MICROBIAL RESPIRATION RESPONSE TO N AND P AVAILABILITY IN LOBLOLLY PINE FOREST SOILS

A Senior Scholars Thesis

by

JUSTIN M. WHISENANT

Submitted to the Office of Undergraduate Research
Texas A&M University
in partial fulfillment of the requirements for the designation as

UNDERGRADUATE RESEARCH SCHOLAR

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Major: Forest Management
Spatial Science

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Director for Honors and Undergraduate Research: Sumana Datta

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ABSTRACT

Microbial Respiration Response to N and P Availability in Loblolly Pine Forest Soils.
(April 2011)

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The complex cycle of nutrients and organic matter in the soil is one of the planet's vital processes for maintaining life. Microscopic organisms, or soil microbes, fill a crucial role in this process, as they decompose organic matter and recycle the nutrients back to forms that plants can readily use. Overall, the nutrient cycle is well understood, but certain aspects remain largely unexplored. Specifically, the effect of nutrient availability on the rate that soil microbes decompose organic matter requires further experimental inquiry. Managed pine forests in the Southeastern U.S. represent a good opportunity for studying the effects of nutrient additions because they commonly receive nitrogen and phosphorous fertilization. Nutrient concentrations in forest soils appear to influence the decomposition rates of organic matter in soils; therefore, fertilization of forest soils can change the rate at which CO_2 is released from the soil into the atmosphere. This experiment looked at the individual and combined effects of nitrogen and phosphorous additions on soil carbon cycling. We performed laboratory incubations on soil samples from two loblolly pine forests in Florida and measured the CO_2 respired by soil microbes

using an infrared gas analyzer. Our results indicate that the addition of nitrogen, alone and combined with phosphorous, to forest soils decreases the rate of soil microbial respiration and overall soil CO_2 efflux. Phosphorous additions alone also suppress respiration but to a lesser extent. This research further supports the idea that forests will be a key tool for ongoing climate change mitigation because certain management practices may increase forest carbon stores. Continued research in forest soil carbon stores is needed to develop specialized practices that maximize the ability of forests to sequester carbon.

ACKNOWLEDGMENTS

I thank my research advisor, Dr. Jason Vogel, for providing the materials and expertise that made this project possible. He was also very patient with my lack of experience and incessant inquisitiveness. I also thank my wife, Vicky, for her patience with my very busy schedule and heavy workload. Finally, I would like to thank the Undergraduate Research program at Texas A&M University for being so accommodating to a first time researcher like myself.

NOMENCLATURE

ACMF	Austin Carrie Municipal Forest site
C	Carbon
CO_2	Carbon dioxide
N	Nitrogen
P	Phosphorus
SAN	Sanderson site
SCE	Soil CO_2 efflux
SOM	Soil organic matter

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CHAPTER I

INTRODUCTION

Carbon (C) cycling in forests is an area of research interest that has gained more attention in recent years due in part to its relationship with atmospheric CO_2. The greenhouse gas CO_2 is the C input for forests and is captured through photosynthesis by plants. The final input of C to an ecosystem by plants is commonly known as net primary production (NPP). Some of this C, having first been converted to sugars, is returned to the atmosphere directly by the plants as a result of normal metabolism (i.e. respiration). Much of the remainder of C fixed by plants makes its way to the soil through various pathways. Root exudates, dead wood, and leaf or needle litter are a few examples of plant C that relocates to the soil.

Soil microbes gradually decompose all organic matter that reaches the soil as plant litter. The rate that C amasses in an ecosystem, in this case the soil, is called net ecosystem production (NEP) and is dependent upon C inputs from plant litter. It is important to note that the difference between the NEP and the NPP of an ecosystem is largely due to soil microbial respiration. Decomposition of soil organic matter (SOM) by microbes is a metabolic process that results in a release of CO_2 gas. Combined with the CO_2 emitted by plant roots, this is known as soil CO_2 efflux (SCE). This is the return stroke of the C

This thesis follows the style of Soil Biology & Biochemistry.

cycle that began with C capture during photosynthesis. Once respired by soil microbes, CO_2 then diffuses back into the atmosphere where it can be fixed by plants to begin the cycle anew.

