

**Soil Organic Matter, Mycorrhizal Fungal Communities, and Nitrogen Cycling in
North American Temperate Forests**

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Research Interests:

My research interests include global change ecology, and the development and application of Earth System models to track the changing climate.

Abstract:

Soil organic matter (SOM) is one of the largest Carbon (C) pools in the biosphere. The ability of these pools to store carbon, and the movement of carbon in and out of these pools, may be affected by the changing climate, specifically the rising CO₂ levels in the atmosphere. A change in carbon storage abilities in these pools can then have negative or positive feedbacks on climate change. The two major mycorrhizal lineages (Ectomycorrhizal [EMF] and Arbuscular Mycorrhizal [AMF] Fungi) are known to interact differently with SOM. Specifically, ECM and AM utilize different forms of nitrogen from the soil. Importantly, this leads to the impactful observation EMF forest soils store more organic carbon than AMF forest soils. In order to explore the interaction of EMF and AMF with the nitrogen cycle, we conducted stable isotope nitrogen pool dilution experiments at various points throughout the year. In these experiments, we sampled soil from temperate forests in southern Indiana and analyzed for net and gross rates of nitrification, denitrification, and mineralization of N. Through the use of net and gross rate measurements, a more developed framework of the mycorrhizae and microbial communities impact on carbon storage can be developed.

Introduction:

Human activity has created an overwhelming effect on major carbon pools and fluxes - leading to global warming. Soil organic matter (SOM) holds more carbon than the biotic and atmospheric pools combined¹. An understanding of how different terrestrial carbon sinks, such as SOM, are being affected by climate change is necessary to predict and understand the overall terrestrial carbon cycle, and the negative and positive feedbacks on climate change that may be occurring¹. A decrease in the size of the SOM pool means that CO₂ is being released into the atmosphere creating a positive feedback on climate change. Alternately, increased carbon storage in the SOM pool means less CO₂ is being released in the atmosphere causing a negative feedback on climate change. Monitoring carbon storage and increasing understanding about the mechanisms governing different levels of soil carbon storage will allow for better prediction of climate change models.

There are many interactions within the SOM that are closely linked with C storage and availability, including symbiotic organisms called mycorrhizae. Mycorrhizae are fungi that live in symbiosis with tree roots. Carbon is transferred from the host tree roots to the mycorrhizae, and in return the fungi uptake essential nutrients that are used by the host tree⁵. There are two major types of mycorrhizal fungi, ectomycorrhizal (EMF) and arbuscular mycorrhizal (AMF) fungi⁵, which dominate a majority of the forests around the world⁵. There are major differences between EMF and AMF dominated soils, primarily that soil carbon storage in EMF dominant soils are greater than AMF dominant soils^{2,3,4,6,7}. This observation has led to the discussion of

possible mechanisms that cause this phenomenon. One explanation is that of the Mycorrhizal Associated Nutrient Economy, or the MANE framework. The MANE framework⁷ has been developed based on the assumption that the leaf litter in tree species associated with EMF are chemically more resistant to decomposition than AMF associated tree species⁷, therefore creating differing nutrient constraints between mycorrhizal species. This difference in leaf litter chemistry ultimately causes a difference in the SOM C and N availability for mycorrhizae, microbes, and plants. However, there is no global difference in leaf litter chemistry of EMF and AMF trees^{8,9}, leading us to question whether other mechanisms may be at work.

Mycorrhizal interactions are closely linked to microbial communities in SOM¹⁰. Microbial communities, which are responsible for the decomposition of organic matter, must maintain a certain ratio of C to N (C:N) in their biomass¹¹. When a microbe is decomposing organic matter, the N in organic matter will either be mineralized (transformation of organic to inorganic N) or immobilized (assimilation of N into the microbial biomass) by the microbe¹¹ depending on the C:N of the organic matter being decomposed. Thus, microbial transformation of N will affect the availability of N to plants. Mineralization transforms N to an available form for plant uptake, while immobilization stores N in the soil in a form that is not readily available¹¹.

