when parcels of water were confined in large plastic mesocosms (60 m^3, 6 m^3), it was possible to follow a classic predator-prey cycle between the nanoflagellates and their bacterial prey. This was particularly obvious when the phytoplankton was fertilized with inorganic nutrients. Then a number of cycles could be traced and we even found that the bacterial productivity was at its highest when the bacteria were grazed so much that their numbers fell drastically (Hobbie and Helfrich 1988). The identity of almost all of these nanoflagellates is unknown although we do have numbers of nanoflagellates (a few thousands per ml) and a species list of algae (H. Kling in O'Brien et al. 1996), generated with the inverted microscope, that states that Chrysophyceae are dominant and that the genera *Chromulina*, *Ochromonas*, *Spiniferomonas*, *Pseudopedinella*, *Pseudokephyrion*, *Paraphysomonas* and *Kephyrion*

are present. Some of these have colorless forms or are mixotrophic but we do not have any solid taxonomy for the nanoflagellates. Bahr's recent experiments (Hobbie et al. in press) solved the problem of measuring grazing rates on bacteria (developed a ^{14}C-labeled bacteria method suggested by Meinhardt Simon) and revealed that the HNAN grazing rate of 16% of the volume per hour removed bacteria at about the same rate (25% of production) as they were produced. Thus in Toolik Lake the nanoflagellates are the dominant grazers of the bacteria.

An additional piece of the puzzle comes from the Rublee and Bettez (1995) finding that the microplankton (20-200 m m, flagellates, ciliates, rotifers, and crustacean nauplii) are the dominant grazers of the nanoflagellates.

The final question concerns the survival of the nanoflagellates in the lake and pond. Do they persist over winter in the water column of the lake or are they reintroduced by streams in the spring. Do they overwinter in the pond and lake sediments?

As a result of the information gathered to date, we conclude that the biggest unknown in our current understanding of the microbial system is the diversity and ecology of nanoflagellates and other protists. We believe this is true for all aquatic environments. For this reason, we have designed a project to use state-of-the-art techniques to study the ecology of nanoflagellates in an arctic system. The information gathered on the identity of the flagellates and their position in molecular based trees (using ribosomal RNA sequences) will provide insights about their evolutionary relationships as well as a data base for designing molecular probes capable of measuring seasonal fluctuations in nanoflagellate populations.

Our goal for future aquatic research is to determine not only the identity, abundance and distribution of all components of the microbial food web, but to understand their physiological capabilities. We want to know what use they are making of these capabilities in the functioning of ecosystems. This will most likely be done via methods, which detect gene expression in the natural environment.

Besides the research being conducted at the lakes and rivers aquatic sites, parallel terrestrial experiments have been ongoing at the ARC LTER. The importance of microbial activity is clearly recognized in the terrestrial environment. However, the soil experiments have been more process-oriented studies of CO_2, methane and DOC fluxes and therefore will not be discussed in this brief report.

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BES - Baltimore Ecosystem Study LTER *Baltimore, Maryland*

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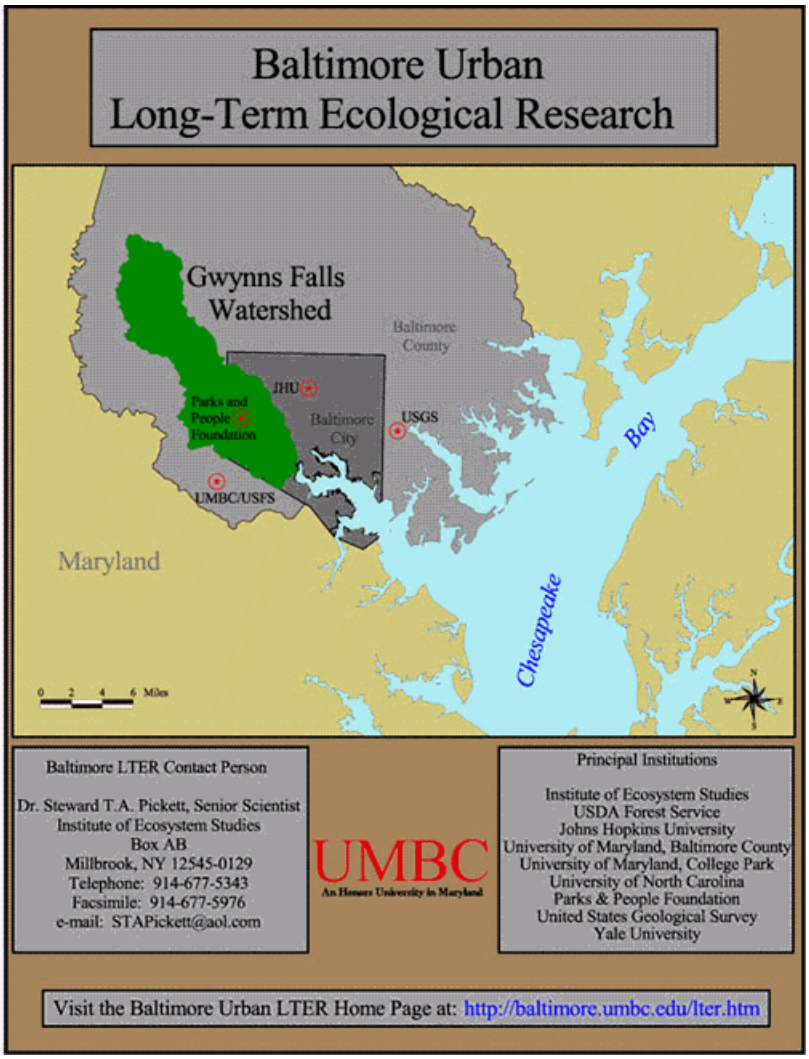
Microbial Ecology in the Baltimore LTER:

We are beginning to establish a group of intensive, permanent monitoring sites where there will be lots of vegetation and soil work, including microbial ecology done. The sites will be distributed to represent land use conditions within the study area as follows:



- 6 forest sites. Located to allow for comparison of the two major forest types (Tulip Poplar, Chestnut Oak) which occur on the two dominant soil types (gneiss, schist) in the area, and for comparison of urban versus rural settings. Exotic species and riparian areas may also be considered in the location of these plots.
- 2 currently agricultural sites. These are included because past land use in our study area was agricultural and we need site-specific calibration of models that can depict agricultural land uses.
- 8 residential sites. Located to allow for comparison of density, age and socioeconomic status.
- 4 commercial/industrial sites. Located to allow for comparison of age and socioeconomic status.
- At each site, we will locate three 20 m x 20 m plots. Each plot will be instrumented for soil solution samplers (lysimeters), trace gas flux chambers, N mineralization tubes, tagged trees, litter collectors, biodiversity surveys, etc., i.e. the whole suite of process-level measurements for the LTER.

Our experimental design has been structured to allow us to compare the influence of natural variation caused by soils with human-induced variation caused by urban air pollution and land use change on microbial nutrient cycling processes.

Specific microbial work that will be done at these sites include routine measurement of nitrogen mineralization, nitrification, denitrification, soil respiration, nitrous oxide flux, methane flux and microbial biomass carbon and nitrogen content. These measurements will be used in simulation models to produce ecosystem, landscape (watershed) and regional scale estimates of microbial processes.

Our sampling design and planned data collection make us well poised to develop our site as a "microbial observatory". We could quickly and easily incorporate the latest techniques for measuring microbial community composition and function into our planned basic microbial monitoring program. Our planned

structure, where microbial data will be collected in the context of a large suite of biogeochemical process measurements, will ensure that new microbial data would immediately be used to help interpret broad patterns of ecosystem structure and function and to stimulate new detailed research on microbial ecology.

BNZ - Bonanza Creek LTER *Fairbanks, Alaska*

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Introduction

Work on microbial processes at Bonanza Creek has ranged across a number of scales. Large scale work has focussed on understanding microbial processes at the ecosystem scale. At the

opposite extreme, work has focussed on understanding the dynamics of soil microbial and faunal communities and how community structure relates to process dynamics.

Controls over carbon dioxide and methane fluxes across the taiga landscape.

Over four years we monitored CO_2 and CH_4 fluxes in a range of taiga forests along the Tanana River in interior Alaska. We studied floodplain alder and white spruce sites and upland birch and white spruce sites. Each site had control, fertilized, and sawdust amended plots. CO_2 emissions decreased with successional age across the sites (alder & birch > white spruce) regardless of landscape position. When soil temperature was below 17° C, flux rates correlated strongly with temperature but not with moisture. Above 17° C, however, fluxes were lower than predicted by temperature and appeared to be limited by soil moisture. Of the manipulations, only N fertilization had any effect on CO_2 production, decreasing flux in the floodplain sites. Landscape position was the best predictor of CH_4 flux. The two upland sites consumed CH_4 at similar rates (~0.5 mg C m^{-2} d^{-1}), whereas the floodplain sites had lower consumption rates (0-0.3 mg C m^{-2} d^{-1}). N fertilization and sawdust both inhibited CH_4 consumption in the birch and floodplain spruce sites but not in the upland spruce site. The biological processes driving CO_2 fluxes were sensitive to temperature, moisture and vegetation, whereas CH_4 fluxes were sensitive primarily to landscape position and biogeochemical disturbances. Hence, climate change effects on C-gas flux in taiga forest soils will depend on the relationship between soil temperature and moisture and the concomitant changes in soil nutrient pools and cycles.

Microbiology of Atmospheric Methane Consumption

Soil consumption of atmospheric CH_4 is an important component of the global CH_4 cycle, yet it remains poorly understood at the microbial level. We investigated the influences of CH_4 supply, soil moisture, dissolved salts, and NH_4^+-fertilizer on the activity of soil CH_4 oxidizers. When starved of CH_4, two upland taiga soils lost their capacities to oxidize CH_4, indicating that the organisms involved were truly methanotrophic. One of the most intensely investigated controls on soil CH_4 consumption has been its inhibition by NH_4^+ and other factors. Our work on the inhibition of CH_4 consumption showed that there are three physiologically different patterns of N-inhibition of CH_4 oxidation that occur in different ecosystems. In some soils, inhibition is immediate and results from competitive inhibition by NH_3; in other soils inhibition is delayed and appears to result from the inhibition of CH_4 oxidizer growth; in yet other ecosystems CH_4 oxidizers are simply insensitive to NH_4^+. On top of these N effects, however, other salts can inhibit CH_4 oxidation. In some soils non-NH_4^+ ions were so toxic that they completely masked the NH_4^+ effect. These results were the first to demonstrate CH_4 oxidizer growth at atmospheric concentrations of CH_4. This work also suggested that the primary landscape-level control over the response of soil CH_4 consumption to NH_4^+-fertilization is the distribution of physiologically distinct CH_4 oxidizers across sites.

Structure of forest floor soil communities

In the forest floor of Alaskan taiga, annual layers of *Equisetum* (horsetail) litter demarcate cohorts of birch litter. Forest floor material was separated into each of the three most recent litter cohorts, plus the Oe and Oa layers. Overall, respiration potential decreased with depth of litter (litter age) and over the growing season. Nitrogen mineralization potential increased with depth, and fluctuated over time. Microbial biomass did not vary with depth, but did increase greatly in September in conjunction with increased litter moisture. Litter C:N ratio decreased with time and varied with depth according to the year-to-year variation in litter chemistry. Different groups of soil fauna show distinctly different patterns of activity with depth and time. For example, larval dipterans are limited by moisture conditions to the deeper strata of the litter layer, and thus are important in fragmenting litter, but only after it has been in the forest floor for 2-3 years, though they are capable of feeding on fresh leaves when the environmental conditions are appropriate. Other fauna, such as nematodes and some mites, migrate from deeper to shallower layers of the litter layer over the course of the summer. Microbial and faunal activities on a particular litter cohort is a function of the litter chemistry (N and labile C content), the vertical position of the litter in the forest floor, and the timing within the seasonal cycle.

Plant Secondary Chemical Effects on Soil Microbial Processes

The vegetation mosaic of the Alaskan taiga is produced by patterns of disturbance coupled to well defined successional patterns. In primary succession on river floodplains, one of the critical transitions in succession is that from thinleaf alder (*Alnus tenuifolia*) to balsam poplar (*Populus balsamifera*). This is the shift from a N_2-fixing shrub to a deciduous tree. Through this transition there are major changes in N cycling including a decrease in N_2-fixation, mineralization, and nitrification. Most models of plant effects on soil processes assume that these changes are caused by shifts in litter quality and C/N ratio. However, our studies have shown that balsam poplar secondary chemicals have major effects on soil nutrient cycling. Balsam poplar tannins inhibited N_2-fixation in alder nodules. Field studies supported this conclusion by showing the lowest rate of N_2 fixation occurs in the successional stage dominated by poplar. Tannins also inhibited decomposition and N-mineralization in alder soils, though different tannin fractions had differing activity, with small tannin molecules (monomers and dimers) acting as microbial substrates. Other poplar compounds, including low-molecular-weight phenolics, were microbial substrates and increased microbial growth and immobilization, thereby reducing net soil N availability. Thus balsam poplar appears to affect nutrient cycling by both inhibiting N_2 fixation and mineralization, and by stimulating immobilization. The combination of these activities may function to both reduce N-supply to alder, and to retain N in the ecosystem as alder is replaced and N-inputs decline.