This role of microbial SOM decomposition in the C cycle is a very important piece of the climate change puzzle. A change in the rate of SCE at large scales could have noticeable effects on the earth's climate. Because of this, researchers are now seeking to better understand what causes soil microbial respiration (i.e. SOM decomposition) rates to change and if these changes are anthropogenic in origin.

In 2007, Magnani et al. presented a paper that found nitrogen (N) additions to forest ecosystems were strongly linked with increased C storage in those systems (Magnani et al., 2007). For their experiment, they used multiple chronosequences to determine C stocks and fluxes for "newly harvested, young and mature stands" (2007). They compared that data with estimates of N deposition in the same areas and concluded that net C sequestration in these forests is directly proportional to the amount of N deposition. Moreover, they concluded that fertilization and atmospheric N deposition are results of human activities, so "mankind is ultimately controlling the carbon balance of temperate and boreal forests" (2007).

Nitrogen limitation to plant growth is the key to the mechanism underlying their explanation. The forests they measured were N limited, so the addition of N by deposition and fertilization led to more forest growth and increased C stocks (Magnani et

al., 2007). Nitrogen is the most commonly limiting macronutrient, so an increase in N supply to a forest will increase growth, which can be directly measured by observation of increased woody biomass. Changes in soil C sequestration are less obvious because of the differential response of soil microbes and plant roots to fertilization. Somewhat counterintuitively, N deposition increases soil C stores (Magnani et al., 2007; Janssens et al., 2010). This is because trees do not need to work as hard mining the soil for N with their roots. Less root production means less C inputs to the soil from root mortality. Besides the decrease in autotrophic respiration from decreased below ground root biomass, SCE may be reduced because the priming of microbial decomposers by root exudates also is decreased (Janssens et al., 2010).

The implications of this new understanding of C and N interaction were difficult to discern. A recent meta-analysis by Janssens et al. (2010) began to clarify some of what Magnani et al. (Magnani et al., 2007) were moving toward. The findings of the meta-analysis indicated that "nitrogen deposition impedes organic matter decomposition, and thus stimulates carbon sequestration in soils" (Janssens et al., 2010). Consequently, forests may increase the amounts of C in their soils proportionately to increases in N deposition from, primarily, air pollution. Notable exceptions where N deposition increased SCE are (1) in immature forest stands, where N deposition increased photosynthesis drastically and (2) in forests with artificially high CO_2 levels where N addition also increased photosynthesis (2010). The second exception is important because it may be a predictor of SCE response to future elevated global CO_2 concentrations.

The metabolic activity of heterotrophic soil microbes affects the amount of SOM and the concentrations of atmospheric CO_2 (Schlesinger, 1997). By decomposing the organic matter deposited by senescing plant tissues or animals, these soil microorganisms 'or heterotrophic microbes' ensure that nutrients will continue to be released in forms that living plants can use. Understanding the controls on SOM decomposition became the focus of intense research during the last several decades because, globally, SOM stores nearly 2x more C than the atmosphere (1997). This C storage occurs because soil microbes do not fully decompose some SOM to CO_2, which if they did, would dramatically increase atmospheric CO_2.

One direct means of estimating changes in SOM is through the measurement of microbial respiration. Increased carbon input as root or leaf litter, N and Phosphorus (P) availability can all increase microbial respiration because these are essential elements for life processes; however, nutrient availability can also suppress microbial respiration because of changes in microbial community structure or the function of specific enzymes (Janssens et al., 2010). With fertilization, increased SOM has been widely observed in forest ecosystems (Johnson and Curtis, 2001), yet it is unclear if this occurs because of changes in microbial respiration or increased inputs from litter.

Southern pine forests are commonly fertilized with N and P and thus could provide insights into how microbial respiration responds to varying nutrient availability. Although increased above-ground growth with fertilization could increase SOM storage,

the effect of heavy fertilization on microbial respiration is unknown in these forests. In previous experiments, anthropogenic N additions to soil have typically, but not always, led to decreased microbial respiration in forest soils (Janssens et al., 2010). Fewer studies have examined P, or the additive effect of N and P on microbial respiration (Thirukkumaran and Parkinson, 2000).

