

# SOIL RESPIRATION RESPONSES TO NITROGEN ADDITIONS AT THE FALMOUTH WASTEWATER TREATMENT FACILITY

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*Abstract.* It is generally assumed that N fertilization should increase soil respiration in most temperate forests because productivity is typically N limited. However, the past three years of measurements at the Falmouth Wastewater Treatment Facility have shown oak control soil respiration to be higher than in oak fertigated plots. The pine fertigated plot respiration rates have been only slightly higher than the control plots. Although N mineralization and nitrification is higher in fertigated plots, laboratory soil respiration measurements show higher respiration rates in control plots for both oak and pine. Possible explanations for the high rates of respiration in the control plots include higher root biomass and microbial activity.

*Key Words:* *fertigation; nitrification; nitrogen mineralization; respiration; soils.*

## INTRODUCTION

Soil respiration is the total of all CO<sub>2</sub> producing activities in the soil including microbial respiration, root respiration, faunal respiration, and chemical oxidation (Singh, 1997). Because CO<sub>2</sub> is produced during decomposition, soil respiration reflects the decomposition rate. Decomposition is linked to the rate of mineralization of nutrients, which in turn affects the quantity of nutrients available for uptake by plants.

Changes in the soil respiration rate affect nutrient storage and the effectiveness of the terrestrial environment as a CO<sub>2</sub> sink. Because of the implications of soil respiration on the carbon cycle, respiration is crucial to understanding ecosystems and global cycles. Despite its importance, many aspects of soil respiration are unknown, and more studies and more accurate estimation techniques are needed. Results of research studying the response of soils to nitrogen addition have varied, and determination of root and microbial contributions to soil respiration has been difficult (Bowden et al., 1993).

Several factors have been discovered to affect soil respiration including moisture and temperature, soil pH, soil nitrogen content, litter quality and content, forest development and soil organic matter content, and management practices (Bowden et al., 1993). Understanding soil characteristics that affect respiration may help explain unexpected soil respiration rates.

Fertigated plots at the Falmouth Wastewater Treatment Facility have higher nitrogen inputs due to the spraying of treated sewage effluent on the plots. Because of added nutrients, treated plots have higher NPP and litter inputs. In addition to the increased availability of decomposable materials in fertigated plots, litter with higher nutrient concentrations is expected to decompose faster, and net mineralization should begin earlier (Schlesinger, 1997). Therefore, higher respiration rates were expected in the fertigated plots. However, during the past three years, students in the Semester in Environmental Science Program at the Marine Biological Laboratory have measured soil

respiration and discovered the respiration rates are often higher in the control plots than in the fertigated plots (Table I).

The goal of the experiment was to determine if the trend of high respiration rates in control plots accurately reflect the soil respiration rates, and the reasons soil respiration is greater in one type of plot than in another. The results improve our understanding of the role of forests in carbon and nutrient storage and also indicate possible future effects of increased nutrient loading.

## MATERIALS AND METHODS

### *Study sites*

Control and fertigated plots already established at the Falmouth Wastewater Treatment Facility in Falmouth, Massachusetts were used for the study. In October 1996, the plant began operation, and starting in June 1988, the treated wastewater was sprayed on the forests from April to December. During the remaining months, infiltration was used to dispose of sewage. (Jordan, 1997) Two types of forests were sprayed: a 36 year old pitch pine forest and a mixed oak-pitch pine forest that is more than seventy years old (Jordan, 1997). For accurate comparisons between plots, the selected control plots are adjacent to the fertigated plots.

### *Field Studies*

In the field, a LiCor infrared gas analyzer (IRGA) was used to determine the surface soil respiration. The technique is similar to that used by Thierron (1996). Four surface soil respiration measurements were taken in control and fertigated, oak and pine plots for a total of 16 measurements. The pine respiration measurements were taken over two days. For the comparisons made between pine plots, only data taken on the second day was used. Heavier weights were placed on the LiCor respiration chamber on the second day of respiration measurements.

Sixteen samples per forest type were collected for four analyses performed by horizon. The O horizon was sampled by cutting a 10cm x 10cm slice from the ground. A corer with a 1.9 cm diameter was used to sample 0-10cm and 10-20cm mineral horizons. Because of the rockiness of the pine soils, mineral cores could not be taken. Samples from each mineral layer were taken with the use of a shovel.