Microbial Stress Ecology

Drying/rewetting and freeze thaw events are common in the taiga, yet we know little about their effects on microbial processes or microbial communities. We have carried out a series of experiments that demonstrate that such stress events cause changes in microbial communities that have long-lasting effects on process dynamics. In an early experiment we showed that a single drying/rewetting event in the lab could reduce respiration by 25% over 2 months following the rewetting. This suggested that stress could reduce the available population of a critical microbial group and that recolonization post-stress could be an important control on microbial activity in litter. To test these ideas, we did a field experiment to evaluate the effect of moisture regime on microbial biomass and activity in birch litter. Litter bags were placed in one of three treatments: continuously moist, watered weekly, and "natural", which experienced two natural dry/wet cycles of two weeks dry followed by rain. There were strong overall correlations between microbial biomass, respiration, and litter moisture content. However, the different treatments had significantly different rates of respiration, biomass, and respiratory quotients (qCO_2) that could not be explained by moisture content directly. The natural treatment had lower respiration rates and biomass C and N than the wet or rewet samples, indicating that the 2-week droughts experienced by the natural treatment reduced microbial populations and activity to a greater degree than did shorter droughts. Metabolic diversity of the bacterial community was reduced by multiple episodic drying and rewetting events, as indicated by a dramatic decrease in the number of substrates used in a BIOLOG plate assay. This experiment showed that the size and functioning of the litter microbial community was strongly affected by its prior stress history.

Freeze-thaw cycling caused a flush of microbial C and N during the first cycle, but over three cycles significantly reduced the microbial communities' abilities to decompose SOM and led to reductions in total C and N mineralized. There differences among soils appear related to differences in the quality of SOM and the native activity of the soil microbial biomass. Multiple stress cycles caused a greater relative reduction in total respiration in high organic-matter-quality than in low quality soils, but recovery of activity is likely faster in high quality soils. The long-term effects of such multiple stresses may depend on the extent of damage to the microbial community and its ability to recover from the stress.

Microbial Activity in frozen soils

We measured microbial respiration in soils from a range of both tundra and taiga communities at temperatures of -2º C and -5º C in a laboratory incubation. For white spruce (*Picea glauca*), we also measured activity at both 50% and 10% of water holding capacity, as these soils tend to desiccate over the winter. Microbial respiration was significant even down to -5º C. However, C and N mineralization appeared to be disconnected from each other in the frozen soils. The controls on activity in frozen soils are still unclear, but unfrozen water content appears to be important; in the moisture experiment, respiration was significantly greater at 50% WHC than at 10% WHC. It is also possible that the nature of microbial substrates shift as soils freeze, from polymeric soil organic matter to dissolved material and dead microbial biomass.

Mycorrhizal dynamics and mammal browsing

Using an exclosure experiment in the willow stage of primary succession on the floodplain of the Tanana River, we tested the hypothesis that browsing can reduce mycorrhizal infection. We measured the effects winter browsing by moose (*Alces alces*) and snowshoe hare (*Lepus americanus*) had on mycorrhizal infection and fine root biomass of willow (*Salix* spp.) and balsam poplar (*Populus balsamifera*). We found that protection from winter browsing increased ectomycorrhizal infection by 10% in the top 5 cm of the soil profile, by 23% at 5-10 cm, and by 42% at the 10-15 cm depth. Mammal browsing in taiga forests is now recognized as a major cause of the shift palatable species such as alder and spruce. We suggest that browsing-induced reduction in ectomycorrhizal infection of salicaceous species plays a central role in this shift in plant community composition from palatable

deciduous species such as willow and balsam poplar to less palatable species such as alder and spruce. We suggest that browsing-induced reduction in ectomycorrhizal infection of salicaceous species plays a central role in this shift in plant community composition.

Microbial community structure and function in oiled taiga soils.

In 1976 two 7570 liter experimental spills (one in February and one in July) of hot Prudhoe Bay crude oil were conducted in an open black spruce (*Picea mariana*) forest at the Caribou-Poker Creeks Research Watershed. In 1994 and 1995, a substantial quantity (ca. 0.3 $g \cdot g^{-1}$ dry soil) of crude oil remained in the soil at the site 18 years after it was spilled, and several microbial parameters showed evidence of long-term oiling effects. Overall, the surviving community in the oiled plot has shifted toward using oil C for growth. Numbers of hydrocarbon degrading microbes, and specific hydrocarbon mineralization potentials, were significantly elevated in the oiled (OIL) plot compared to an adjacent oil-free, reference (REF) plot. Glutamate mineralization potentials and soil C mineralization, on the other hand, were not different between treatments, suggesting that OIL plot heterotrophs were well-acclimated to the oil. Despite the similarity in C mineralization, net N mineralized was lower and net nitrification was absent in OIL soils. Biomasses of total fungi and bacteria, and numbers of protozoa, showed no consistent effects due to oiling, but metabolically active fungal and bacterial biomasses were uniformly lower in OIL samples. Community-level multiple substrate metabolism (Biolog) was assessed using a new technique for extracting kinetic data from the microplates. This analysis suggested that the microbial population diversity in the OIL soils was lower than in REF soils. Further, these data indicated that the surviving populations in the OIL plot may be considered metabolic generalists. Some evidence of crude oil biodegradation was seen in the chemistry data, but enrichment of the oil residue in higher molecular weight components, duration of contact with soil organic material, and slow rates of C mineralization indicate the crude oil will persist at this site for decades. Contamination of Alaskan taiga soil at this site has yielded observable long-term microbial community effects with larger-scale consequences for ecosystem function.

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MICROBIAL ECOLOGY IN THE CENTRAL ARIZONA - PHOENIX LTER

Microbial processes are key elements of changes in organic matter storage and biogeochemistry of both terrestrial and aquatic systems in the urban environment. The Central Arizona - Phoenix (CAP) LTER project will incorporate microbial ecology studies in several of its core monitoring efforts as well as its short-term experiments. Planned measurements of soil respiration and trace gas flux from soils and other urban surfaces on a patch-specific basis will give information on magnitude and spatial distribution of microbial processes. The current scheme of urban patch types includes:

1. open ground (vacant lot, graded soil, "natural")
2. water (streams, rivers, canals, lakes)
3. riparian zones (vegetated, unvegetated)
4. agriculture (fallow, active)
5. recreational (golf courses, parks)
6. residential (mesic, xeric)/commercial/industrial
7. transportation corridor

Research will characterize these patch types in terms of several ecological variables; those of relevance here include soil organic matter, soil gas fluxes, dissolved organic matter, protist/bacterial biomass of aquatic ecosystems, arbuscular myccorhizal communities, and selected biogeochemical transformations (e.g., denitrification, nitrification, methanogenesis).

Investigations of materials transport and transformation represent an attempt to construct whole-metropolis budgets for nutrients, major ions, salts, and metals. Analysis of input-output balance (using existing data and data collected from the new monitoring project) will give us an idea of where process studies should be focused to understand controls over microbial material transformation in the urban system.

In addition to these planned core elements of research, CAP scientists began a series of short-term studies or "resurveys" in January 1998, several of which have microbial components:

Lichen resurvey with heavy metal analysis

Lichens are a classic bioindicator of air pollution. Twenty-five sites surveyed in Maricopa County in 1974 for the presence or absence of different lichen species will be resurveyed. The new survey will compare the present concentration of trace metals in current lichen tissues with those in samples collected from the original survey.

Comparison of soil respiration among residential patches

Evolution of CO_2 from soils in residential lots of four types is being measured as an index of microbial respiration. Permanent soil plots were established in yards of one of four possible residential water-use options (xeriscaped or watered)/patch history (agriculture or desert) combinations.

Biodiversity of AM fungal communities

A preliminary study is being conducted to elucidate arbuscular mycorrhizal (AM) fungal diversity along a temporal gradient (time since landscape establishment) in residential landscaped areas in Phoenix. Rhizosphere soil was collected from ash trees (Fraxinus sp.) at each sampling site in June 1997 and used to establish trap cultures to determine AM species richness and composition. Preliminary findings revealed that AM fungal species richness was greater at more established landscape sites (at least 45 years since planting) compared with recently installed sites (less than 5 years since planting). Species in the Glomaceae predominated at all sites.

Resurvey of urban lakes

Urban lakes of the Phoenix metropolitan area were characterized in the 1970s as to water chemistry, sediment chemistry, and phytoplankton populations. These lakes are currently being resurveyed; surveys will likely be repeated every 10 years.

Rio Salado Town Lake

A pilot project incorporating university classes (field geology, limnology) will examine hydrogeology and limnology of the Tempe Town Lake, an impounded, >300-ha urban lake that will be created in the now dry Salt River bed in the City of Tempe. Pending funding, microbial studies will include: 1) microbial transformations in water column bottom and subsurface substrata; 2) formation and mitigation of noxious algal blooms; 3) microbial biofilm biomass and N and organic C transformations in the 0-1 in stratum of the groundwater recharge zone; and 4) microbially mediated transformations of N and organic C in fringing wetlands receiving surface water inputs (both storm flows and effluent).

CDR - Cedar Creek Natural History Area - *Minnesota*

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PAST AND CURRENT ACTIVITIES:

More microbial work is needed at Cedar Creek in spite of early studies pointing quite clearly to the strong role of microbes in ecosystem processes at Cedar Creek. Although not directly microbial, (McKone and Biesboer 1986) and (Pastor, et al. 1987, Pastor, et al. 1987) invoked microbial processes in explaining the patterns they observed concerning nitrogen fixation and mineralization and litter decomposition.

Zak (Zak and Grigal 1991) (Zak, et al. 1990) conducted considerable research on microbial biomass in old fields, swamps and forrests. He showed, for example, that N processing can differ by an order of magnitude over short distances in the heterogeneous landscapes of Cedar Creek. Microbial biomass ranged from 80 g C m2 to 165 g C m2 (swamp sites) to as low as 3.4 g N m2 to 9.5 g N m2 (old field, pin oak forest, and savanna sites). Based on relationships between microbial biomass and n-mineralization and denitri, Zak concluded that microbial population dynamics may be exerting control over N availability. Given that most of the hypothetical mechanisms for plant community dynamics at Cedar Creek are nitrogen based, Zak's work clearly points to the potential importance of microbes as the mechanistic agents. His conclusion (Zak, et al. 1990), "Carbon (C) and nitrogen (N) cycles within terrestrial ecosystems are intimately linked through patterns of plant and microbial activity," clearly stated the need to examine both plants and microbes at Cedar Creek.

Johnson, who is again working at Cedar Creek as of this year, examined mycorrhizal fungi at Cedar Creek. Her 1991 paper (Johnson, et al. 1991) showed that VAM mycorrhizal diversity (Shannon-Wierner index, not spp. richness) declines with succession, though density shows modal relationship.

Johnson also tested the hypothesis that nutrient-stress plants release more carbohydrates which leads to selection for mycorrhizae that are less efficient resource provisioners -- turns out to be true in a fertilizer experiment (Johnson 1993).

Although not directly studied, microbes were invoked by Wedin in his 1995 paper on decomposition (Wedin, et al. 1995). Wedin argued that microbes are probably the responsible agents for the increase in litter C which, by stable isotope analysis, proved to originate from soil organic material.

Finally, Terry Chapin and Val Evinger came to Cedar Creek natural history area last summer. They did Biolog plates, short term N15 incubations to estimate microbial N pools, and FAME. We've not heard anything back except that no patterns were observed in Biolog.

CDR is especially interested in getting microbial information (bacterial, protistan, and fungal) from FACE and small biodiversity plots. This involves over 600 plots.

A LTER microbial observatory effort could provide funds to collect bacterial, protistan, and fungal biomass, density, and some measure of diversity from FACE, biodiversity, and smaller, multi-trophic level experiment. A full time technician who would process the samples in collaboration with others at CDR. Data would be collected and dissemination to the investigators and others via Cedar Creek's web page.

Our purpose would be to identify the below ground mechanisms for the biodiversity, CO2, and N fertilization effects we observe and to conduct this work for the next 5 to 10 years. I think this has some very exciting potentials for providing insight into the biodiversity/ecosystem functioning debates.

Measurements would include:

- Microbial density (bacteria (DAPI), fungi (hyphal counts), protists DAPI))
- Bacterial diversity (we would have samples done at a lab where such facilities exist, either nucleic acids or FAME, whichever appears to be the best for these purposes)
- Bacterial C functional diversity (Biolog)
- Microbial N (bacteria, fungi, and protists, we would use chloroform fumigation/extraction methods and use ratios to determine fungi and protits based on density)

Our hypotheses would be that biodiversity effects are mediated by rhizospheric microbial processes. We would get at this by examining microbial responses to the manipulations of plant diversity, CO2, and N in conjunction with measures of soil respiration, n-mineralization, decomposition, and many other variables we keep track of.

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CWT - Coweeta Hydrologic Laboratory, *Otto, North Carolina*

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There are several long-term studies underway which rely extensively upon microbial research and microbial processes. These include measurement of fluxes of nitrogen to the air and to water from dozens of watersheds with varying vegetation covers and of varying successional age, and also studies of human-caused versus natural (hurricane)-caused disturbances.

