# EFFECTS OF FERTILIZATION ON $CO_2$ EFFLUX IN A TWO-YEAR-OLD LOBLOLLY PINE STAND ON THE VIRGINIA PIEDMONT

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**Abstract—**Fertilization is becoming a common, cost effective treatment within managed forests of the Southeastern United States. However, there is little known about how fertilization will affect the belowground processes that drive soil $CO_2$ efflux. A thorough understanding of belowground carbon (C) dynamics is necessary for the estimation of net ecosystem productivity and the C storage potential of these managed systems. In April 2004, we began monitoring total soil $CO_2$ efflux and heterotrophic respiration. Respiratory components were measured prior to fertilization, weekly following fertilization, and bi-weekly after respiratory components stabilized. We found that total soil $CO_2$ efflux did not differ consistently between fertilized and unfertilized plots over the 8 months. Heterotrophic respiration was significantly ($P<0.0001$) lower in fertilized plots starting from 8 days after fertilization throughout the duration of the study. We hypothesize that a corresponding increase in root respiration is offsetting any decrease due to microbial suppression.

## INTRODUCTION

Schlesinger (1997) estimates that forests account for up to 75 percent of all carbon (C) stored in terrestrial ecosystems and are responsible for about 40 percent of the C flux between the atmosphere and the biosphere. This makes $CO_2$ flux from soils the second-largest flux in the global C cycle, an order of magnitude greater than $CO_2$ emissions from fossil fuel burning (Raich and Schlesinger 1992). Forests have the capability to function as giant C sinks by removing inorganic C from the atmosphere and fixing it as biomass. The C finds its way to the forest floor as fallen plant material, where it is released back into the atmosphere by either soil microbes or fire, or it is incorporated into the soil for long-term sequestration.

Total $CO_2$ efflux at the soil surface is a combination of: (1) root (autotrophic) respiration resulting from maintenance and growth and (2) heterotrophic respiration produced during the decomposition of organic matter by soil micro and macro fauna found within the soil profile. Forests and forest soils can act as either C sinks or C sources; and only by understanding the different mechanisms that determine the shift between source and sink can forests be managed to sequester C long-term.

Delcourt and Harris (1980) reported that forests of the Southeast have acted as C sources from 1750 to 1960 due to the deforestation of virgin forests during the industrial revolution. Recently (1960 to present), reforestation of agricultural fields and the intensive management of secondary forests have made the Southeast function largely as a C sink. With 13 million ha of *Pinus taeda* L. (loblolly pine) stands intensively-managed in the Southeastern United States, there is the potential to sequester large amounts of C in both plant biomass and as organic matter in forest soils (Jokela and Long 2003, Maier and Kress 2000).

Fertilization in southeastern pine forests has increased approximately 800 percent since 1990 to just over 500,000 ha of planted pine being fertilized in 2000 and 2001 (NCSFNC 2002, Wear and Greis 2002). Wear and Greis (2002) estimated that the use of fertilizer in the United States exceeds use by the rest of the world.

The effects of above-ground responses to nutrient additions are well understood. Fertilization has been shown to increase net primary productivity (Albaugh and others 1998, Axelsson and Axelsson 1986, Gough and others 2004), but there are still questions concerning the impact of fertilization on belowground C evolution. The overall objective of this research is to examine how fertilization initially impacts belowground C fluxes in a 2-year-old clonal plantation of *P. taeda* located on the Virginia Piedmont. Specifically, we will determine the effects, over time, of fertilization with diammonium phosphate, supplemented with ammonium nitrate, on total soil $CO_2$ efflux and heterotrophic respiration.

## MATERIALS AND METHODS

### Site Description

This study was installed at Reynolds Homestead Forest Resources Research Center located in Patrick County, VA (latitude 36°40' N, longitude 80°10' W). The Reynolds plantation was intensively-farmed with row crops and tobacco from the early 1800s to the mid 1900s. In 1969, this property was donated by tobacco manufacturer R.J. Reynolds to Virginia Tech to study forest biology.

The elevation is approximately 300 to 500 m with a gently sloping topography, and soils are mapped as Lloyd clay loam, Louisa loam, and Hiwassee loam series. These are deep, well-drained Ultisols derived from granite, gneiss, and schist. Past farming practices led to erosion and the removal of most of the A horizon, resulting in a truncated soil profile with clayey B horizons incorporated into surface Ap horizons. The climate is warm and humid, receiving 1,310 mm of precipitation spread evenly throughout the year. The mean temperature is 14 ºC with an average minimum of -1.4 ºC, usually occurring in January, and an average maximum temperature of 29.2 ºC, occurring in July.