Project description

Dr. Jason Vogel, the faculty advisor for this project, previously collected the soil to be used for this experiment from forest research sites in Florida. The sites are part of an experiment examining the effects of fertilization and genetic selection of loblolly pine on the C cycling of soils. The laboratory experiment for this project supplemented in-situ measurements of root biomass, root respiration, and microbial respiration. There were 2 sites in Florida being examined, and at each site, 2 levels of previous fertilization and 4 replicate blocks (16 plots total). Trees at the two sites were 9 and 10 years old at the time of soil collection. With this lab experiment, we determined whether microbial respiration and biomass were enhanced by nutrient additions similar to those made during the in-situ experiment (Thirukkumaran and Parkinson, 2000). If microbes were limited by either N or P, the addition of this nutrient with fertilization should increase microbial respiration, and if microbes were limited by C availability, the addition of N or P should have no effect on microbial respiration.

CHAPTER II

METHODS

Samples for this experiment were taken from two loblolly plantations in Florida. The two sites, henceforth referred to as ACMF and SAN, were part of a larger experiment by Dr. Jason Vogel et al. that sought to explore the effects of forest management practices on nutrient cycling with particular respect to the influence of N and P additions to C cycling and how it varied with different loblolly pine families. After soil cores were collected in the spring of 2010, they were cold-shipped to Texas A&M in College Station, Texas and immediately frozen. Samples were divided by genetic horizons, OeOa and A_1, for this experiment. They were individually thawed and coarse wood, needle litter, and live and dead roots removed. Since this initial preparation, the soils remained frozen or refrigerated.

For this laboratory incubation, the soils were removed from cold storage and 3 to 5 replicates, depending upon plot, placed in 237-mL canning jars that had two stopcocks in an airtight lid from which to sample microbial respiration. For each jar, 2.5 cm of the A_1 horizon were added followed by 1.5 cm of OeOa horizon. In this manner, each jar simulated the forest floor from the site and plot of its origin. Mass was measured for A_1, OeOa, and the filled jar. Mass was measured again at the end of the experiment and the difference used to calculate the total C respired by microbes. Next, the pH for both horizons of each plot was measured as described in *Methods of Soil Analysis* (Sparks, 2005). The soils from each plot having low levels of field fertilization had N (as NH_4Cl)

and P (as triple superphosphate) added to the soil both singly and together in amounts equivalent to the fertilization on the high fertilization plots. The jars fertilized in the lab were compared to the controls for the low in-situ fertilization, and jars containing soil from the high fertilization plots. A total of 48 jars were monitored throughout the experiment.

Samples for the lab incubation corresponded to four plots from SAN and eight plots from ACMF. Each SAN plot was distributed amongst 5 jars. The two controls for each SAN plot were a high in-situ fertilization and a low in-situ fertilization. The remaining 3 jars were combinations of lab fertilizations meant to replicate in-situ levels of N and P additions. They were high N and high P addition (540N, 100P), high N and no P addition (540N, 0P), and no N and high P addition (0N, 100P).

Two levels of field fertilization also represented the ACMF site. Four plots with high fertilization levels were used with each plot having a control, high N and high P addition (310N, 80P), and high N and no P addition (310N, 0P). Four plots with mid-level field fertilization were represented by 4 jars with a control, high N and high P addition (170N, 60P), high N and no P addition (170N, 0P), and no N and high P addition (0N, 170P).

Capping process

Prior to each measurement, jars were scrubbed of CO_2 and allowed to sit for several hours. During this time, microbially respired CO_2 accumulated in the jars. To cap the jars, both stopcocks in each jar lid were left open and air was pumped through them for

30 seconds. Between the air compressor and the jars was a CO_2 filter that removed all CO2, so the jars would start each incubation from a near zero concentration of CO_2. At the end of thirty seconds the stopcocks were closed sealing the jars for the duration of the incubation. Following the baseline respiration measurements, jars were fertilized with N and P according to Appendix 1. Control jars received no fertilization.

CO_2 measurement

Using the access ports on the jar lids, microbial respired CO_2 was extracted from the headspace of the jar with a 30-ml syringe and filtered CO_2-free air drawn into the jar to replace the volume removed. The CO_2 collected in the syringe was then injected into an infra-red gas analyzer (LI-820, Licor Biosciences, Lincoln NE) attached to a Vici Valco Instruments (Houston, TX) sampling loop. At the beginning and end of the experiment, soil pH also was determined.