We have measured the dynamics of carbon, nitrogen, phosphorus and sulfur (Stanko-Golden et al. 1994) into and out of labile organic (microbial) pools (Coleman 1994) in uplands, hillslope/riparian, and streams both within the Coweeta basin (Maxwell and Coleman 1995) and more generally in the 53,000 km^2 region of the southern Appalachians. In the Coweeta basin, we have measured small, but significant increases in nitrate export from our upland sites into the > 73 km of upland streams as nitrogenous inputs from the Atlanta metropolitan airshed region moves steadily upward (W.T. Swank, pers. comm.).

In long-term recovery studies after massive windthrows on hillslope sites after Hurricane Opal hit Coweeta in October 1995, there have been large 100-fold increases of nitrate-N in tipup areas into tension lysimeters in our impacted sites (Yeakley, et al., in prep.). In contrast, in a nearby Rhododendron extirpation plot, when all aboveground Rhododendron maximum was removed from the site, but the soil surface was minimally-disturbed, there was only a gradual increase in soil respiration over 2-3 years' time post-cut (from August 1995 onward). This is attributed to gradually increasing losses of carbon from decaying root systems. No increases in nitrate losses into the lysimeters were noted in the extirpation site.

Coweeta investigators are using a diversity of approaches to assess the trophic significance of bacteria in streams and to assess the role of microbes in nitrogen cycling in streams. Early research investigated the significance of bacteria in the diets of aquatic insects in streams using 3H-thymidine to follow the incorporation of bacteria into animal tissue (Findlay and Meyer 1984, Findlay et al. 1986, Meyer et al. 1987, Meyer 1994). Additional studies used microscopy to examine copepod feeding on bacteria associated with decomposing leaf material (Perlmutter and Meyer 1991) and using fluorescently labeled bacteria to measure the rates at which they were consumed by filter feeders (Hall et al. 1996). Recent microbial work has relied upon stable isotopes to investigate the role of microbes in stream ecosystems.

We have assessed the trophic significance of bacteria in stream food webs by adding tracer quantities of 13C-acetate to streams, one of which had all leaf litter excluded from it for two years (e.g. Hall and Meyer in press). Bacteria in the litter-excluded stream had 7-10 times more labile than those in the reference stream during both summer and winter, showing their higher relative use of streamwater DOC. The fraction of invertebrate carbon derived from bacteria was significantly related to the fraction of amorphous detritus in invertebrate guts, suggesting that the bacterial carbon supporting higher trophic levels was associated with amorphous detritus particles. Invertebrates in the litter-excluded stream did not derive a greater fraction of their carbon from bacteria despite a lower standing crop of detritus in the litter-excluded stream. The standing stock of colloidal carbohydrates was 5 times greater than cellular bacterial biomass; hence the high use of bacterial carbon by invertebrates may be a consequence of the availability of these polymers.

To measure microbial nitrogen in streams, we have also adapted a microbial technique commonly used in soils for estimating microbial biomass nitrogen and carbon. We have measured microbial carbon and nitrogen in fine benthic organic matter, leaves and wood in streams at Coweeta, Hubbard Brook and Walker Branch (Oak Ridge National Lab) using this technique (Sanzone et al. in prep.). We are using this technique to assess the del 15N of microbes in 15NH4 addition experiments in several LTER and other stream sites (e.g. Hall et al. in press).

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Microbial Ecology at the Hubbard Brook LTER

We instituted a long-term monitoring program of microbial biomass and activity at HB in 1993 (see www.hbrook.sr.unh.edu under Data - Soil). The objectives of this program are 1) to provide background data on microbial parameters to help interpret spatial and temporal patterns in our long-term biogeochemical records of stream and soil solution chemistry, litterfall and tree growth and 2) to provide baseline information for more detailed studies of microbial processes (e.g. trace gas fluxes, N mineralization and nitrification, denitrification) and how they respond to ecosystem change (e.g. snow depth and soil freezing, base cation addition). The microbial program at Hubbard Brook could benefit quickly and greatly from an infusion of funds. A recent NSF site review specifically recommended expansion of the microbial program at Hubbard Brook. Moreover, we have begun collaborating with several individuals who could immediately bring new dimensions to the program. Melany Fisk (Cornell University) is doing novel work with carbon substrate use at Hubbard Brook, Mary Arthur (University of Kentucky) has measured fungal:bacterial ratios, Linda Pardo (USFS) is applying state-of-the-art stable isotope techniques, and Andria Costello, an incoming new faculty member at Syracuse University is interested in applying the the latest methods of molecular characterization of microbial communities at Hubbard Brook. Sharon Parker (USFS) may also be interested in molecular work at Hubbard Brook. In summary, the HB LTER is well poised to function as a "microbial observatory". We have an existing basic monitoring program and could quickly incorporate the latest techniques into this program. Our existing structure, where microbial data is collected in the context of the long-term biogeochemical record at HB, would ensure that new microbial data would immediately be used to help

interpret broad patterns of ecosystem structure and function and to stimulate new detailed research on microbial ecology.

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There are three long-term experiments underway which provide unique opportunities for microbial research, and at least two affiliated projects which use newer techniques. The three projects are the soil warming experiment, the DIRT (or litter input alteration) experiment, and the chronic N addition experiment. Paul Steudler is working with a colleague on a project which used modern biochemical methods to identify changes, and Wric Davidson is working with colleagues at Penn St. on ^{15}N and ^{13}C NMR on the chronic N plots. We would be interested in pursuing the "observatories" path

Unique results have been observed in the Chronic N plots. Over the first 9 years we have added up over 1300 kg N per hectare to a hardwood stand at the Harvard Forest. Only in year 9 did we begin to see measurable nitrate leaching losses, meaning that the system retained nearly 100% of this very large amount of N (annual addition rates of 150kg N/ha/yr are twice the annual internal cycling rate). More than ¾ of this N is retained in soils, suggesting that microbial N immobilization is a major process for N retention. However, two different types of measurements show that there is no significant increase in CO_2 evolution from soils accompanying this increased N incorporation. Two alternative hypotheses are being considered: 1) that abiotic (chemical) processes are an important component of the N retention capacity, and 2) that immobilization occurs through Mycorrhizal uptake, assimilation and release, using C from photosynthesis directly. Understanding the capacity of the actual mechanisms responsible for N retention is a critical part of predicting the rate at which forests will approach N saturation and the elevated nitrate leaching losses and potential for forest decline which can result.

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JRN - Jornada Basin, *Las Cruces, New Mexico*

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A large number of microbial investigations are underway by the Jornada research team which can be separated into two types of activities. The first is integrated into the long-term ecological research program itself and part of the core data collection activities of the site. This includes collection of biomass N, viable heterotrophic counts (by MPN) and plate counts of a Nitrogen Efficient Guild (NEG) that is adjacent to the 15 permanent plots (3 in each of 5 vegetation types). The NEG is made up of organisms which are either free living N fixers or very efficient N scavengers. We are also looking at AMF.

The second sort of work involves either site PIs or visitors whose independent projects are either microbial ecology or which have microbial ecology

components. For example, there are several projects on the Jornada which are not part of the core research. One looks at microbe distribution associated with shrub invasion and resource island formation (NSF international Programs/Long Term Studies funding) comparing Swedish pasture shrub invasion with that in the Jornada's grassland sites. We (Yossie Steinberger at Bar Elan in Israel and I) just got word that we will be funded for a project which will measure how the total nitrogen, soluble nitrogen and biomass nitrogen pools of in desert soils change with time, particularly what happens after natural wetting events. It will also elucidate how these changes correlate with or control plant productivity and it will follow the population levels of soil organisms including arbuscular mycorrhizal fungi, heterotrophic bacteria and soil nematodes which, because they can be divided into feeding groups, provide a mirror of the soil biota on which they feed. Other researchers like Ross Virginia and Jim Reynolds have looked at various microbial ecology components that impact their work with shrub dynamics, Bill Schlessinger's students have measured microbe mediated processes as part of biogeochemistry work and Curtis Monger has ongoing work which looks at various microbially mediated deposition carbonate processes and cryptogamic crusts as part of soils studies.

Peter Herman is surveying microbial diversity, biomass, and activity in microbial guilds among the various habitats that we study in the Jornada Basin. Several of his papers appear as Herman et al. 1993, 1994, and l995 (all appearing in Applied and Environmental Microbiology). The references are listed in the JRN site bibliography.

Within efforts of Bill Schlesinger to quantify trace gas production at the Jornada, we have measured microbial biomass, nitrogen-fixation and related variables have been measured in different habitats. Several of these papers have appeared as Gallardo and Schlesinger l992, l995 in Biogeochemistry.

On the whole, microbial ecology has a fairly robust presence on the Jornada LTER site and that presence is likely to grow because all of our investigators recognize the connections between macrobiologial and microbiological processes. The limit has been resources (both human and monetary) to make the connections, not a lack of will or interest.

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Microbial Research at the KBS LTER Site

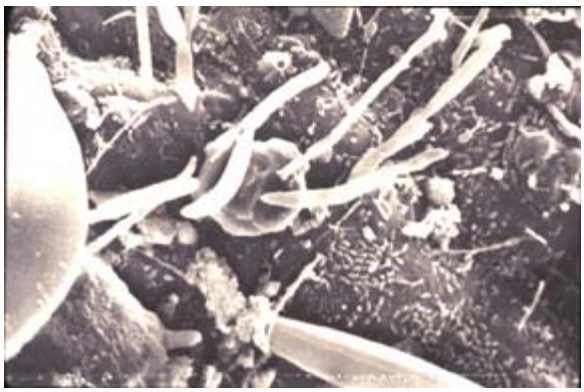
Soil microbial ecology is a central part of KBS LTER research. Understanding the ecological interactions underlying the productivity of field crop agriculture is the central focus of LTER research at KBS, and microbes comprise one of our most intensively studied taxa, together with vascular plants and insects. Microbial studies at KBS take a variety of forms, with most studies directed towards questions about the patterns, causes, and consequences of microbial diversity and microbial biomass for ecosystem processes in intensively

managed ecosystems.

Our studies to date have centered on examinations of microbial growth rates, biomass, and fungal: bacterial ratios. We have also focused on population-level questions using direct microscopy, classical pure-culture techniques, and, for the multitude of unculturable microbes in soil, molecular analyses of phenotypes and genomes. Many of these latter techniques provide whole-soil signatures of community composition, and have been particularly useful for examaning community-level differences among sites and experimental treatments. For questions related to specific populations we have focused our efforts on examinations of specific functional groups such as denitrifiers, nitrifiers, lignin and 2,4-D degraders, and the rhizobacteria, linking these groups to specific microbial processes. Much of this research has been collaborative with the NSF Center for Microbial Ecology (www.cme.msu.edu) at Michigan State; a number of KBS LTER co-PI's are also co-investigators in the CME.

We provide below background information on our current studies of microbial community structure. Other microbial work is also underway at KBS but not described here due to space limitations – including detailed biogeochemical and population-level investigations of microbial processes such as denitrification, trace gas fluxes, and soil organic matter turnover and DOC and DON fluxes. Following this section we highlight specific analytical procedures now in use at KBS.

Microbial Community Structure



The diversity and complexity of soil microbial communities present a major challenge to our efforts to understand how biological processes can be managed in agricultural systems. Soil microbial communities are arguably the most diverse communities on earth, and the factors that determine this extraordinarily high diversity are not well understood (Caldwell et al. 1997). Torsvick et al. (1994) have provided evidence that in one gram of soil there are billions of individual organisms and thousands of species. What are the ecological consequences of such high diversity at such a small spatial scale? And how does this change across the range of scales that we consider to be important for other organisms (e.g. plants and consumers) and biogeochemical processes? To determine how to manage the biological processes controlled by soil microbes, it is important to understand the patterns, causes, and consequences of microbial diversity and the scale at which microbial communities are structured. Understanding the link between the scale at which the microbial community is structured and the scale at which ecosystem processes occur may itself tell us a great deal about the role of microbial diversity in ecosystem functioning.