### Experimental Design

This study was a randomized complete block design with a split plot with repeated measures, replicated four times. Four blocks, each consisting of two plots, received two levels of

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*Citation for proceedings:* Connor, Kristina F., ed. 2006. Proceedings of the 13th biennial southern silvicultural research conference. Gen. Tech. Rep. SRS–92. Asheville, NC: U.S. Department of Agriculture, Forest Service, Southern Research Station. 640 p.

fertilizer (fertilizer and no fertilizer), with 25 unique *P. taeda*. clones (SP) repeated within each plot. The clonal seedlings were donated by the Forest Biology Research Cooperative, University of Florida, Gainesville, FL, for use in this project. The site was prepared by spraying the planting rows with glyphosphate (Round Up, Monsanto Co., St. Louis, MO) followed by a shallow tillage of the rows. The seedlings were planted May 19, 2003, at 3.2-m (row) x 2.6-m (seedling) spacing with 25 clones per plot and a buffer strip of stock seedlings surrounding each plot. In spring of 2004 (prior to fertilization), all vegetation was removed (chemically and mechanically), and plots remained free of all vegetation except for desired *P. taeda* clones. After each sampling date, any emerging vegetation was removed mechanically to insure a clean surface for the following sampling date.

May 6, 2004, one randomly-chosen plot within each of the four blocks received a single application of fertilizer in the form of diammonium phosphate [$(NH_4)_2PO_4$] supplemented with ammonium nitrate ($NH_4NO_3$), which was banded at a rate of 225 kg $ha^{-1}$ and 186.5 kg $ha^{-1}$, respectively. This rate is equivalent to 112 kg nitrogen (N) $ha^{-1}$ and 23 kg phosphorous $ha^{-1}$.

Total soil and heterotrophic respiration of six selected clones were measured per plot, prior to fertilization, to determine a baseline respiration rate. After fertilization, measurements were repeated weekly until response to fertilization leveled off, then every other week or monthly for the duration of the experiment (approximately 1 year).

### Total Soil Respiration Measurements

Total soil $CO_2$ efflux was measured at the soil surface using the LiCor 6200 infrared gas analyzer (IRGA) (LiCor Inc., Lincoln, NE) with a dynamic closed cuvette chamber constructed from a PVC pipe for walls, a plexi glass top (25.5 cm internal diameter, height at center 13.5 cm) with a total system volume of 67,044 $cm^3$ (Janssens and others 2001, Selig 2003). The bottom of the chamber was fitted with a stainless steel edge to create a seal with the ground.

The LiCor 6200 was recalibrated before each sampling date and the system zeroed between every block. Respiration measurements were made in the same sequential blocking order at approximately the same time of day for each sampling date. The chamber was placed at the soil surface, next to the seedling stem ($\leq$ 0.25 m), where no living, photosynthesizing, plant material was present. $CO_2$ evolution was measured over a 30-second period and respiration rate (μmol $m^{-2}$ $s^{-1}$) calculated on a per unit land area with the following equation:

$$\text{Soil CO2 efflux} = \left[\left(\frac{\Delta C}{\Delta t}\right)\left(\frac{PV_t}{RT}\right)\right] \div \text{surface area of soil} \quad (1)$$

where

C=[$CO_2$],

t=time,

P=atmospheric pressure,

$V_t$=system volume,

R=universal gas constant, and

T=temperature (ºC).

### Heterotrophic Respiration

Following total soil respiration measurements, heterotrophic respiration was measured using the LiCor 6200 with a 0.25-L cuvette chamber, with a total system volume of 429 $cm^3$. Soil was extracted to 10 cm in depth with a 2.5-cm diameter push tube at the base of each seedling sampled. Roots were carefully removed from the soil sample and the soil placed into an aluminum weight boat (10 cm x 2 cm), which was immediately placed into the 0.25-L cuvette chamber. Heterotrophic respiration was measured over a 30-second sampling period. Following respiration measurements, soil was sealed into a labeled envelope and brought back to the lab for drying. Soil was oven-dried for 48 hours at 105 ºC and weighed to the nearest 0.01 g. Microbial respiration was calculated and expressed on a per soil mass basis (μmol $g^{-1}$ $s^{-1}$).