### *Nitrogen Analyses and Incubations*

Initial analysis of exchangeable nitrogen was done on 48 samples (2 samples from each horizon in each plot) by KCl extraction (Sparks, 1996). 5g of wet organic soil and 10g of wet mineral soil were placed in a plastic jar, and 50mL of 1 M KCl was added. The bottle was shaken for 1 h on a shaking table. Within several hours of the extraction, the supernatant was decanted and filtered using gravity filtration. The filtrate was frozen for later analysis. During the freezing process, the scintillation vials broke and may have been exposed to some contamination.

Samples of the collected soils were placed in glass Ball jars for incubation and loosely fitted with lids to allow aeration. After incubating the samples at 15-16°C for 13-14 days in a Ball Jar, soil respiration measurements and KCl extraction were done. Measurements of CO<sub>2</sub> flux for the incubated samples were made by attaching the LiCor

to the Ball jar in which the soils were incubated. In addition, the final 48 KCl extractions were completed on the incubated samples.

The procedure used to determine ammonium content from the KCl extracts is similar to that described by Solorazano (1969). Samples were diluted in a 1:5 ratio and treated with alkaline citrate medium, sodium hypochlorite, and phenol. Sodium nitroprusside is used as a catalyst. Blue indophenol is formed and absorbance is measured using a spectrophotometer. Concentration is calculated with a standard curve.

Nitrate from KCl extracts was measured by using a Lachat Flow Injection Analyzer (FIA) QuikChem 8000 machine which uses a similar method to that described by Wood et al. (1967). Nitrate in the sample is converted to nitrite with a cadmium reduction column. After the sample passes through a reaction manifold, color is produced and concentration calculated.

#### *Additional measurements*

In addition, moisture content, bulk density, and root mass, and the C:H:N content were determined. Moisture content and bulk density were determined by weighing samples of a known volume before and after drying. Root content by horizon was measured by weighing dried roots that had been separated from organic matter. However, due to the great abundance of roots in the organic horizon, and the small number of roots in the mineral horizon, a greater percentage of roots were weighed from the mineral horizons than from the organic horizon. Using a Perkin Elmer C:H:N analyzer, percent carbon and nitrogen in the samples were determined.

The soil pH, and bulk density for the pine plots were taken from the data found during the Semester in Environmental Science's Forest Plant and Soil C and N Balances Lab .

## RESULTS

	Class 1998 Data		Class 1997 Data		Class 1999 Data		2nd 1999 Data	
	By Plot	By Trt/Forest Type	By Plot	By Trt/Forest Type	By Plot	By Trt/Forest Type	By Plot	By Trt/Forest Type
OC1	276	<b>242</b>	163	<b>167</b>	212	<b>186</b>	66	<b>53</b>
OC2	207		171		161		60	
OF1	207	<b>200</b>	70	<b>88</b>	105	<b>117</b>	33	<b>43</b>
OF2	193		106		129		53	
PC1	235	<b>200</b>	96.5	<b>104</b>	169	<b>118</b>	68	<b>92</b>
PC2	166		112		68		117	
PF1	181	<b>208</b>	95.5	<b>131</b>	110	<b>145</b>	101	<b>105</b>
PF2	235		167		180		110	

Table I: Soil respiration data for control and fertigated forest plots during the last three years

During the past three years, soil respiration has been higher in the control oak forest than in the fertigated oak forest while fertigated pine plots have had consistently higher respiration rates than the control (Table I). Data collected for this experiment is labeled 2<sup>nd</sup> 1999 Data.

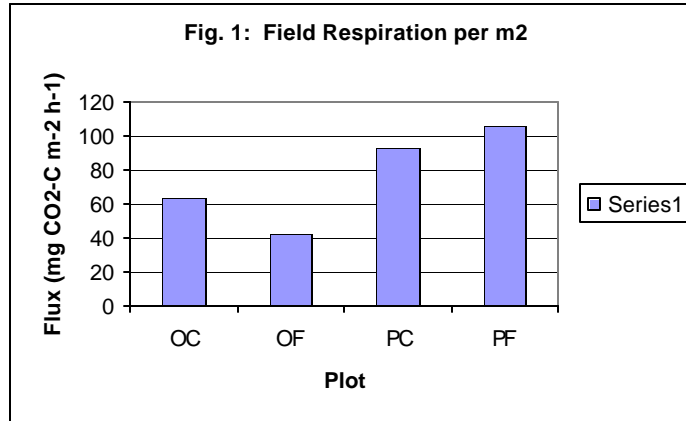


Fig. 1: Field respiration measured per m2 per g C.