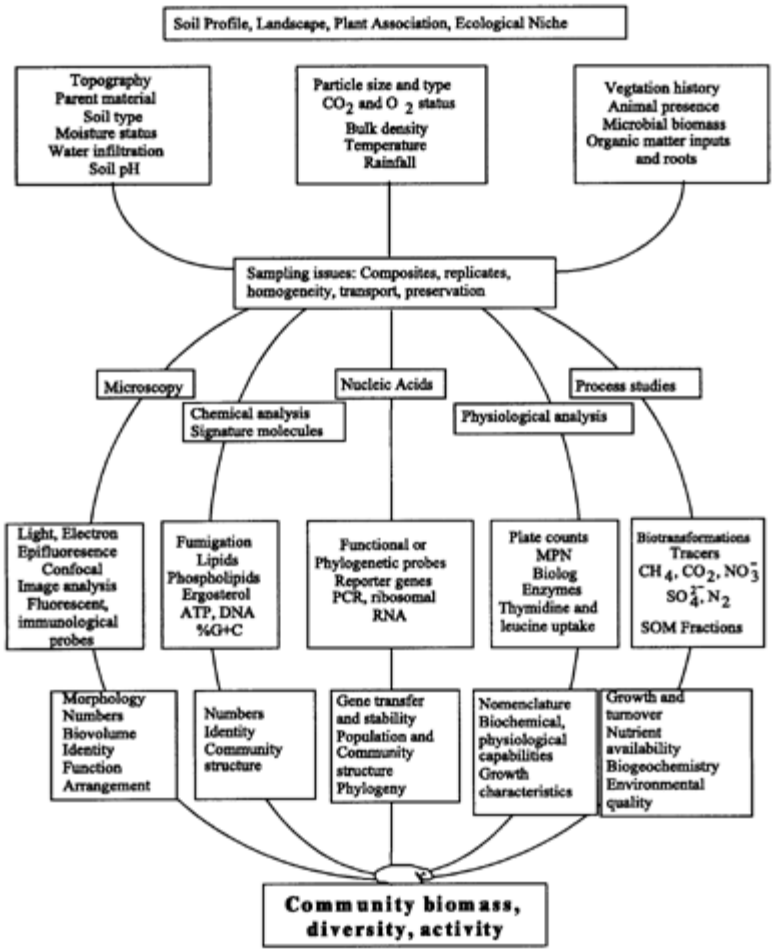
The high spatial heterogeneity of soil in an ecological context is well documented (Robertson and Gross 1994, Paul and Clark 1996). Differences among habitats in the degree of soil heterogeneity may influence the diversity of microbes that occur there and their function (Gross et al. 1995). For example, our results with nitrifiers and soil C dynamics are best interpreted in relation to differences among treatments in soil heterogeneity (reflecting the availability of microhabitats) and soil organic fractions (reflecting resource heterogeneity; Paul et al. 1998a). Spatial heterogeneity in soil microbial communities occurs at a broad range of scales, from soil particles (e.g. soil macroaggregates), to plant rhizospheres, to field plots, and to the ecosystem and global levels (Tiedje 1994).

At KBS we have documented that there is spatially-structured dependence in microbial processes at both a macro- (e.g 10's of meters, Robertson et al.1997) and micro- (cm, Cavigelli et al. 1995) scale. We have shown that microbial activity (measured by short-term microbial respiration) varies among and within plant communities; in some sites samples taken only centimeters apart vary by a factor of >2. The among-community scale component of this nested variation may be

attributable to differences in primary productivity and soil physical properties (e.g. depth to the Bt horizon). At the within-community scales, the doubling of microbial activity may be attributable to the distance to the nearest plant. However, we suspect that these differences may also be due to heterogeneity in soil structure, leading to discontinuous resource availability at the millimeter-scale. This small-scale heterogeneity may be driven by the interaction of plant-derived substrates, such as roots and decaying plant particles, and within-aggregate habitats differences due to clay content, pore sizes, and aeration.

To date, our investigations of soil microbial communities have primarily concentrated on the level and pattern of microbial diversity among the different plant communities that occur on the KBS LTER site. These communities range from the intensively managed row crops under different input intensities to native communities at different successional stages. Our results, generated by a variety of phenotypic and genetic approaches, have documented differences in the apparent diversity of whole-soil microbial communities (patterns of bacterial fatty acids, FAMEs), as well as differences in the diversity of key functional groups, notably denitrifiers (Cavigelli 1998) and nitrifiers (Bruns et al. 1998).

In the past decade we have concentrated on documenting the level and patterns of microbial diversity among ecosystems using strategies such as those outlined in the figure at right. We have now begun more intense investigations of the regulation, maintenance, and consequences of microbial community structure. We hypothesize that the majority of soil microbial diversity is driven by the heterogeneous distribution of resources and habitats in soil. For example, we have found a variety of autotrophic nitrifier genera in our never-tilled successional plots (Bruns et al. 1998), all of which grow in the laboratory only at low NH_4^+ concentrations. In contrast, in our agronomic plots there is a single dominant genus (*Nitrosomonas*) that is able to grow across a wider range of NH_4^+ concentrations. These differences in nitrifier diversity could be due to differences in resource availability, and therefore competitive interactions. However, this pattern may also be due to greater diversity of protective soil habitats in the never-tilled community. Heterogeneity in soil structure, which may lead to higher levels of microbial diversity, is affected not only by cultivation regime but also by the presence and activity of plants that create biopores and habitat for the mesofauna that are directly responsible for much of the soil structure (Oades 1993).



To improve our knowledge of how microbial community structure interacts with the functioning of ecosystems we must obtain a more quantitative knowledge of the interaction between microbes, plant residues and disturbance, at a variety of spatial scales. This will require examining the availability of specific resources (at the substrate level) across multiple spatial scales.

We are currently concentrating our research on soil microbial communities at the KBS LTER in three areas:

- Investigations of the availability of microbial resources (especially substrates) through continued studies on the pools and fluxes of soil

organic matter;

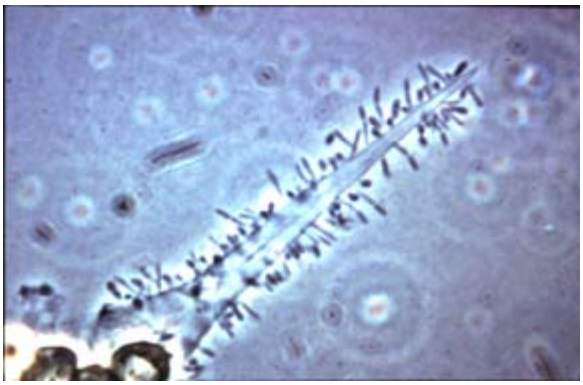
- Examination of the scales at which carbon turns over in soils from the microaggregate (mm) to the landscape (km); and
- Investigations of the diversity and structure of specific groups of soil microbes across the 11 different communities on the KBS LTER, with an initial emphasis on Basidiomycete fungi, a microbial group that is responsible for significant carbon turnover in soil.
- Investigations of the linkage between plant diversity, disturbance, soil structure, microbial diversity, and key ecosystem functions such as primary productivity, nitrogen cycling, and nutrient retention in general.

Analytical Procedures Used for Microbial Ecology at KBS

Microbial Biomass
Microbial biomass is enumerated at KBS using the chloroform incubation technique calibrated with direct microscopy. Specific techniques are documented in Howarth et al. (1994, 1996) and Paul et al. (1998). Results are available on the KBS LTER web site.

Fungal Biomass and Fungal: Bacterial Ratios
Ergosterol is a steroid found in most fungi, but absent in other microorganisms. We have found that the concentration of ergosterol in soils (Stahl and Parkin 1996) is directly related to the growth rate of fungi and provides an estimate of the fungal biomass in soil. Comparisons of fungal and bacterial ratios, and the size of bacterial biomass are also useful for documenting changes within soil microbial communities. Computerized fluorescence microscopy has greatly aided our ability to examine these characteristics.

Culturable Microorganisms
During the establishment of our main cropping systems site a culture collection of bacteria was established to provide a benchmark collection. From over 1000 isolates a 100-isolate subset was selected for intensive study ("the KBS 100"). These isolates have been characterized using a variety of polyphasic taxonomic tools (see figure above) and are maintained as a long-term reference collection. Additional collections include lignin decomposing Basidiomycetes from the site (molecular techniques show that many have previously not been described; Thorn et al. 1996) as well as collections of denitrifiers (Cavigelli 1998) and nitrifiers (Bruns 1996).



Non-Culturable Microbes: Community-Level Signatures
We have used a variety of phenotypic tools to characterize soil microbial community composition as related to ecological change (Klug and Tiedje 1993, Sinsabaugh et al. 1998). These include fatty acid methyl ester (FAME) and phospholipid fatty acid (PLFA) analyses (Peterson and Klug 1994, Haack et al. 1994, Cavigelli et al. 1995, Corlew-Newman and Klug 1998), as well as Biolog™ carbon utilization signatures. We are also using G+C analysis to examine the distributions of low G+C populations (e.g. Pseudomonas) vs. high G+C populations (e.g. Arthrobacter), and L-asparaginase activity to resolve differences in rhizosphere populations.

Population-Level Signatures: Gene Probes
We have collaborated with the NSF Center for Microbial Ecology (CME) at MSU in the development and testing of several gene probes for assaying specific soil populations at KBS. Particularly successful has been the deployment of probes for 2,4-D metabolism (Holben et al. 1992, Ka et al. 1994a,b,c,d, 1995), and for nitrifying bacteria (Zhou et al. 1995, Bruns 1996, Bruns et al. 1998). We are beginning to design population-specific rRNA oligonucleotide probes to determine the contribution of these various fractions of rRNA to total prokaryotic community rRNA. The advantage of working with RNA is that it allows detection of the most active (highest ribosome content) populations, which are also probably the most dominant populations.

Population-Level Signatures: Phenotypic Techniques
We have used lipid analysis (fatty acid markers) to track changes in fungal communities in different soils (Stahl and Klug 1996, 1998, Stahl et al. 1998), changes in mycorrhizal associations (Calderon 1997), and differences in denitrifier community composition (Cavigelli 1998). These techniques have been combined with techniques for culturable microbes and community-level signatures (above).

Bacterial Growth Rates
Microbial biomass provides an estimate of the pool size of microorganisms, but not of biomass turnover. We have examined bacterial turnover dynamics using ^{3}H, thymidine, and ^{14}C-leucine incorporation kinetics (Harris 1994, Harris and Paul 1994).

Microbial Process Measurements
Measurements of key microbial processes such as nitrification, carbon mineralization, and carbon and nitrogen gas fluxes are coupled to those of microbial and plant community structure to provide insight into the functional significance of microbial diversity at KBS. Processes examined include CH_4 oxidation and N_2O production (Robertson 1993, Paustian et al. 1995, Ambus and Robertson 1998a,b), carbon oxidation (Paul et al. 1994, 1998a,b, Paustian et al. 1995), denitrification (Cavigelli 1998), and nitrification (Bruns 1996, Knoke 1997).

Microbial Predators
Nematodes are important fungal and bacterial consumers that can affect the distribution and abundance of microbial populations. We have examined changes in nematode groups among cropping system treatments (Freckman and Ettema 1993) as well as the distribution of various nematode trophic groups (Robertson and Freckman 1995). These studies, in combination with our data on biomass and biomass turnover measurements, provide evidence on the controls in the distribution of key microbial groups in soils.

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The research at Konza Prairie focus on several aspects of microbial ecology. This includes investigation of microbial biomass and activity as affected by fire, water, and nutrients on Konza tallgrass prairie LTER, microbial responses to elevated CO_2 on the site, and using Konza Prairie as a benchmark for biological aspects of soil quality.

A major focus has been the ecology and diversity of denitrifiers in the surface and subsurface profiles of tallgrass prairie in comparison with agricultural ecosystems. We have used both molecular techniques and physiological techniques to assess denitrifier diversity, and the results of a recent study on microbial enzymes is now in review.

Aquatic Microbial Ecology:

We do not monitor aquatic microbes regularly at Konza, but have done some work on this previously. This work includes two main projects on groundwater and several independent projects on periphyton.

The groundwater work involved monitoring bacterial numbers flowing from a spring for two years, and relating those to groundwater invertebrates (Edler, C. and W. K. Dodds 1996. The ecology of a subterranean isopod, Caecidotea tridentata. Freshwater Biol. 35:249-259). A second groundwater project was to compare nitrogen cycling in shallow subsurface below cropland and prairie fields (several references with a basic description in Dodds, W. K. et al. 1996. Biological properties of soil and subsurface sediments under abandoned pasture and cropland. Soil Biol. Biochem. 7:837-846.)

If funds were available for observing (monitoring) aquatic microbes at Konza LTER, the sampling could be prioritized as follows. 1) Algal biomass in streams, 2) Bacterial counts at several groundwater sites, 3) activity measurements of specific groups (e.g. denitrifiers and nitrifiers) in stream and groundwater, and 4) monitoring coliform and giardia from pristine sites.

These initiatives would be based upon using Konza as a pristine baseline for water quality of prairie streams, and helping describe ecosystem function of autotrophic streams. We need data to assess human effects on streams. Even though streams draining grasslands and wooded grasslands are common worldwide, little is known about their ecology.

Research on Arbuscular Mycorrhizal Fungi:

1. Mycorrhizal fungal community composition: Spatial and temporal patterns of mycorrhizal fungal community composition and diversity are assessed via AM fungal spore isolation from soil subjected to various treatments, including burning, mowing, phosphorus fertilization, nitrogen fertilization, grazing, and fungicide application (mycorrhizal suppression). In addition, field experiments and greenhouse trap culture experiments are evaluating patterns of host-plant specificity of mycorrhizal fungal species.

Representative Publications:

- Eom, Hatrnett, Wilson, and Figge 199- Am. Mid. Nat., submitted
- Eom 1998 (Ph.D. Dissertation)
- Bentivenga and Hetrick 1992 Mycologia

2. Assessment of interspecific variation in mycorrhizal colonization among tallgrass prairie grasses and forbs:

Representative Publications:

- Hetrick, Kitt, and Wilson 1988 Can. J. Bot.
- Hetrick, Wilson, and Todd 1992 Can. J. Bot.
- Miller, Hetrick, and Wilson 1997 Can. J. Bot.
- Wilson and Hartnett 1998 Am. J. Bot.