### Soil Temperature and Moisture Measurements

Soil temperature and volumetric water content were taken, concurrently with respiration measurements, at the base of each clone, to be used as covariates to normalize respiration rates to a common temperature and moisture. Soil temperature was measured to the nearest 0.1 ºC at 7 cm using a Digi-sense temperature gauge (model no. 8528-20, Cole-Parmer Instrument Co., Niles, IL). Soil moisture was measured at a depth of 15 cm using a time domain reflectometer (Soil Moisture Equipment Co., 6050X1, Golena, CA). Soil moisture content was expressed as a volume percent and determined to the nearest 1 percent.

### Analysis

The effect of fertilization on total soil and microbial respiration was analyzed using ANOVA to detect significant differences between treatments using the GLM procedure in SAS version 9 (SAS Institute, Cary, NC). Efflux rates were normalized to a common temperature and volumetric water content by ANACOVA when within block variation of temperature or moisture was significant. A time series analysis was performed to test for treatment effects over time using PROC MIXED in SAS. Clonal differences were not evaluated as part of this analysis.

## RESULTS AND DISCUSSION

### Heterotrophic (Microbial) Respiration

Heterotrophic respiration rates in the fertilized plots were significantly ($P<0.0001$) lower relative to plots that did not receive fertilizer when measured over time. Microbial respiration rates in fertilized plots decreased below control levels starting 8 days following fertilization, and remained depressed throughout the duration of the study (fig. 1a). Microbial respiration showed a positive relationship to volumetric water content of soil and, to a lesser extent, soil temperature (fig. 1c).

It has been well-documented early on in the literature (Bååth and others 1981, Kowalenko and others 1978, Nohrstedt and others 1989, Roberge 1976, Smolander and others 1994, Söderström and others 1983), and more recently (Gough and Seiler 2004, Lee and Jose 2003, Thirukkumaran and Parkinson 2000), that the addition of N to the soil affects heterotrophic respiration. N fertilization may have a direct affect on respiration rates by a change in soil pH. Ammonium nitrate ($NH_4NO_3$), and other ammonium salts, have been shown to lower soil pH, accompanied by a decrease in microbial activity (Kowalenko and others 1978, Söderström

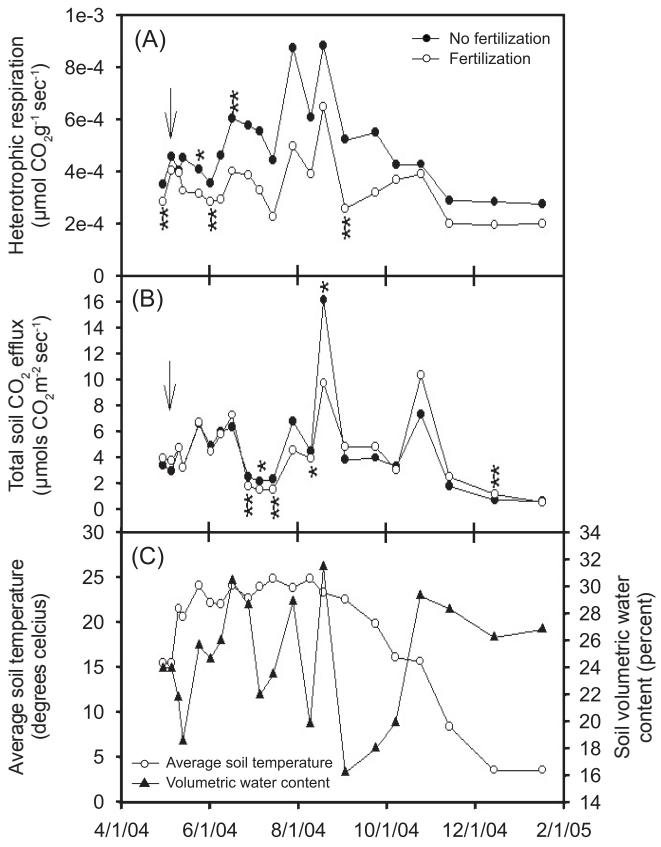


Figure 1—(A) Heterotrophic respiration in a two-year *Pinus taeda* plantation located on the Virginia Piedmont; (B) total soil $CO_2$ efflux at the soil surface; and (C) average temperature and volumetric water content of the soil. The arrows indicate time of fertilization, single asterisk (*) represents significance at $\alpha$=0.10, and double asterisk (**) represents significance at $\alpha$=0.05 (n=24). Fertilized plots received 112 kg N $ha^{-1}$ and 23 kg P $ha^{-1}$ in the form of diammonium phosphate and ammonium nitrate (225 kg $ha^{-1}$ and 186.5 kg $ha^{-1}$, respectively).