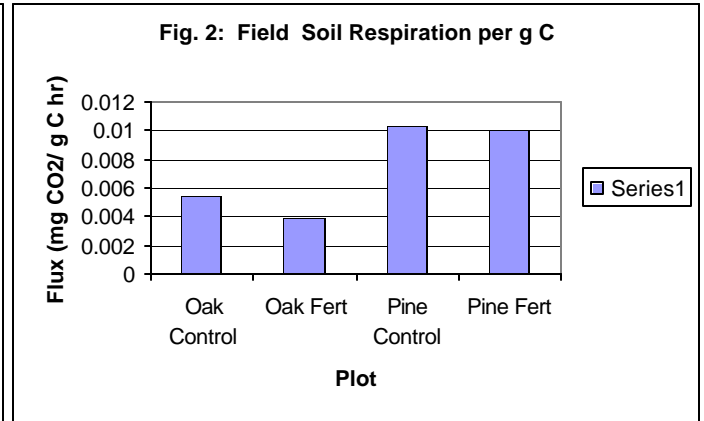


Fig. 2. Field respiration measured

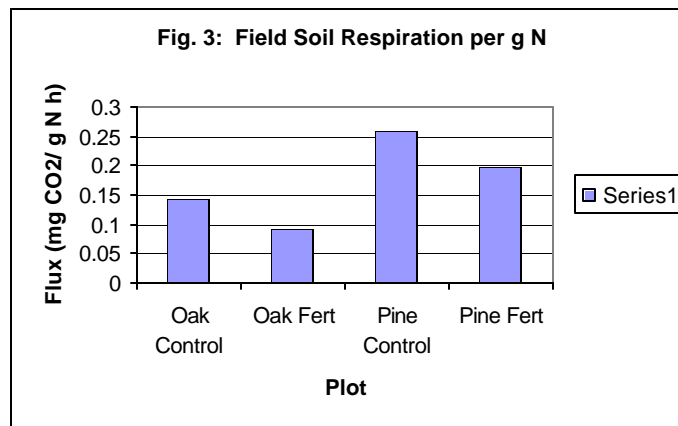


Fig. 3. Field respiration measured per g N.

For the field experiments, soil respiration was found to be slightly higher in the oak control plot ( $53 \text{ mg CO}_2\text{-C m}^{-2} \text{ hr}^{-1}$ ) than the fertigated plot ( $43 \text{ mg CO}_2\text{-C m}^{-2} \text{ hr}^{-1}$ ). In the pine plots, the fertigated plots showed slightly higher respiration rates ( $105 \text{ mg CO}_2\text{-C m}^{-2} \text{ hr}^{-1}$ ). The pine plots showed slightly higher respiration than the fert plots (Fig. 1). However when the units were converted from  $\text{mg CO}_2\text{-C m}^{-2} \text{ hr}^{-1}$  to  $\text{mg CO}_2\text{-C /g C h}$  respiration was practically the same for pine control and fert. When the units are expressed in  $\text{mg CO}_2\text{-C/g N h}$ , respiration is higher in the control plots than in the fertigated for both forest types (Fig. 2&3). With the exception of the 2<sup>nd</sup> 1999 data set, during the past three years, the oak forest soil respiration has been higher than the pine. Pine fert respiration ( $48 \text{ mg CO}_2\text{-C m}^{-2} \text{ h}^{-1}$ ) on the first day of measurements was comparable to the oak plots (oak fert:  $43 \text{ mg CO}_2\text{-C m}^{-2} \text{ hr}^{-1}$ , oak control:  $63 \text{ mg CO}_2\text{-C m}^{-2} \text{ h}^{-1}$ ). However, pine respiration on the first day was not used since it is not comparable to respiration on the second day.

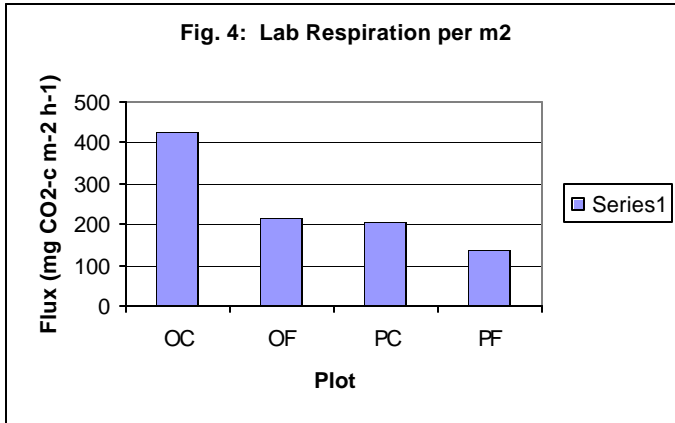


Fig. 4. Incubation respiration per m2.