3. Effects of mycorrhizae on plant population dynamics and community structure:

Ongoing field studies are evaluating the influence of mycorrhizal fungi on plant demography, competitive relationships, and species composition and diversity.

Representative Publications:

- Hartnett, Hetrick, Wilson, and Gibson 1993 J. of Ecol.
- Hetrick, Wilson, and Hartnett 1989 Can. J. Bot.
- Hartnett, Samanus, Fischer, and Hetrick 1994 Oecologia
- Wilson and Hartnett 1997 Am. J. Bot.

Hartnett and Wilson 199- Ecology, submitted

4. Assessment of AM fungal nutrient uptake in tallgrass prairie:

Representative Publications:

- Hetrick, Wilson, and Schwab 1994 Can. J. Bot.
- Bentivenga and Hetrick 1992 Can. J. Bot.
- Fischer Walter, Hartnett, Hetrick, and Schwab 1996 Am. J. Bot.

Other Related Publications:

Groffman, P. M., C. W. Rice, and J. M. Tiedje. 1993. Denitrificationin a tallgrass prairie landscape. Ecology 74:855-862.

Rice, C. W., and F. O. Garcia. 1994. Biologically active pools of soil C and N in tallgrass prairie. pp. 201- 208. In J. Doran et al. (ed) Defining soil quality for a sustainable environment. Spec. Pub.No. 35. Soil Sci. Soc. Am., Madison, WI.

Garcia, F. O., and C. W. Rice. 1994. Microbial biomass dynamics in tallgrass prairie. Soil Sci. Soc. Am. J. 58:816-823.

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Sotomayor, D., and C.W. Rice. 1996. Denitrification beneath grassland and cultivated soils. Soil Sci. Soc. Am. J. 60:1822-1828.

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LUQ- Luquillo Experimental Forest, *near San Juan, Puerto Rico*

Jean Lodge [/S=D.J.LODGE/OU1=S32A@mhs-fswa.attmail.com]

In addition to the LTER researchers, there are two microbial ecologists (Dr. Gary Toranzo & Dr. Arturo Massol) and three mycologists (Drs. Paul Bayman, Steve Rehner & Carlos Betancourt) at the University of Puerto Rico who have contributed to or are planning on initiating studies in the Luquillo forest. They greatly enhance our critical mass and capabilities in microbial ecology research at the LUQ-LTER site.

D.J. Lodge (1993) monitored total fungal biovolume at two-week intervals for one year at El Verde (LUQ-LTER). Fungal biovolume in the litter layer could triple in two weeks in response to frequent rains, and decrease by half during the following two weeks in response to drying cycles. The critical factor forfungal volume in litter was frequency of days with rainfall exceeding 3 mm (sufficient to wet the canopy and reach the forest floor) rather than total rainfall during the previous two weeks. Three consecutive days without rainfall reaching the forest floor was sufficient to dry out the thin litter layer and cause massive crashes in fungal mycelia. Soils were more buffered against fluctuations

in rainfall, and six weeks of low rainfall was requiredto reduce the soil moisture sufficiently to cause crashes in the fungal populations in soil. Fungal nutrient stocks in the litter layer were calculated based on the biovolume estimates above and fungal specificdensities and nutrient concentrations obtained from field-collected mycelia at the site (Lodge 1987). Fungal stores represented a large fraction of the potassium and phosphorus in the litter layer, but not much of the nitrogen (Lodge 1993). Pre-fruiting mycelia were found to concentrate so much phosphorus through short-circuited nutrient cycling (translocation of phosphorus from partly decomposed litter to form biomass in fresh leaf litter with low phosphorus content) that they accounted for virtually all of the phosphorus in the litter in some patches of forest floor.

We hypothesize that nutrients are released in pulses from the litter layer, and that nutrients released in pulses are more available to higher plants than if they had been released at a constant rate (Lodge, McDowell & McSwiney 1994). Pulsed nutrient release should result from fungal accumulation and internal recycling of nutrients and subsequent crashes in fungal populations in response to drying cycles, followed by rains that then leach the released nutrients into the soil. Trees cannot compete well against decomposer microorganisms for limiting nutrients if there is an ample supply of labile carbon for the microbes, as demonstrated by a post-hurricane litter removal/fertilization experiment conducted at El Verde (Zimmerman et al. 1995). However, the release of concentrated pulses of nutrients that are synchronized with crashes in microbial biomass should allow windows of opportunity for

plant roots to obtain nutrients under reduced microbial competition (Lodge, McDowell & McSwiney 1994). Microbial nitrogen retention during dry- wet cycles in subtropical evergreen forests was compared between the LEF and southern China by Fu, Zou, Lugo, Yi and Ding (in press). Rootlike structures of basidiomycete fungi that are used to translocate nutrients and colonize new resources in the litter layer also hold litter together in mats which prevents it from being exported down steep slopes (Lodge & Asbury 1988). In addition to preventing the loss of nutrients contained in leaf litter from being exported by streams during heavy rains, the retention of leaf litter on steep slopes apparently protects the soil surface from erosion by pounding rain thereby conserving soil nutrients.

The relationship between soil development, earthworm density and both active and total fungal and bacterial biomass were studied in landslide and adjacent forest soils by Li, Xou and Myster (in press). They found that earthworm density and biomass was positively correlated with total bacterial biomass,leaf litter, and the light-carbon fraction of the soil organic matter pool.

Effects of carbon and phosphorus additions on loss of nitrogen as nitrate and total soil respiration predominantly microbial) in a simulation of post hurricane conditions - (McDowell, Lodge & Massol, in prep.)

Surveys, Inventories and other Microbial Diversity Studies:

Basidiomycetes of the Greater Antilles, Especially the Luquillo LTER Site: This is a 4-year program funded by NSF Biotic Surveys & Inventories, and is approximately at the half-way point. A monograph of Alboleptonia by T.J. Baroni & D.J. Lodge is in press, as well as a paper describing a new genus,

`Macrocybe' to accommodate a species found in Puerto Rico and other species in the tropics (D.N. Pegler, D.J. Lodge & K.K. Nakasone). Another paper on Calocybe species is also in press (T.J. Baroni, R. Vilgalys, D.J. Lodge & N.V. Legon). A monograph on the Hymenochetaceae and Ganodermataceae by Leif Ryvarden is ready to go to press. Monographs nearing completion are Puteaceae, by E. Horak, and Hygrophoraceae by S.A. Cantrell & D.J. Lodge.

Previous fungal surveys:

All historical and recent records of fungi identified from the Luquillo LTER site through 1995 were published by Lodge (1996) according to their substrate and habit in the appendix of the chapter on microorganisms in the book, The Food Web of a Tropical Rain Forest. A review of literature on fungi of Puerto Rico and the Virgin Islands was also published in 1996 by Lodge.

Factors important in structuring fungal communities and species diversity

Microfungi in decomposing leaves:

Several hundred species of microfungi were cultured and identified from decomposing leaf litter of two tree species that occurred together at two sites at El Verde in the Luquillo forest (Polishook, Bills & Lodge, 1996). A list of species was included in the publication. As we hypothesized based on studies at the same site in the 1960's, there is a strong preference among microfungal species for different types of leaves. Species composition of fungi isolated from the same leaf species in two widely separated plots was more similar than fungal species composition in leaf litter of different species at the same site.

Slime Molds:

Drs. Steven Stephensen and John Landolt cultured slime molds from leaf litter in the 16-ha gridded forest site at El Verde LUQ-LTER site. They found a higher diversity in the part of the grid that was more highly disturbed by clearcutting and agriculture over sixty years previously as compared to the little-disturbed forest in the other part of the grid. This corresponds to higher densities of their prey items, bacteria, in the more disturbed part of the grid (see Willig et al., bacterial functional diversity below). There were also difference in litter entrained in the understory. This led to further studies on canopy slime mold communities along the elevational gradient in the LUQ-LEF, and the degree of isolation of canopy litter communities and modes of slime mold dispersal.

Stream fungi and algae:

In the 1960's, Padget identified fungi on decomposing leaves in a stream at El Verde. Recently, Carlos Santana & Carlos Betancourt (1997) published a survey of spores trapped in foam in streams of Puerto Rico, including the LTER site. Pringle (1996) studied the effect of atyid shrimp on spatial heterogeneity of algal communities in montane streams at the LUQ-LTER site. In another study published by Pringle, Blake, Covich, Busby & Finley in 1993, the sediment removal activity of shrimp was found to increase algal biomass as compared to shrimp exclosure plots.

Nitrogen fixing cyanobacteria, lichens, and bacteria associated with plants:

Rates of nitrogen fixation by plant epiphyllous microbes were found to be very high by Joe Edmisten in the 1960's using the acetylene reduction technique. Recently, Dr. Li of the US Forest Service research lab in Corvallis Oregon has been culturing and obtaining molecular sequences of nitrogen fixing bacteria that live INSIDE of plant roots at the LEF. These bacteria are abundant, and there is a high diversity, including some that are previously unknown.

Bacterial functional (enzyme) diversity:

Willig, Moorehead, Cox and Zak (1996) found that high anthropogenic disturbance of part of the forest within the current 16-ha study grid at El Verde was correlated with an increased bacterial enzyme diversity and bacterial abundance as compared to the lightly disturbed part of the grid. They hypothesized that the secondary forest tree species that dominate the more disturbed part of the grid provide more labile carbon substrates, which provide the energy for bacteria to degrade more resistant compounds.

Spatial variation and calibration of sampling :

Lodge & Cantrell (1995 a,b) studied spatial components of diversity in litter agaric fungi and developed recommendations for plot number and sampling intensity calibration for tropical rainforests using studies in the LUQ-LTER and Amazonian Ecuador.

Wood inhabiting ascomycetes:

Sabine Huhndorf studied wood inhabiting ascomycetes for one year in the 16-ha grid at El Verde in the LEF. She found (Huhndorf & Lodge, in press) strong partitioning among size classes of wood, seasonality of fruiting, and effects of anthropogenic disturbance more than 60 years previously on species composition. A new application of probability statistics is being explored for_ surveys of highly diverse groups of organisms that have low frequencies of individual species (Huhndorf & Lodge, in prep.).

John Paul Schmidt, a doctoral student at the University of Chicago, is currently studying the relationship between initial carbon & nutrient density in wood and diversity of higher fungi during decomposition. The surveyed logs are at El Verde in the LUQ-LTER site.

Endophytes:

Lodge, Fisher & Sutton (1996) found 25 species of fungal endophytes in leaves of Manilkara bidentata at El Verde, and estimated that there were an additional 3 species not encountered. One leaf from each of three trees were sufficient to find 80% of the fungal endophytes of that tree species in the leaf blades, but not in the petioles. Endophytes in petioles & leaves were different. Bayman, Lebron, Tremblay, and Lodge (1997) found variation in endophytic fungi isolated from the roots and leaves of epiphytic orchids, Lepanthes spp.

MCM - McMurdo Dry Valleys, *McMurdo Station, Antarctica*

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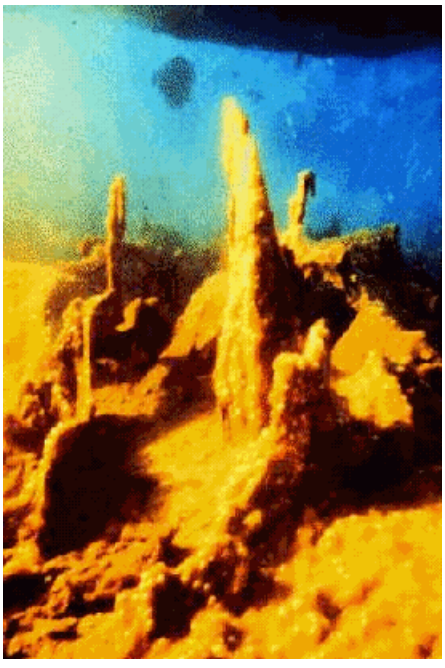
Diana H. Wall [Diana@NREL.ColoState.EDU]

Introduction:



The Antarctic McMurdo Dry Valley ecosystems are driven by the same basic processes, such as microbial utilization and re-mineralization of nutrients found in all ecosystems, but they lack many confounding variables, such as higher plants and animals, found in other ecosystems. McMurdo LTER research contributes to general ecological understanding through studies of processes in a less diverse and complex ecosystem. The extreme environment found at MCM is an analog to primordial microbial growth and for potential microbial growth on other planets.

The McMurdo LTER site would be a reasonable choice for more intensive microbial studies given that these ecosystems only have a microbial-micro-invertebrate community. The microbial mats found throughout the aquatic environments of the Antarctic dry valleys are among the most well-developed and complex communities of their type. The soil communities are based on microbes and are the simplest food webs occurring in any ecosystem.