and others 1983, Thirukkumaran and others 2000). A study by Leckie and others (2004) suggests that microbial composition and activity in the long term may be influenced by fertilization to a greater extent by indirect effects on plant growth and litter input as opposed to direct effects on microbial populations. Another possibility is a shift in population dominance within the soil community after the addition of N, and changes in C allocation to the roots. However, it is unlikely that either of these long-term effects explain our rapid decrease after just 8 days.

Gough and Seiler (2004) found that microbial respiration was significantly (P<0.05) depressed throughout the entire 197 days in potted *P. taeda* seedlings grown in a greenhouse. The authors found that microbial activity per gram of soil was only 44 and 66 percent of that measured in control pots at 49 and 197 days following fertilization, respectively. In contrast, Haynes and Gower (1995) found no difference in heterotrophic respiration between fertilized and control trenched plots in a 31-year-old *P. resinosa* plantation in northern Wisconsin.

## Total $CO_2$ Efflux at the Soil Surface

There was no significant (P=0.3263) difference between fertilized and control plots in total $CO_2$ efflux when analyzed over the duration of the study, but the treatment date interaction was significant (P<0.0001). There was no consistent trend in total $CO_2$ efflux over the 8 months measured. However, on two dates, total $CO_2$ efflux was significantly (P<0.05) lower in fertilized plots and on one date significantly (P<0.05) greater in fertilized plots relative to controls (fig. 1b).

A review of the current literature has shown mixed responses of $CO_2$ efflux to N fertilization. Pangle and Seiler (2002) found that fertilization did not have a significant (P<0.05) effect on soil $CO_2$ efflux in a 2-year-old *P. taeda* stand located on the Virginia Piedmont. Lee and Jose (2003) also found no significant effect of fertilization in *P. taeda*. Gough and Seiler (2004) found a significant (P<0.05) increase in soil respiration compared to control 4 days after applying fertilizer (rate 50 kg N $ha^{-1}$ and 106 kg P $ha^{-1}$; in the form of DAP) in 1-year-old loblolly pine seedlings grown in a greenhouse. Four to 13 days from fertilization, the authors found a significant (P<0.05) increase in soil respiration in fertilized treatments compared to control treatments. From day 13 to 47 (post-treatment), soil respiration rates reversed and were significantly (P<0.05) lower in fertilized pots compared to controls; and after day 47, fertilized pots were, again, significantly (P<0.05) higher than controls. Mattson (1995) measured three forested stands that had received three different N fertilization treatments (control, single, and double fertilized sites). The author measured $CO_2$ efflux as 29 percent lower in the 2x fertilized site when compared to the control. Similarly, Maier and Kress (2000) found a decrease in $CO_2$ efflux following N fertilization in an 11-year-old *P. taeda* stand.

This difference in responses could be due to a number of things. One proposed by Gough and Seiler (2004), and by Lee and Jose (2003), is that a decrease in heterotrophic respiration combined with an increase in total autotrophic respiration may have a cancellation effect on total C evolution at the soil surface. Other possible reasons are the rate of N fertilization and the time from initial fertilization that measurements were taken (Gough and Seiler 2004).

## CONCLUSIONS

Shortly after fertilization, heterotrophic respiration decreased relative to plots that received no fertilization and remained depressed throughout the 8 months measured. We were unable to find a consistent trend in $CO_2$ efflux between treatments. Fertilized plots were significantly (P<0.05) lower on a number of occasions, but later rose above control plots. Our hypothesis is that an increase in specific root respiration and/or root mass balanced the observable decrease in microbial activity leading to no significant (P<0.05) difference in $CO_2$ efflux over the course of the study.

## ACKNOWLEDGMENTS

We would like to thank John Peterson, Nathan King, and Erica Fritz for their assistance in data collection. Also, we would like to thank Roger Harris and Bob Jones for the use of their labs, and the Forest Biology Research Cooperative, University of Florida, Gainesville, FL, for donating the seedlings.

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