

# Quantifying Nitrogen Cycling on Surface Mined Land Using Natural Abundance and Fungal Relationships: An Exploratory Study

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## Introduction

Nutrient cycling plays a vital role in the regeneration of drastically disturbed soils, such as those found in reclaimed surface mining areas. These soils represent a great potential for carbon sequestration efforts. Mycorrhizal fungus is an important factor in nutrient cycling, especially nitrogen. The role of these fungi in carbon cycling may have important implications for carbon sequestration efforts. However, little is known about the development of mycorrhizal systems on disturbed soils or their role in nutrient cycling and reclamation. This study uses stable nitrogen and carbon isotopes to examine the impact of surface mining on soil nutrient cycling. This information is applied to fungi to illustrate the role of fungal cycling of nitrogen within an ecosystem.

## Background

Many recent studies on the disturbance in nutrient cycling caused by surface mining have used naturally occurring isotopic signatures of carbon and nitrogen. Isotopic signatures are defined as:

$$\delta X\text{‰} = (R_{\text{sample}}/R_{\text{std}} - 1)10^3$$

where X is a stable isotope and R is the isotope ratio (i.e.  $^{15}\text{N}/^{14}\text{N}$ ) (Campbell et al. 2009).

In the process of uptaking and transferring nitrogen to plant hosts, mycorrhizal fungi preferentially transfer  $\delta^{14}\text{N}$ , thereby becoming enriched in the isotope  $\delta^{15}\text{N}$  (Hobbie et al. 1999). In this study, the mass balance equation is:

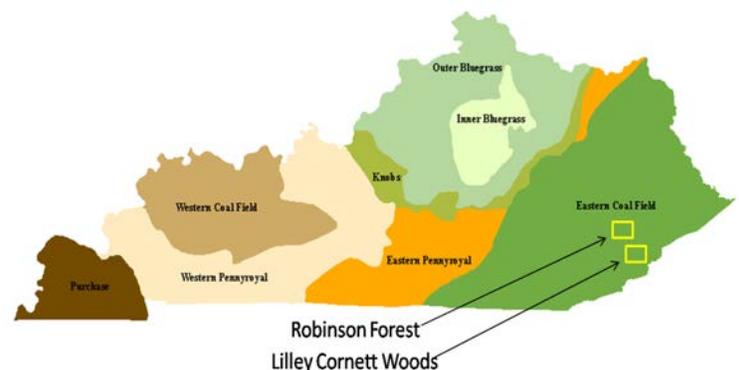
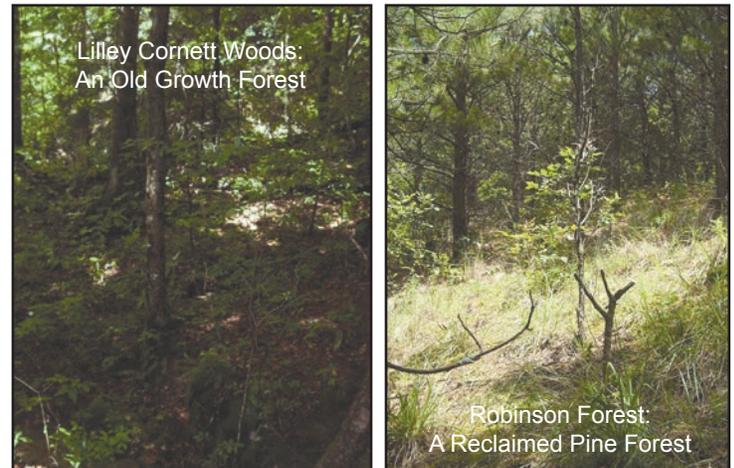
$$\begin{aligned} 100(\delta^{15}\text{N}_{\text{av}}) &= (100 - T)(\delta^{15}\text{N}_{\text{fun}}) + (T)(\delta^{15}\text{N}_{\text{tr}}) \\ 100(\delta^{15}\text{N}_{\text{pl}}) &= (f)(\delta^{15}\text{N}_{\text{tr}}) + (100 - f)(\delta^{15}\text{N}_{\text{av}}) \\ \Delta &= \delta^{15}\text{N}_{\text{av}} - \delta^{15}\text{N}_{\text{tr}} \end{aligned}$$

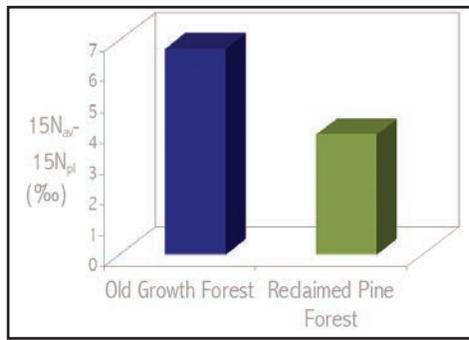
where  $N_{\text{av}}$  is N available to plants (in this study, the soil N),  $N_{\text{fun}}$  is fungal N,  $N_{\text{tr}}$  is N transferred from fungi to plant,  $N_{\text{pl}}$  is plant N, T is the percentage of N taken up by the fungal hyphae that is transferred to the plant, f is the percentage of plant N obtained from the fungal hyphae, and  $\Delta$  is the fractionation that occurs in the formation of compounds in the fungi by which N is transferred to the plant. The parameters  $f \leq 100$  and  $\Delta \geq 8\text{-}10\text{‰}$  (Hobbie & Hobbie 2006) are used to compare mycor-

rhizal fungal N cycling in an approximately 10-year-old pitch pine reclaimed surface mined forest and an old growth forest.