Soil Microbial Ecology Research at MCM:

Antarctic dry valley soils have a long history of microbiological research, originating with studies using this ecosystem as a Martian analogue. Early results suggested reduced microbial biomass and diversity in dry valley soils and even suggested that some expanses of soil in this ecosystem were sterile. MCM LTER soil research has applied modern methodology

and innovative experiments and is showing that dry valley soils contain bacteria, fungi, protozoa, rotifers, tardigrades and nematodes, frequently in abundances comparable to hot desert soils. Currently, several investigations of microbial processes are underway:

Soil Ecosystem

(1) Long-term monitoring of soils for algal biomass at several sites (2) Long-term soil manipulation of soils (moisture, temperature, carbon sources): This experiment is investigating the responses of soil biota to changing environmental conditions. Variables measured include nematode species diversity, abundance and age structure. Soil respiration and carbon and soil nitrogen cycling are also being monitored. Results from this ongoing experiment have demonstrated the sensitivity of the omnivore-predator nematode species to soil manipulation, as this species was reduced with soil warming. (3) Measurement of soils in situ for respiration and photosynthesis rates. Studies of isotopic signatures for carbon sources in dry valley soils suggest that the probable carbon source in this soil may be relict sediments from a lake present across the valley floor 10-25,000 ybp. (4) Long-term measurement of in situ decomposition by use of cotton strips at two field sites in Taylor Valley. This experiment includes soil manipulations (water amendment, precipitation blocking, and soil warming) to see what factors limit microbial processes in these soils. A completed microcosm study concurrently measured decomposition, microbial biomass, protozoan biomass, nematode communities, and nitrogen and carbon mineralization which showed that under constant temperature, microbial processes in dry valley soils are limited by moisture. (5) DNA studies of the endemic nematode species, Scottnema lindsayae, have shown genetic isolation of this species between valleys. (6) Protozoan distribution and abundance has been analyzed for several sites.

(7) Nematode distribution and abundance has been compared within and between valleys, showing relationships to soil elevation, depth, chemistry, carbon, and proximity to sources of moisture. (8) Models are being developed that examine factors inducing the survival state and activity of the nematodes and their food source, microorganisms.

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Blair, J. M., P. J. Bohlen and D. W. Freckman. 1996. Soil Invertebrates as Indicators of Soil Quality. In: Pages 273-291, J. W. Doran and A. J. Jones (eds.). Methods for Assessing Soil Quality. Soil Science Society of America, Madison, WI.

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Brussaard L., V.M. Behan-Pelletier, D.E. Bignell, V.K. Brown, W.A.M. Didden, P.I. Folgarait, C. Fragoso, D.W. Freckman, V.V.S.R.

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Marion, G.M., G.H.R. Henry, D. W. Freckman, G. Jones, M.H. Jones, J. Johnstone, E. Levesque, U. Molau, P. Molgaard, A. N. Parsons, J. Svoboda, and R.A. Virginia. 1997. Open-Top Designs for Manipulating Field Temperature in High-Latitude Ecosystems. Global Change Biology 3 (Suppl. 1): 20-32.

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Powers, L. E., M. Ho, D. W. Freckman, and R. A. Virginia. 1998. Distribution, Community Structure and Microhabitats of Soil Biota Along an Elevational Gradient in Taylor Valley, Antarctica. Arctic and Alpine Research 30:133-141.

Bargagli, R., D. Wynn-Williams,F. Bersan, P. Cavacini, S.Ertz, F. Frati, D. Freckman, R. Lewis-Smith, N. Russell and A, Smith. 1997. Field Report BIOTEX I: First BIOTAS Expedition (Edmonson Point-Bais Terra Nova, Dec 10, 1995 - Feb 6, 1996). In: M. Tamburrini and R. D'Avino, (eds). Newsletter for the Italian Biological Research in Antarctica. Universita degli Studi di Camerino, Gennaio, Italy.

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Freckman, D. W. and R. A. Virginia. 1993. The Ecology of Nematodes in Antarctic Dry Valley Soils. Antarctic Journal of the United States. 28:10-11.

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Powers, L.E., D.W. Freckman, and R.A. Virginia. Depth Distribution of Soil Nematodes in Taylor Valley, Antarctica. Antarctic Journal of the United States.

Treonis, A.M., D.H. Wall and R.A. Virginia. Invertebrate diversity in Taylor Valley soils and sediments. Antarctic Journal of the United States.

Submitted Papers related to microbial ecology:

Courtright, E.M., D. W. Freckman, R. A. Virginia, L. M. Frisse, J. T. Vida and W. K. Thomas. Nuclear and Mitochondrial DNA

Sequence Diversity in the Antarctic Nematode Scottnema lindsayae. Molecular Ecology

Wall, D. H. and J. C. Moore. Interactions Underground: Soil biodiversity, mutualisms and Ecosystem Functioning. Bioscience

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NWT - Niwot Ridge/Green Lakes Valley, *near Boulder, Colorado*

Elizabeth Ann West [westa@ucsu.Colorado.EDU]

Steve Schmidt is one of the principle researchers at Niwot Ridge. The unifying theme for all of the ongoing projects in my lab is soil microbial ecology. We are interested in the role that soil microorganisms play in biogeochemical cycles, plant-microbe interactions and mineralization of toxic chemicals in soil. We work at scales ranging from the cellular to the ecosystem.

Current projects include:

1) Controls on methanotrophic and methanogenic processes in alpine tundra soils (Neff et al., 1994; West et al., 1998).

2) Population dynamics of pentachlorophenol-mineralizing bacteria in soil (Colores et al., 1995, 1996; Radehaus and Schmidt, 1992; Schmidt et al., 1995).

3) The fate and effects of atmospheric nitrogen in alpine tundra soils (Brooks et al., 1996, 1997, 1998; Fisk and Schmidt, 1995, 1996).

4) Modeling the population dynamics of soil microorganisms (Colores et al., 1996; Hess and Schmidt, 1995; Schmidt, 1992; Schmidt et al., 1996).

5) The role of soil microorganisms in the attenuation of allelopathic interactions between plants (Schmidt and Ley, 1996; Schmidt, 1988, 1990; Schmidt and Reeves, 1989).

6) Mycorrhizal uptake of phosphorus and organic nitrogen compounds in natural systems (Mullen and Schmidt, 1993; Mullen et al., 1998).

7) Diversity and functioning of ectomycorrhizal fungi in alpine and subalpine systems (Link to Chris Schadt for more information).

More information can be found at: http://spot.colorado.edu/~schmidts/research.html

A survey of graduate student work shows a large focus on microbial ecology work at the Niwot LTER. A brief summary of the student projects follow:

Heidi Steltzer will be looking at the spatial variability of microbial biomass N within the moist meadow tundra this summer. The goal

of the project is to associate (using geostatistics) variability in plant species, relative abundances with different aspects of community structure. The question, which relates to the microbes is: Does spatial variability in plant species relative abundances cross-correlate spatially with microbial biomass N? This would suggest that there is a direct effect of litter quality on the spatial pattern of N in microbial biomass.

Dave Oline (mailto:david.oline@colorado.edu) is measuring the diversity of specific groups of low temperature terrestrial crenarchaeota from the surface soil on Niwot Ridge. An NSF proposal grant under the Biotic Surveys and Inventories Program has been submitted to identify and sequence the 16S rRNA of groups at Niwot Ridge and at three other sites at lower elevations in the Front Range, and I will be using the diversity data for a comparative study.

Gamelyn Gerald Dykstra, a previous 1997 summer REU student, and performing an undergraduate study of the dynamics and composition of protozoan and nematode populations in high-altitude talus soils.

David Lispon, a graduate student (lipson@culter.Colorado.EDU) is focused on plant-microbe competition for amino acids in alpine tundra dry meadows. One study investigated the effect of freeze-thaw and dry-rewet events on competition for amino acids (1). Current studies in this area include the differential preferences for different amino acids between plants and microbes. He has also studied the links between microbial population dynamics and plant N availability (2). The microbial community undergoes regular seasonal changes, which lead to a pulse of available N early in the growing season. I am currently studying the mechanisms that bring about these changes in microbial biomass, and the functional changes in the community structure between winter and summer. Relevant references include:

Lipson, D.A., and Monson, R.K. (1998) Plant-microbe competition for soil amino acids in the alpine tundra: effects of freeze-thaw and dry-rewet events. Oecologia 113:406-414.

Lipson, D.A., Schmidt, S.K., and Monson, R.K. Links between microbial population dynamics and N availability in the alpine tundra. (in review).

Ruth Ley (ley@ucsu.Colorado.EDU) is a Ph.D. candidate investigating microbial functional groups across an organic matter gradient in extremely high altitude soils (3000+ meters) found in talus slopes. The work includes describing the energy budgets of these soils: quantifying energy inputs from microbial chemoautotrophy, aeolian C inputs, and photosynthesis along the organic matter gradient. The soils range from mineral to developed tundra soils and are derived from the same parent material. This work should provide basic information on the development of microbial communities (shifting dominance of functional groups) and biogeochemical processes with soil development.

Ann West (westa@ucsu.colorado.edu) Started out analyzing the controls on field CH4 fluxes of the different plant communities on Niwot Ridge (now submitted to Biogeochemistry "Landscape patterns of CH4 fluxes across an alpine tundra ecosystem") and is now investigating the microbial ecology of methane fluxes in both Kobresia and Carex plant communities with results in a resent paper: West, A.E. and S.K. Schmidt. 1998. Wetting stimulates atmospheric methane oxidation in alpine soils. FEMS Microbiol. Ecol. (In Press).

NTL – North Temperate Lakes - *near Boulder Junction and Madison, Wisconsin*

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David Armstrong [amrstrong@engr.wisc.edu}]

SUMMARY OF MICROBIAL RESEARCH at the NORTH TEMPERATE LAKES LTER - ***June 1998***

Research on microorganisms at the North Temperate

Lakes LTER (NTL) has focused on microbial ecology and processes involving the phytoplankton and bacterioplankton. Investigations of the roles of primary and secondary producers on ecosystem cycles of carbon and phosphorus have been facilitated by long-term data sets on nutrient elements in the eleven NTL lakes. The design of the NTL site has also been important to the types of research questions addressed. Thus, the diversity of the eleven lakes at the NTL site have enabled comparative studies linking microbial activity to differences in chemistry, morphometry, and other characteristics among lakes. Similarly, data on all levels of the metazoan food web have been used in assessing food web relationships between phytoplankton and secondary consumers. Our long-term data on microbial biological has focused on the phytoplankton. In addition, pre-LTER phytoplankton and bacterial information spanning a period of 100 years was collected through the work of E. A. Birge, T. Brock, C. Juday, E. B. Fred, C. Pedro-Alio, T. J. Phelps, J. G. Zeikus and others.

ALGAE

Until recently, microbial research at the NTL LTER has emphasized the phytoplankton. This focus stems from the importance of the phytoplankton as the autotrophic base of the temperate lake food web. Phylogenetic data based on phytoplankton pigments has been collected biweekly in all NTL lakes since 1981. Concurrently, phytoplankton samples have been collected and preserved. From 1986 to the present, primary production by the phytoplankton has been measured on three lakes during the ice-free period using C_{14} Techniques. Some of the research based on our long-term data sets and other measurements is described briefly as follows:

Algae production and water clarity are closely related to nutrient loading, availability, and internal cycling (c.f., Armstrong et al., 1987; Lathrop, 1992). The importance of recycled production of algae was assessed in three lakes oligotrophic to mesotrophic lakes which receive low nutrient influent waters mainly as groundwater inflow and/or direct precipitation. Recycled production was evaluated using measurements of primary production (Adams et al. 1990), nutrient sedimentation (Poister et al. 1994), and estimates of nutrient external inputs and outflow. The analysis showed that recycled production was the main source of phosphorus that drives annual primary production, representing about 85% of the annual production in Crystal, Sparkling, and Trout Lakes (Poister et al. 1994).

Linkages between algae and fish communities were observed in our research on Crystal Lake. Our long-term data revealed strong inter-annual fluctuations in water clarity that are caused by changes in the phytoplankton community as measured by chlorophyll concentration. The dynamics in the phytoplankton community are related to a cyclic pattern in the population of yellow perch, whereby years with lowest chlorophyll concentrations are also years with strong year classes of planktivorous young-of-the-year of yellow perch (B. Sanderson, in prep). We were able to detect these linked, cyclic population patterns through our long-term data sets.

The role of life history processes in regulating microbial populations was revealed in our research on population dynamics of dinoflagellates in two bog lakes that differ in morphometry; one shallow and one deep. Differences in population abundance of *Peridinium limbatum* were explained by differences in emergence rates of cells from the lake sediments (Sanderson and Frost, 1996). In the deep bog, emergence rates were low from hypolimnetic sediments, whereas in the shallow system, emergence rates were high across the entire basin. The linkages of life history to lake morphometry enabled higher abundance of the dinoflagellate to develop in the more shallow bog.

BACTERIA

Community level research on aquatic bacteria at the NTL LTER site has been conducted using total bacterial counts (DAPI and AO techniques) and bacterial production measurements (thymidine and leucine uptake methods). Information on bacterial ecology has been obtained through comparative studies among lakes, including the NTL LTER lakes. For example, anoxic bacteria in some of the LTER lakes were found to be 2-10 times larger in size and to maintain a higher biomass per unit volume than aerobic bacteria

in temperate lakes (Cole et al., 1993).