CHAPTER III

RESULTS

Before the experiment began, we hypothesized that N and P additions affect soil microbial respiration rates negatively, so adding these nutrients would lower the CO_2 efflux rates. The results of the CO_2 measurements from the lab incubation correlated favorably with the predicted results.

Microbially respired CO_2, for all samples from the SAN site, remained at a relatively steady state for the four measurements preceding fertilization (Fig. 1). At ACMF, the initial measurement was high but steadied for the three subsequent pre-fertilization measurements (Fig. 1). Following the first measurement, average deviation from the control respiration measurements remained within 2.2% for all samples from both sites during the pre-fertilization period.

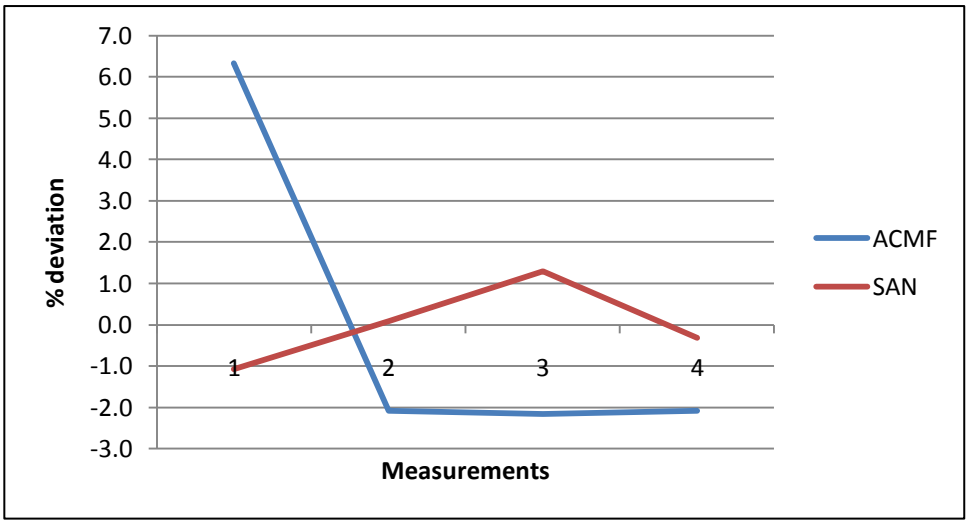

Figure 1
Pre-fertilization % deviation from average respiration of control samples

Following fertilization, CO_2 respiration rates spiked for all plots while the controls continued at a relatively steady rate (Figs. 2A and 2B). Samples fertilized with N alone or N combined with P spiked the most while the P only fertilization increased respiration by half as much.

For the two months following fertilization, control samples remained fairly steady with individual respiration rates typically well under 10% deviation from control averages. The fertilized samples all measured a distinct decrease in respiration following fertilization. Nitrogen addition alone had the lowest respiration rate but was closely mirrored by the N and P combined addition. The rates of respiration for N and for N and P together were -45.9% and -46.9%, respectively, by the end of the experiment for SAN (Fig. 2A). The addition of P alone suppressed respiration as well but less significantly than additions that included N. P alone finished at -27.3% off the control respiration average.