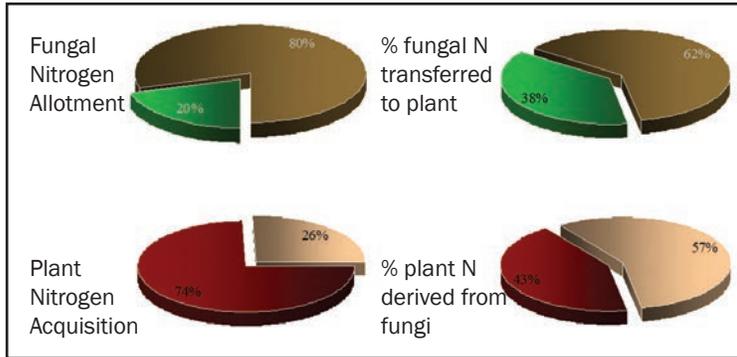
## Methodology

Isotopic relationships between soil, plants, and fungi were examined for a 10-year-old pitch pine reclaimed valley fill in Breathitt County, Kentucky (Robinson Forest) and for an old growth mixed mesophytic forest in Letcher County, Kentucky (Lilley Cornett Woods). A quantitative inventory of sporocarps was performed to measure their size and substrate at each site. Isotopic signatures were determined for fungal and presumed substrate samples, as well as dominant foliage samples (beech, *Fagus grandifolia*, in the old growth forest; pitch pine, *Pinus rigida*, in the reclaimed site). Bulk soil samples were analyzed to quantify the nitrogen flux occurring between soil, mycorrhizal fungi, and trees at each site.





$\delta^{15}\text{N}$  differences between soil and dominant trees, a measure of tree reliance on fungal N sources.



## Conclusions

It was found that 20% of mycorrhizal fungal N is transferred to host trees in the old growth forest and 74% of host plant N comes from mycorrhizal fungi. In the reclaimed forests, 38% of mycorrhizal fungal N is transferred and 43% of plant N is fungi-derived. While reclaimed site trees obtain less of their total N from fungi than an old growth forest, the mycorrhizal fungi that is present in a reclaimed area give more of their N to plants, indicating a shortage of mycorrhizal fungi relative to the old growth forest. This interpretation is consistent with the quantity of sporocarps observed at each site.

The results suggest that the mycorrhizal fungi provide a larger total source of N in the old growth while those on the reclaimed site are more utilized by the plants relative to their total assimilated N. Assuming the old growth forest represents an equilibrium of the plant-soil-fungi system, it is concluded that the reclaimed site is not at equilibrium and that the addition mycorrhizal fungi is needed to obtain optimum N cycling within the system.

	Old Growth	Reclaimed Pine
Humus	77	19
Wood	35	2
Leaf Litter	6	0
Total	118	21
Total/m <sup>2</sup>	0.59	0.07

Total sporocarps counted within 10m x 10m plots (Old Growth:2, Reclaimed Site:3)

	Old Growth	Reclaimed Pine		Old Growth	Reclaimed Pine
$\delta^{15}\text{N}_{\text{fun}}$	6.0	6.1	$\delta^{15}\text{N}_{\text{tr}}$	-5.3	-8.4
$\delta^{15}\text{N}_{\text{pl}}$	-2.9	-3.3	$T$	20.4	37.9
$\delta^{15}\text{N}_{\text{av}}$	3.7	0.6	$f$	74.5	43.2

Mass balance equation variables: a)measured; b)calculated assuming  $\Delta=8-10\%$

## Reclaimed Pine

Mycorrhizal	$\delta^{15}\text{N}$	$\delta^{13}\text{C}_{\text{fun}}$	$\delta^{15}\text{N}_{\text{sub}}$	$\delta^{13}\text{C}_{\text{sub}}$
Boletaceae	6.7	-26.9		
<i>Amanita*</i>	5.0	-25.5		
<i>Pisolithus tinctorius*</i>	6.6	-26.5		
Saprotrophic				
UNID A*	-3.7	-24.5	-2.1	-27.0
UNID B	-3.7	-24.2	-2.4	-27.3
Undetermined				
<i>Lycoperdon</i>	-0.5	-24.7	-0.4	-26.6
<i>Lycoperdon</i>	1.5	-23.2	-0.3	-27.2
<i>Lycoperdon</i>	7.5	-23.2	0.2	-25.8
UNID C	2.3	-23.3	-0.9	-25.7

## Old Growth

Mycorrhizal	$\delta^{15}\text{N}$	$\delta^{13}\text{C}_{\text{fun}}$	$\delta^{15}\text{N}_{\text{sub}}$	$\delta^{13}\text{C}_{\text{sub}}$
Boletaceae	8.7	-24.7		
<i>Russula</i>	3.1	-25.7		
<i>Tremellodendron pallidum</i>	4.5	-24.8		
<i>Strobilomyces</i>	7.2	-26.1		
<i>Strobilomyces</i>	6.5	-26.3		
<i>Strobilomyces</i>	6.1	-26.7		
Saprotrophic				
<i>Galiella rufa</i>	-3.7	-24.6	-4.416	-26.0
Undetermined				
<i>Lycoperdon</i>	-2.9	-24.3	-0.2	-27.1
UNID D	3.2	-25.2	0.6	-27.5
UNID D	2.6	-26.4	0.8	-26.4
UNID E	-0.4	-23.5	-0.8	-27.4

Isotope data for sampled sporocarps

## References

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