Two of the LTER lakes, Trout and Crystal, were also used in conjunction with Paul Lake of the nearby University of Notre Dame Environmental Research Center lakes to determine whether bacterial production is coupled to primary production over depth (Pace and Cole, 1994). Bacterial production and primary production were expected to be closely coupled in space and time if bacteria are limited by labile carbon supply. Vertical distributions of bacterial and primary production differed among the three lakes, and overall, coupling was not observed. Other factors, such as nutrient recycling, allochthonous loading and phytoplankton loss rates were suggested as alternate reasons for differences in vertical distributions.

We are currently conducting experiments on bacterial response to changes in nutrients and growth substrates (Lauster, 1998). We are also exploring the implications of resource limitation on the trophic relationship between bacteria and algae, and on the ecosystem processes of recycling and primary production. Experiments are conducted across a diverse subset of six lakes of the NTL LTER, including two high DOC lakes and open-water fens.

Microbial Biogeochemistry

The role of biogeochemical processes in regulating the chemical composition of lakes has been explored in several investigations. Levels of silica were found to be coupled closely to groundwater inputs and internal cycling by siliceous algae (Hurley et al., 1985). Similarly, N and P levels are coupled to uptake and regeneration cycles associated with phytoplankton (Poister et al., 1994). Other investigations have explored the role of sulfate reduction and related processes in lake acidification (Brezonik et al., 1993; Sherman et al., 1994), production of methyl mercury (Bloom et al., 1991; Hurley et al., 1994), and the preservation and degradation of algal pigments in lake sediments (Hurley and Armstrong, 1990,1991).

Our LTER data provided information on the relative importance of the microbial-dominated processes of primary production and respiration. Curiosity about the consistent, annual cycle of pH values detected in the NTL lakes led to a reconsideration of the connection between lakes and drivers of lake chemistry. Data on lake pH, alkalinity, DOC, cations and anions were used to show that CO_2 accumulates in lakes under the ice and is quickly vented into the atmosphere after spring melt (Kratz et al., 1987). These initial findings of CO_2 accumulation at NTL and elsewhere lead to a program of direct measurement of CO_2 and collaboration with other scientists (Toolik Lake LTER and others) on the dynamics of CO_2 in lakes from a variety of regions around the world. The collaboration, along with data from other lakes, led to the conclusion that lake CO_2 is not in equilibrium with the atmosphere, and despite utilization of CO_2 by primary producers, most lakes are net sources of CO_2 to the atmosphere (Cole et al., 1994).

The collaborative work on CO_2 distribution led researchers to reconsider commonly held assumptions of in-lake processes and their interactions with external drivers from the land and air. A study of 27 lakes in the Northern Highland district of Wisconsin examined whether saturation with respect to partial pressure of CO_2 (P_{CO2}) in surface waters during summer was related to several watershed and lake variables (Hope et al., 1996). Dissolved organic carbon (DOC) concentration in the surface waters was a good predictor of P_{CO2}. Thus, DOC and P_{CO2} increased with increasing ratio of watershed to lake area and with increasing percentage of wetland area in the watershed. Two complimentary mechanisms of CO_2 accumulation in the lake were proposed, namely, that lakes receive the excess CO_2 directly from groundwater or that DOC from the watershed is converted to CO_2 by in-lake microbial respiration, producing super saturation levels of CO_2 in lakes.

INITATIVES

We are initiating additional research on bacterial community structure. The approach will include use of genetic techniques to explore differences in bacterioplankton diversity associated with the landscape position of a lake (high to low in the landscape) (Kratz et al. 1997), and the heterogeneity of conditions within a lake (oxic versus anoxic; water versus sediment, etc.). Experiments will also be conducted on genetic diversity responses to resource manipulation. We have a long-term interest in linking the microbial community to biogeochemical cycling in lakes.

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PAL - Palmer Station, *Antarctica*

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Several microbial studies involving biodiversity, biomass and productivity have been ongoing at the Palmer Station LTER over the last seven years. Most of

this work is being done simultaneously in the South Polar waters of the LTER grid and at a biogeochemical time-series station in the oceanic Pacific near Hawaii. The latter project, also supported by NSF, is part of the Joint Global Ocean Flux Study (JGOFS) program. We are just completing a separate Cross Ecosystem project as well.

Like most marine ecosystems worldwide, the Southern Ocean food web is supported by the photosynthetic activities of microorganisms. In Antarctica, most of the production results from the growth of eukaryotic phytoplankton (floating algae). One important difference is the community structure. The dominance of eukaryotic algae is in sharp contrast to the unicellular cyanobacteria and Prochlorococcus like cells which dominate most temperate and tropic ecosystems.

Another unique aspect of our microbial-based research efforts is the apparent uncoupling between microphotoautotrophic and microheterotrophic activities. Even before the start of the LTER program in Antarctica we had observed that the relationships between bacterial biomass and phytoplankton biomass in the southern ocean are fundamentally different from other marine and freshwaters worldwide. In Antarctica there is an approximately one order of magnitude fewer cells (and accordingly lower biomass) than is found elsewhere at comparable photosynthetic biomass. This affects both the structure and function of the ecosystem and, perhaps, the efficiency of carbon fluxes. We have already published several papers on this phenomenon though no intellectually satisfying explanation has been suggested.

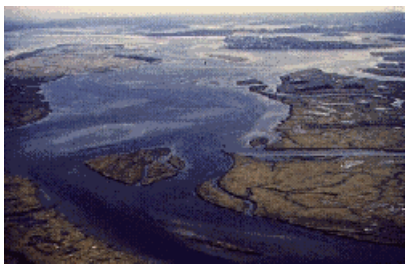
Another comparative aspect of these investigations involves dissolved organic matter turnover by heterotrophic bacteria and specifically the role of ectoenzymatic activity as a crucial first step in microbial metabolism. We have found that the relative activities of the enzymes leucine aminopeptidase (LAPase) and beta-galactosidase (BGase) varied significantly among different ocean regions. A strong latitudinal effect was observed in bacterial C and N assimilation pathways such that the favored compounds in one region appear to be microbiologically-inert in others. These observed patterns have major implications for the parameterization of secondary production and nutrient regeneration models.

Finally, we are actively involved in a major study of the presence and ecological significance of planktonic Archaea in Southern Ocean waters. As you probably know, Archaea is now recognized as one of three major domains of life. A few years ago, DeLong and others working within the LTER program discovered a relatively large abundance of Archaea in surface waters near Palmer Station (up to 30% of the total bacterial number). Subsequent work has confirmed this unexpected result and we have a paper submitted which provides evidence that this is a circumpolar phenomenon in the Southern Hemisphere. By comparison, in subtropical waters at our cross site station near Hawaii, planktonic Archaea comprise only a negligible portion of the total population. How this relates to the biochemical processing of organic matter (enzyme activities, above) and to the population structure (the so-called "deficit" of bacteria inpolar waters) are foci of our ongoing investigations.

PIE - Plum Island Sound Ecosystem LTER - *Massachusetts*

Charles S. Hopkinson, Jr. [chopkins@lupine.mbl.edu]

Background:



The Plum Island group has as one of its focus areas, microbial food web structure and function. With funding as a Land-Margin Ecosystem Research site, the Plum Island researchers led an all-LMER intersite comparison of the degradability of riverine dissolved organic matter using a microbial bioassay technique. Marsh dominated estuaries are detrital systems and the overwhelming majority of allochthonous and autochthonous organic matter inputs to the system are processed by microbes prior to reaching higher trophic levels.

Research Questions Concerning Microbial Ecology in the Plum Island LTER

Overarching Question:

How does planktonic community structure and production respond to changes in organic matter, nutrients, and water fluxes?

Specific Research Questions:

What is the spatial and temporal variation in the source of dissolved and particulate organic matter (from land, oceans, macrophytes, and algae) supplied to and used by pelagic bacteria? Requires measures of bacterial populations, bacterial activity, bacterial growth and growth efficiency, controls on bacterial growth, grazing on bacteria, N nutritition of bacteria, microbial food web structure, effect of UV light, nutrients, and organic matter quality of bacterial populations and bacterial dynamics, identification of sources of organic matter, and chemical characterization of organic matter.

How does the quality and quantity of organic matter mediate competition between bacteria and phytoplankton for nutrients? Requires measures of bacterial and phytoplankton production, nutrient and organic matter uptake dynamics by bacteria and algae, interaction between organic matter quality and inorganic nutrient availability on bacterial growth dynamics, effect of nutrient concentration on algal population dynamics and growth, importance of algal leachates for bacteria and the relative importance to overall energy flow and to higher trophic levels of various food webs originating from phytoplankton vs bacteria.

How does production of zooplankton and fish differ according to changes in water residence time, organic matter inputs, and nutrient supply? Requires measures of how bottom up controls influence higher trophic level production and production efficiency. Water residence time, organic matter inputs and nutrient supply dictate the relative production rate at the food web base. How does the production of higher trophic levels differ from one trophic structure to another?

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SEV - *Sevilleta National Wildlife Refuge, near Albuquerque, New Mexico*

Jim Gosz [jgosz@sevilleta.unm.edu]

Sevilleta Microbial Ecology Efforts

The Sevilleta LTER program has a number of research efforts that involve microbial ecology. The following represent those broad categories and names of individuals involved. Finally, these areas represent potential areas of concentration that would be pursued in future LTER work, especially if there were focused funds in the form of developing Microbial Observatories.

Microbial Ecology Topics

Microbial Processing of Carbon and Nitrogen:

This research involved carbon and nitrogen dynamics as affected by plant species, bare ground versus below plants, soil depths. Carbon mineralization, nitrogen mineralization, nitrification processes have been quantified in a number of vegetation types.

Plant litter decomposition studies also have been performed to accompany the more detailed processing studies of carbon and nitrogen. Studies of fungal communities and decomposition ratesfor woody material in packrat middens contribute to some specific issues on the role of vertebrates on carbon andnitrogen processing in this system.

In addition to collaboration with the U.S. Forest Service, the Sevilleta has interactions with the Microbial Ecology Lab at the Ecology Center of Kiel, Germany. Based on analyses of microbial biomass, microbial activities and specific microbial (enzymatic) potentials in soils, mathematical models and data analysis techniques are being developed to define the role of microbial populations in transformations and fluxes of elements.

Tom Kieft, New Mexico Technological Univ.

Carl White, UNM

Carol Klopatek, US Forest Service, Flagstaff, AZ

Jeff Klopatek, Arizonia State Univ.

John Zak, Texas Tech University

Mike Willig, Texas Tech University

Bai-Lian Li, UNM

Mycorrhizal Ecology:

The role of mycorrhizae are important in nitrogen limited systems and their influences are being studied with respect to increased nitrogen deposition from anthropogenic activity. Studies are designed to investigate nitrogen enrichment effects on plant growth and mycorrhizal species composition. If N enrichment alters the functioning of mycorrhizae along the mutualism-parasitism continuum, mycorrhizae may play a role in restructuring plant communities during N eutrophication. It is important to separate this effect from other effects such as climate change.

Nancy Collins Johnson, Northern Arizona Univ.

Mike Allen, San Diego State Univ.

Edie Allen, UC Riverside

Diane Rowland, UNM

Cryptogamic Crust Ecology:

These studies relate to the role of cryptogamic crusts on small mammal community species richness, role of small mammals on these crusts, nitrogen fixation capabilities, effects on soil erosion (wind, water), effects on soil moisture, and regeneration rates after disturbance. Remote sensing studies are now being performed using 1 m^2 imagery for greenness index values that allow the detection of these areas. We are developing interactions with studies on the Jornada LTER, the Negev Desert in Israel, and Great Basin Desert areas in Utah.

Ursula Shepard, UNM

Carl White, UNM

Dave Lightfoot, UNM

Dave Evans, Univ. of Arkansas

Elisheva Crowell, UNM

Gordon Johnson, UNM

Curtis Monger, New Mexico State Univ.

Giora Kidron, New Mexico State Univ. (from Israel)

Arian Pregnezer, Sandia National Lab

Parasitology:

The Sevilleta has an extensive collection of parasites of rodents from the rodent sampling efforts over the last decade. The taxonomy and host-parasite relationships have been worked on for specific areas (habitat types) on the Sevilleta. These studies have provided a catalog (survey) of the parasites with reference specimens for future studies.

Donald Duszynski, UNM

Murrey Daley, Northern Colorado College

Michael Patrick, UNM

Paulette Ford, New Mexico State Univ.

Wade Wilson, UNM

John Hnida, UNM

Kim Hechscher, UNM

Soil microfauna:

General surveys have been performed for several habitat types and site specific conditions, e.g., under plant canopies, between plant canopies, with soil depth.

Diana (Freckman) Wall, Colorado State Univ.

Clifford Crawford, UNM

Roman Zlotin, Indiana Univ. and Russian Instit. Of Biogeography

Sandra Brantley, UNM

R. Ruess, Institute of Soil Biology, Czech Republic

Future Research:

A recent paper on microbial processing on the Sevilleta has been published in Ecology Tom Kieft and Carl White The studies to date have been independent efforts to understand each of these aspects. Future activities will involve the integration of efforts in microbial processing, nitrogen fixation, mycorrhizal activity, cryptogamic crust ecology and microinvertebrate processing to allow the modelling of carbon, nitrogen, and water for this semiarid ecosystem at the transition of multiple biomes.

