**Documents for the solutions in freezer B9** (McFarlane?)

**Introduction to the Study**

Acid rain has been shown to deplete base cations and consequently decrease soil pH and buffering capacity in many northeastern forests. In this study, we looked at three sites that were experimentally amended with lime within the past 30 years in order to restore base cation and pH levels. Lilly (unpublished 2004) found that important differences persisted in the pH of the amended and unamended plots at these sites.

The activity and stability of soil bacteria is pH sensitive, as certain species can exist only under specific soil conditions. N mineralization is the process by which organic N in soils is converted to the plant available forms of NO3- and NH4+, which are by-products of microbial metabolism.

We hypothesized that mineralization rates would be higher in lime amended soils versus unlimed soils because soil bacterial community would be greater. By analyzing the initial amount of NO3- and NH4+ after a period of incubation (28 days), we can tell how fast the process of mineralization is taking place.

**Experimental Design**

We looked at the difference in soil mineralization rates in limed and unlimed areas at three different sites: Harvard Forest (MA, Proctor Maple Research Center (VT) and Bartlett Experimental Forest (NH).

At each site, two treatment areas exist, one where soils were limed, and one where soils remained untreated. In each treatment area, three thirty meter measuring tapes were laid out ~10 m from each other. Soil samples were collected every 5 m along each transect at 6 sampling points per transect. We pounded a PVC corer into the ground to 20 cm at each of these sampling points to obtain soil samples to analyze for initial NO3- and NH4+. Simultaneously, 20 cm PVC cores were installed near each sampling location and removed after 28 days to obtain post-incubation values for NO3- and NH4+.



Figure 1. Diagram of experimental design. Each 12 samples inluceds 6 from the 0-10 cm layer and 6 from the 10-20 cm layer.

Soil samples for analysis were collected at a total of 18 sampling locations per treatment from two depths I nthe soil profile (0-10 cm and 10-20 cm). These samples were then composited by depth in soil profile and transect from which they were sampled to produce one sample per transect per depth (we also produced three replicates of each sample to look at variability using this method).

**Field Methods**

1. Lay out three thirty meter transects 10 m apart from each other.
2. Flag every 5 m from the start of the transect (starting with 5m instea of 0 so you end up with 6 and not 7 sampling locations). Pin flags should be left to mark each 5m.
3. At each flag, install a 23 cm core into the soil to the right of the pin flag. Try to stay within one meter of the pin flag when coring.
4. Cap PVC cores with gas permeable plastic and secure with a rubber band, leaving room for air flow.
5. Sample soil to the left of the pin flag with the soil corer for a 20 cm soil sample. Samples must represent the full 20 cm increment when pulled out of the ground. If there is less soil, resample near the original location.
6. Move the original pin flag next to the mineralization core. Cover the plastic bag on top of each core with litter to prevent excessive heating of soil in the core.
7. Repeat steps at each sampling location.
8. When finished, there should be 18 samples from the 0-10cm soil and 18 from the 10-20cm for each site (for Limed plus the Control you should have 72 total samples).

**Laboratory Methods**

These methods follow those for mineralization in *LTER Standard soil methods for long-term ecological research.*

1. Weigh 100 g of fresh soil into glass jar of known weight. Samples will be kept in the oven at 105°C until they reach a constant weight.
2. Pass fresh soil through a 2 mm sieve (wear gloves)
3. Weigh duplicate 10 g subsample of soil into each of two extraction cups.
4. Weigh soil samples for gravimetric moisture analysis
5. Add 100 mL 1M KCL extractant to each cup, then cap and shake for 1 minute.
6. Make one blank per 9 samples with KCL.
7. Allow to equilibrate overnight (12-24 hrs)
8. Reshake extraction cups and allow to settle for at least 45 minutes.
9. Remove 10 mL of solution from extraction cup into syringe.
10. Place preloaded filter-holder on syringe and filter solution. Solution will pass into a clan scintillation vial. Solution may be stored in vial in a refrigerator until analysis.
11. Weigh samples kept in the oven for gravimetric moisture to constant mass and record final weight.