EMF and AMF fungi have different strategies for nitrogen uptake. Through these different uptake strategies, EMF and AMF alter carbon cycling^{4,6,7,10,12}. AMF uptake inorganic nitrogen, while EMF are able to directly uptake organic N¹³. AMF, therefore, are dependent on the bacterial mineralization of organic N to create an inorganic nitrogen pool available for uptake. Since EMF can directly uptake the N in organic matter, and because they obtain Carbon from their host tree, EMF decompose organic molecules to obtain nitrogen¹⁰. Thus, they mine nitrogen from organic molecules and leave behind carbon-rich, nitrogen-depleted molecules which are difficult for other organisms to decompose. These nutrient acquisition strategies create differential N environments in EMF and AMF dominant soils, which impacts carbon storage^{6,7,10,12}.

To date, previous studies have used net rates to quantify differences in Nitrogen cycling between EMF and AMF soils. Net rates are a calculation of net change to inorganic nitrogen pools. Net rate calculations have been informative, but it is clear that there are underlying characteristics of these pathways that cannot be adequately quantified with net rates. By conducting a stable isotope pool dilution experiment, we will be able to calculate the individual N cycling pathways that can be used to answer more mechanistic questions.

Methods:

Study Site

The sample site is located in Bloomington, Indiana in Moore's Creek Research and Teaching Preserve, a mixed deciduous hardwood forest. There are 28 plots where all soils were collected for this experiment (7 EMF unfertilized, 7 EMF fertilized, 7 AMF unfertilized, 7 AMF fertilized).

Sampling

Soil was collected 4 times during the growing season - once in mid-May, early July, early September, and late October. Sampling took place 2 weeks after fertilization in order to avoid a possible pulse in microbial activity directly after fertilization. Five soil cores were taken within each sample site (one at each corner of the plot, and one in the center). The soils were collected in Indiana, and then transported to the lab space in Urbana, Illinois. The pool dilution experiment started within 24 hours of soil collection.

Pool Dilution Experiment

The soil cores were homogenized and then subdivided into 3 treatments: 1.) control, 2.) ^{15}N -labeled KNO_3 addition for measurement of gross nitrification rates¹⁴ and, 3.) ^{15}N -labeled NH_4Cl addition (at three different concentrations) for measurements of gross N mineralization rates¹⁴. Soils were sampled 15 minutes, and 4 hours, after ^{15}N -label addition for extraction in KCl . Additional soil subsamples from the ^{15}N treatments were used to determine microbial NH_4^+ and NO_3^- assimilation using chloroform fumigation. The soil extracts will be analyzed colorimetrically for NO_3^- and NH_4^+ concentrations on a Lachat flow injection auto-analyzer and analyzed for N isotopes on an isotope ratio mass spectrometer (IRMS) interfaced to an elemental analyzer in the Yang Lab¹⁵. Additionally, a subsample of the labeled sample were sealed in a mason jar with a stopcock. Four hours after label solution addition, gas samples were taken from the headspace to capture $^{15}\text{N}_2\text{O}$ and $^{15}\text{N}_2$ produced during the incubation period. Gas sampled from this headspace underwent N isotope analysis on the IRMS for calculation of denitrification rates¹⁵.

Results and Future Directions:

Processing all samples for every experiment is not finished – therefore a clear map of results from the pool dilution, including gross and net rates, will be done in the near future. Issues concerning machine reliability have come up while processing results. Specifically, the EMF sites had extremely low concentrations of nitrate, as was expected. Special methods are needed to detect such low concentrations which we are currently working to develop.

Preliminary data does show a higher concentration of inorganic N (especially NO_3) in AMF soils compared to EMF soils.

Once all samples have been analyzed, and the data has been reviewed, net and gross rates calculations can be made. We predict, once all rates have been calculated, that all N cycling rates will be lower in EMF soils than AMF soils.

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