Experiments testing the effects of climate dynamics, nitrogen enrichment (i.e., air pollution), human activities, grazing and native fauna will allow the integration of microbial studies with plant and fauna research in understanding the ecology of the Sevilleta LTER. These studies, performed over time, and done in a way that allows comparisons among LTER and non-LTER sites represents the real contribution of LTER sites in a network of microbial observatories.

SGS *- Shortgrass Steppe, Nunn, Colorado*

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Summary of Microbial Ecology at the Shortgrass Steppe LTER

The studies at the SGS can be summarized into 4 areas:

1. the natural history of microbes as a function of soil processes
2. fundamentals of microbial mediated processes
3. the influences of natural and anthropogenic disturbances on microbial

mediated process
4. development of an array of models that include microbes and their activities

Modeling Soil Microbial Community Structure and Activity:

The SGS/LTER team has coupled the models traditionally used by community ecologists with those used by ecosystem ecologists. The studies listed below have provided insight into the roles of productivity and dynamics in structuring ecosystems, the estimation of interactions strength using field data, and common features of natural ecosystems and the impacts of agricultural practices on the structure and function of soil communities.

Our work has demonstrated that the resilience of simple food chains was directly related to the level of primary productivity and the rates of detritus inputs from external sources. The key to this study was in the development of Lotka-Volterra based models that were expressed in terms of ecological energetics. This result would seem to support the "dynamics hypothesis" that proposes that food chain length was a function of the limitations that increased length placed on the likelihood of the system recovering from a minor disturbance. A second important result was that the feasibility (ability of the system to maintain positive population densities at steady state) of the food chains was a function of the level of productivity and detritus inputs. Higher levels of productivity were able to sustain longer food chains. This had been suggested by Hutchinson (1959 Am. Nat. 93:145) and subsequently termed the "energetics hypothesis." The conclusion of our work was that system dynamics and energetics were inextricably inter-related. Food chain length was a function of both energetics and dynamics.

Considerable progress has been made in the development of procedures used to estimate the interaction strength among organisms with a food web. Paine (1992 Nature 355:73) proposed measuring interaction strengths among species through a series of species removal experiments. As an alternative, we estimated interaction strength with models and the field average population densities substituted for the theoretical equilibria. The elements of the Jacobian matrix (interaction strengths) are estimated by sampling physiological parameters, consumption coefficients and population densities, from ranges established by microcosm and field data.

An interesting pattern emerged when we estimated the interaction strengths (as above) and the trophic position of each consumer-prey interaction within food webs in North America and Northern Europe. The negative effects of consumers on prey decreases with increased trophic position and the converse was true for the positive effects of prey on consumer. Communities that possess this asymmetric patterning of interaction strength with increased trophic position are more likely to be stable than matrices depicting the same trophic structure but no pattern in the distribution of interaction strength.

The work has established regularities in the structure of soil food webs. An important finding was that soil communities are organized as assemblages of communities that include roots and their consumers, fungi and their consumers and bacteria and their consumers. These assemblages can be viewed as pathways of material and energy transfers. One observation from the agricultural studies was that the fungal energy channel is less resistant and less resilient to tillage practices than the bacterial energy channel. Disturbances induce shifts from one pathway to another. Our current efforts (see below) include modeling components that will compare soil food web structure and stability in disturbed and native prairie.

Effects of Grazing on Microbial Community Structure : This project involves manipulating cattle grazing in historically non-cattle grazed and cattle grazed pastures and tracking the changes in above and belowground communities. We moved the fences in 1991 and have sampled the sites annually. There has been a marked shift in the microbial community. Grazed pasture that was taken out of grazing now possesses a community structure that is similar to the non-cattle grazed pasture, and non-cattle grazed

pasture that was allowed to be grazed is similar in structure to the historically grazed pastures. The microbial communities began to diverge from one another two years following the manipulations. The complete shift has taken six years to materialize, and was only evident after the last growing seasoning. We intend to continue sampling these sites over the next few growing season to see if the pattern holds.

Our work with arbuscular mycorrhizal fungi holds much promise. We currently assess the vesicular-arbuscular mycorrhizal (AM) symbiosis by assaying soils for inoculum potential using corn as a bioassay plant. Corn is grown in soils and after 4 weeks the percentage of the length of corn seedling roots that is infected by AM fungi is used as measure of mycorrhizal inoculum potential (MIP). This technique is robust, but has come under a lot of criticism. The alternative would be to harvest plants and assess the degree to which the native plants are infected and/or use the native plants to assess inoculum potential. We plan to compare the results using the native plants to those obtained using the corn bioassay.

Effects of elevated CO2 on plant growth, microbial activity and trace gas emissions: Opentop chambers were established in 1997 at the site to study the influence of CO2 enrichment on plant growth, soil nutrient dynamics, soil food web structure and the exchange of trace gasses across the soil/atmosphere interface. The manipulation provides an excellent opportunity to study the effects of a press disturbance on community structure and dynamic stability.

The plan includes sampling soils from each treatment, and then modeling community structure and nutrient dynamics. These tasks will be achieved in three phases.

Phase I - Data Collection: Soils from the large cylinders in each treatment will be parsed by depth and distributed to the different team members for their respective. This work will provide estimates of the densities and biomasses of each of the taxa within the soil food web, the nutrient quality . The data will be used in two ways. First, standard univariate and multivariate statistical procedures will be used to assess treatment effects. Second, the data will be used to initialize simple and complex models.

Phase II - Modeling Community Structure: Soil food webs will be modeled using estimates of the biomasses of the soil biota, the diversity and productivity of plants, decomposition rates of the dominant vegetation (or substrate) and nitrogen mineralization rates. Estimates of nutrient retention will be obtained from the trace gas measures and from estimates of nitrate concentration from soil collected below the rooting zone of the vegetation. The output of these models will include, the contributions of each taxa to total N-mineralization and trace gas fluxes, the partitioning of these processes through different pathways (i.e., bacterial, fungal and root), and the relative proportions of each taxa within the food web. Each of these measure will assist in determining whether the soil community structure has changes under elevated CO_2.

Phase III - Modeling Interaction Strengths and Stability: The stability and resilience of the soil food webs constructed above will be assessed. Each of the state variables will be estimated from replicate samples from the field. Hence each will have a mean and variance associated with them. The mean value will be used as our estimate of the steady-state values of the state variable. This will enable us to estimate the elements of the Jacobian matrix of the communities for each treatment. The stability and resilience of the communities is obtained from the eigenvalues of the Jacobian matrix. We will use the field data and the variances associated with each variable to perform Monte Carlo simulations to gauge the stability and resilience of the communities. Each simulation will include at least 1000 independent samplings of the variables and estimates of the eigenvalues. The stability of the communities will be expressed as the proportion of the 1000 runs that are stable (all eigenvalues are negative), and the resilience will be expressed as the average of inverse of the absolute values of largest negative eigenvalues of the stable runs.

Impacts of Burrowing Mammals on Soil Biota and Processes: Burrowing mammals are an important component of the SGS landscape. One project is investigating the influence of the den activity of the Swift Fox (*Vulpes velox*) on soil community structure. Dens that are active (active dens) and those that possess pups (active/natal dens) have been compared to inactive dens and undisturbed native areas. The dens, the entrances to the dens, and 1 and 10 meters from the entrances have been sampled over the past two years for arthropods, vegetation and overall occupancy/activity by the foxes. We expanded the study this year to include soil bacteria, fungi, protozoa, nematodes and mycorrhizae.

If funds permit, we would like to investigate the impact of prairie dogs on soil microbial community structure and processes. These studies would parallel those for the Swift Fox and the work conducted in the 1980's at Wind Cave National Park, South Dakota and more recently at Badlands National Park, South Dakota.

Recommendations:

- The SGS group should continue its efforts in linking microbial community structure to ecosystem function. This should be formalized by making current connections more explicit.
- We should initiate studies that catalog microbial species diversity using the traditional and molecular techniques. The effort should include bacteria, actinomycetes, fungi (saprobic and mycorrhizal), and protozoa.
- Link microbial species diversity to function. These studies would use techniques that characterize substrate utilization by individual isolates or communities (e.g., Biolog or PFLA).
- We would like to initiate studies that focus on the linkage between microbial community structure and the ecosystem exchange of CO2, NOx and NH3. These studies might particularly focus on the response of microbial community structure and trace gas flux to disturbances.

VCR - Virginia Coast Reserve, *near Oyster, Virginia*

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There are four scientists focusing on microbial ecology at the Virginia Coast Reserve LTER site, including Iris Anderson (VIMS), Bob Christian (ECU), Aaron Mills (UVA) and Linda Blum, (UVA). A wide variety of microbiological techniques are employed to address a number of research questions. Most of the work is funded by other agencies beyond the core VCR LTER funding, although the VCR provides logistical support for the work.

The type of questions being addressed by students in the Laboratory of Microbial Ecology at the University of Virginia are available at http://www.evsc.virginia.edu/~alm7d/. Interests range from a wide variety of questions including controls on microbial abundance, activity, and community structure and function in the environment; microbial interaction with higher organisms; and the dispersal and distribution of microorganisms in the environment. The environments that we work in (aquatic or terrestrial) and the methods that we use are determined by the specific questions we ask.

We use a variety of techniques from cultural counts to advanced molecular techniques. We are however, dependent on our colleagues in the Biology Department and at the Medical School for access to equipment for the more sophisticated molecular methods applications. We use cultural, microscopic, and biochemical methods to measure microbial (bacteria, fungi, protozoa, and micro algae) abundance/biomass. We use a variety of methods to measure microbial activities including radioisotopes and stable isotopes, and other traditional methods. We also use PCR-based DNA fingerprinting and DNA-hybridization techniques to examine microbial communities. Currently we are limited in our work by access to the equipment for some of the molecular genetic techniques.

SPECIFIC QUESTIONS CURRENTLY BEING ASKED BY UVA MICROBIAL ECOLOGISTS INCLUDE:

1. Spatial and temporal distributions of microbial communities in heterotrophic and autotrophic estuaries and community response of disturbance.
2. Chemical and physical factors affecting the transport of bacterial in coastal plain aquifers.
3. Relative importance of bacterial secondary production to higher trophic levels in heterotrophic and autotrophic estuaries.
4. Role of denitrification processes in removal of N from ground water systems.
5. Characterization of spatial and temporal variability in microbial community structure using PCR-based DNA fingerprinting techniques

6. Comparisons of groundwater bacterial communities based on measures of community structure, functional potential, diversity, and environment.

PAST QUESTIONS INCLUDE:

1. Belowground dynamics in a Virginia salt marsh.
2. Bacterial dynamics in marsh creeks.
3. Microbial response to Spartina alterniflora plant roots.
4. Microbial metabolism of marsh sediment DOC.
5. Protozoan grazing effects on bacterial dynamics.
6. Comparison of bacterial-based and phytoplankton-based grazing food chains.

GENERAL QUESTIONS THAT HAVE BEEN ADDRESSED BY OUR COLLEAGUES AT ECU AND VIMS INCLUDE:

1. Nitrification response to daily tidal fluctuations in O2 content.
2. Characterization of N-cycling processes in a Spartina alterniflora salt marsh.
3. Sediment respiration response to wrack deposition and increased tidal flooding.
4. Denitrification response to marsh plant disturbance in a high marsh.
5. Interaction between microbial N-cycling and macro-algae production.

With potential for new funding, LTER's are ideal places for location of Microbial Observatories for several reasons. First is that there are number of microbial ecologistsalready working at LTER's – e.g., VCR, Toolik, Plum Island, KBS, SEV,NTL, and probably several more sites. A second advantage of placing microbial observatories at LTER's is that the microbial ecologists at LTERs work with macroecologists. This interaction facilitates scale and temporal comparisons that microbial and macro ecologists working in the absence of one another often ignore. At the VCR our interaction with physical scientists provides us with unique opportunities to examine the feedback between physical processes and microbes.

In the context of the tiered approach of a suggested model for LTER network observatories, the capabilities and needs of the Laboratory for Microbiology at the VCR LTER are outlined in the following table. Our capabilities and needs are technique-based. The our ability to address questions of interest to microbial ecologists, the VCR LTER, and the LTER network are based on our ability to have access to appropriate techniques.

Technique	Currently in Use at VCR LTER	Equipment Present	Needs
Microbial Abundance and Biomass - Microscopic	yes	partially	Additional scope and digital imaging capability
Cultural and Archiving Capabilities – traditional methods	yes	partially	Additional ultra-low freezer on shore
Individual identification & phylogeny – genetic probes	no	no	Skills and equipment
Microbial Abundance and Biomass – biochemical methods (ergosterol, lipid PO_4)	yes	partially	Additional GC
Microbial Activity – radioactive & stable isotopes	yes	yes	
Microbial Activity – gene probes	no	no	Related skill and equipment
Microbial Community Analysis –	yes	no	All related

PCR-based DNA/RNA finger printing			equipment
Microbial Community Analysis – DNA hybridization	yes	no	All related equipment
Microbial Community Analysis - PFLAs	yes	no	Better access to analysis (cost)
Gene Analysis	yes	no	Better access to analyses (cost)