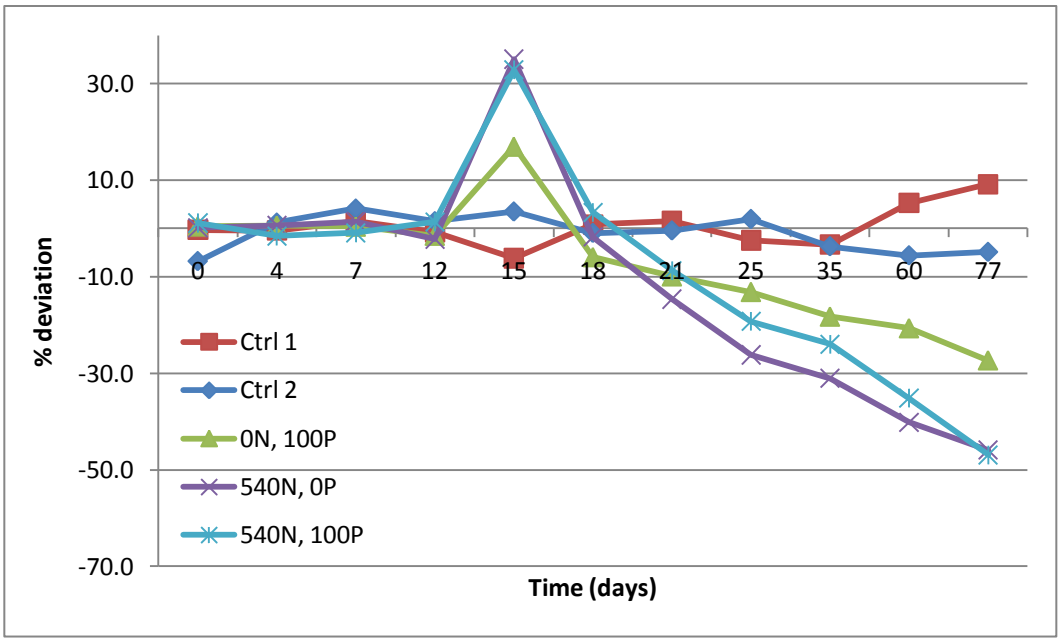

Figure 2A
Deviation (%) from average control sample respiration rate at SAN

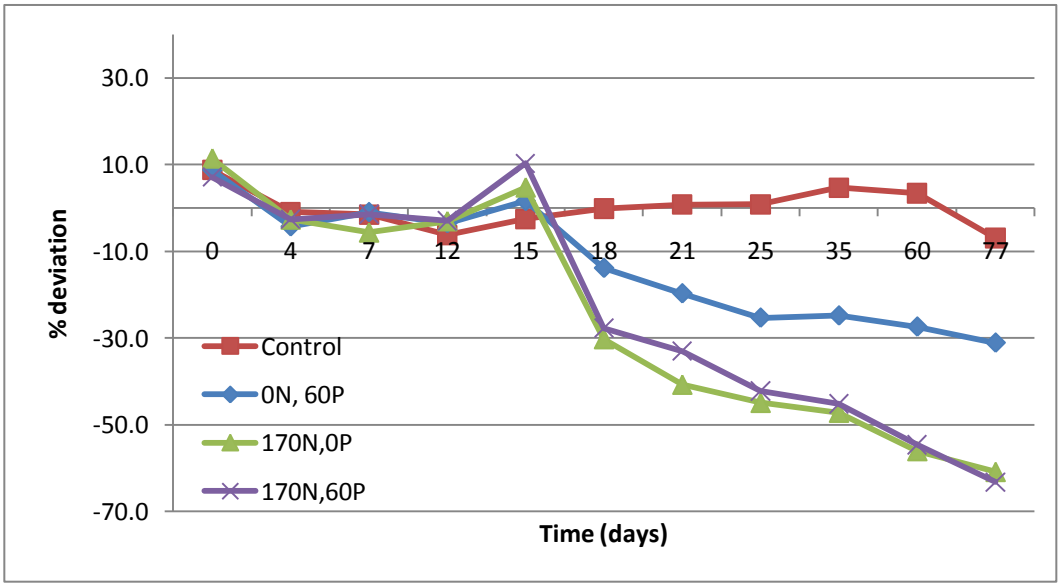

Figure 2B
Deviation (%) from average control sample respiration rate at ACMF

Looking at the data with respect to total C respired (Fig. 3) shows similar suppressive effects to what figures 2B and 2b showed. For all three sites, the highest amounts of C respired came from samples receiving no additional fertilization (i.e. controls). The lowest amounts of C respired came from samples with the maximum amount of N addition and no P addition. Combined N and P addition respired more C than N addition alone. P addition alone showed similar respiration amounts to N and P addition.

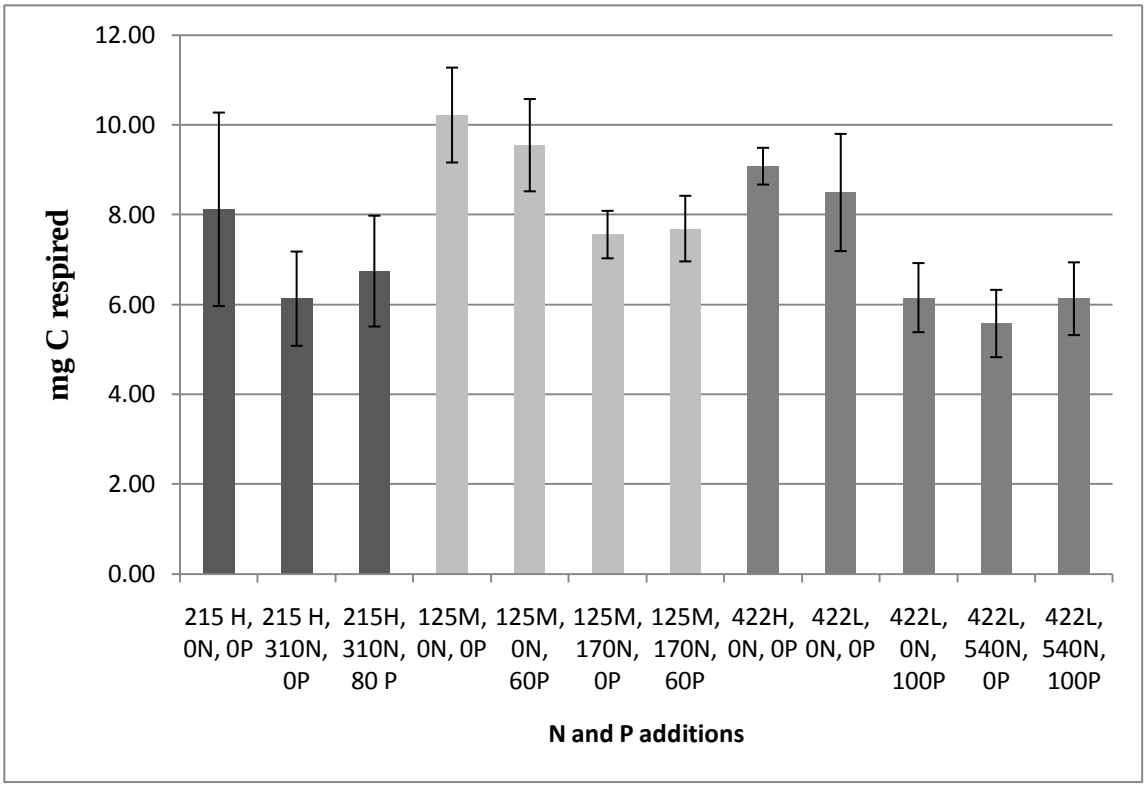

Figure 3
Total C respired

CHAPTER IV

CONCLUSIONS

This experiment sought to explore the effects of N and P fertilization on the rate of CO_2 respired by soil microbes. We believed that measurements from the lab incubations would mirror those from field measurements where fertilized plots produced lower SCE readings than plots receiving less or no fertilization. Lab incubations allowed the microbial portion of SCE to be isolated. We added N and P in amounts that mirrored those in the field and found the results reinforced our hypothesis. N and P additions, alone or in combination, suppress microbial respiration. This decrease is completely separate from SCE decreases that result from lowered root growth and respiration resulting from fertilization.

The mechanisms behind the decrease in microbial respiration with fertilization are still poorly understood. The literature presents several explanations; any combination of which may explain what we observed in this experiment. Soil microbes may react to the increase in available N and P by switching their focus from respiration to reproduction which would decrease measured respiration. The presence of additional nitrogen also may inhibit production of specific enzymes, and thus decrease the rate at which soil microbes can decompose organic matter (Fog, 1988). This too would decrease respiration. A final explanation for decreased respiration may be that the populations of microbes change with the different concentrations of N and P. Those present under

higher concentrations may be more efficient but previously limited by low available quantities of N (Janssens et al., 2010).

Implications

Decreased microbial respiration, due to fertilization, suggests managed forests will have greater levels of soil carbon storage than unfertilized forests. Until recently, many studies of fertilized forests (Janssens et al., 2010) attributed decreased soil respiration to decreases in root growth alone with the primary hypothesis being that fertilized soils do not require the tree roots to extensively mine the soil because nutrients can be attained more easily in fertilized soils. Fewer roots would result in less C input to the soil and potentially reduced soil carbon storage (2010). However, this project found suppressed microbial respiration with fertilization could counteract decreased root growth.

Specialized practices may be developed that maximize both tree growth and the ability of forests to sequester C in the soil. Notably, while N additions in this experiment reflected common managed forest fertilization levels, anthropogenic air pollution causes more widespread deposition of N in the world's forests. Many forests near urban areas receive N originating from industrial and transportation emissions (Denman, 2007). These additions are much more widespread than forest fertilization and could affect microbial respiration similar to the fertilization study reported here although it is unclear how much N is needed to suppress microbial respiration. Increased understanding of the influence of intentional fertilization on soils may eventually be extended to those forest soils receiving added N from air pollution.

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APPENDIX

Appendix 1

Fertilization (equivalent to kg ha-1) applied in-situ and addition fertilizer applied in-lab incubation. H, M, and L represent high, medium, and low quantities respectively.

Jar #	Site	Plot	In-situ Fertilization	Lab Fertilization	Jar #	Site	Plot	in situ Fertilization	Lab Fertilization
1	SAN	134	H (760 N, 180 P)	CTRL	25	ACMF	336	H (450 N, 100P)	310N, 80P
2	SAN	134	L (220 N, 80 P)	CTRL	26	ACMF	336	H (450 N, 100P)	310N, 0P
3	SAN	134	L (220 N, 80 P)	540N, 100P	27	ACMF	365	H (450 N, 100P)	CTRL
4	SAN	134	L (220 N, 80 P)	540N, 0P	28	ACMF	365	H (450 N, 100P)	310N, 80P
5	SAN	134	L (220 N, 80 P)	0N, 100P	29	ACMF	365	H (450 N, 100P)	310N, 0P
6	SAN	334	H (760 N, 180 P)	CTRL	30	ACMF	215	H (450 N, 100P)	CTRL
7	SAN	334	L (220 N, 80 P)	CTRL	31	ACMF	215	H (450 N, 100P)	310N, 80P
8	SAN	334	L (220 N, 80 P)	540N, 100P	32	ACMF	215	H (450 N, 100P)	310N, 0P
9	SAN	334	L (220 N, 80 P)	540N, 0P	33	ACMF	256	M (50 N, 60P)	CTRL
10	SAN	334	L (220 N, 80 P)	0N, 100P	34	ACMF	256	M (50 N, 60P)	170N, 60P
11	SAN	122	H (760 N, 180 P)	CTRL	35	ACMF	256	M (50 N, 60P)	170N, 0P
12	SAN	122	L (220 N, 80 P)	CTRL	36	ACMF	256	M (50 N, 60P)	0N, 60P
13	SAN	122	L (220 N, 80 P)	540N, 100P	37	ACMF	346	M (50 N, 60P)	CTRL
14	SAN	122	L (220 N, 80 P)	540N, 0P	38	ACMF	346	M (50 N, 60P)	170N, 60P
15	SAN	122	L (220 N, 80 P)	0N, 100P	39	ACMF	346	M (50 N, 60P)	170N, 0P
16	SAN	422	H (760 N, 180 P)	CTRL	40	ACMF	346	M (50 N, 60P)	0N, 60P
17	SAN	422	L (220 N, 80 P)	CTRL	41	ACMF	225	M (50 N, 60P)	CTRL
18	SAN	422	L (220 N, 80 P)	540N, 100P	42	ACMF	225	M (50 N, 60P)	170N, 60P
19	SAN	422	L (220 N, 80 P)	540N, 0P	43	ACMF	225	M (50 N, 60P)	170N, 0P
20	SAN	422	L (220 N, 80 P)	0N, 100P	44	ACMF	225	M (50 N, 60P)	0N, 60P
21	ACMF	166	H (450 N, 100P)	CTRL	45	ACMF	125	M (50 N, 60P)	CTRL
22	ACMF	166	H (450 N, 100P)	310N, 80P	46	ACMF	125	M (50 N, 60P)	170N, 60P
23	ACMF	166	H (450 N, 100P)	310N, 0P	47	ACMF	125	M (50 N, 60P)	170N, 0P
24	ACMF	366	H (450 N, 100P)	CTRL	48	ACMF	125	M (50 N, 60P)	0N, 60P

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