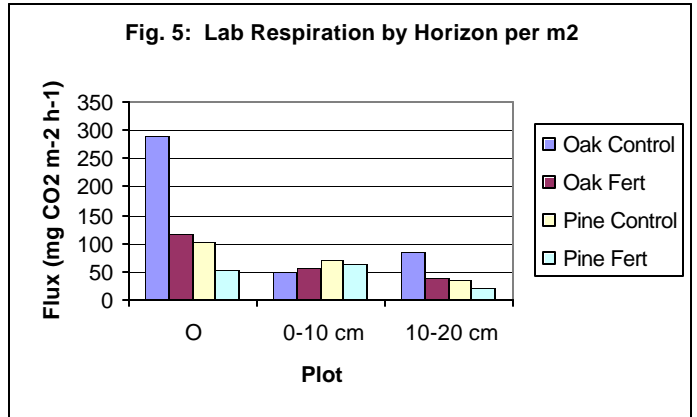


Fig. 5. Incubation respiration by horizon

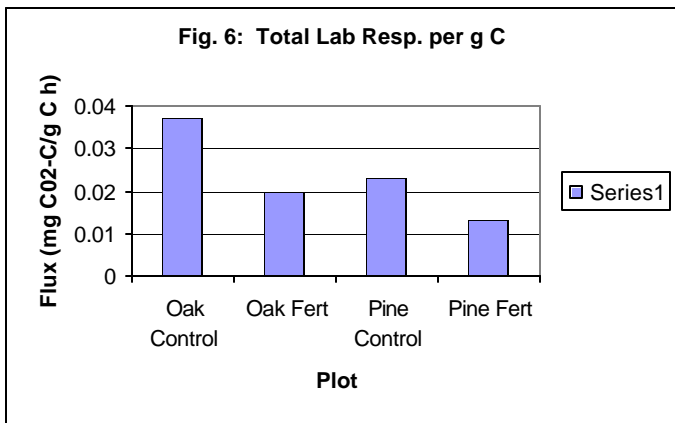


Fig. 6. Incubation respiration per g C.

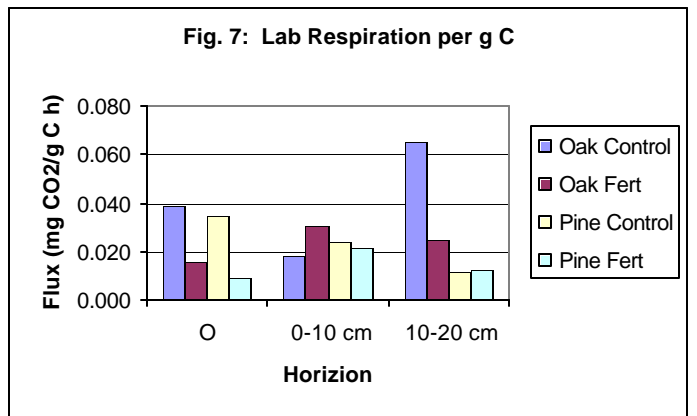


Fig. 7. Incubation respiration per g C

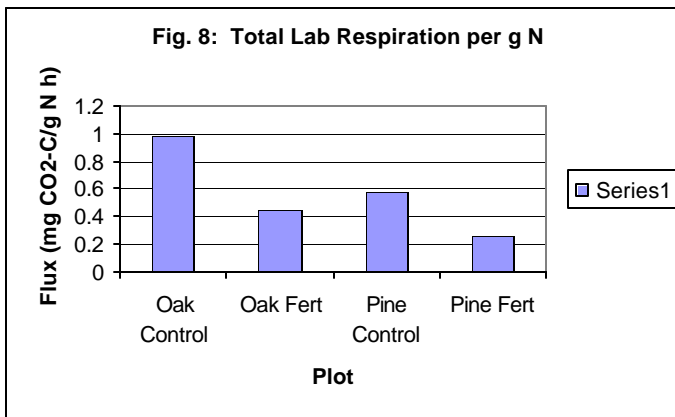


Fig. 8 Incubation respiration per g N.

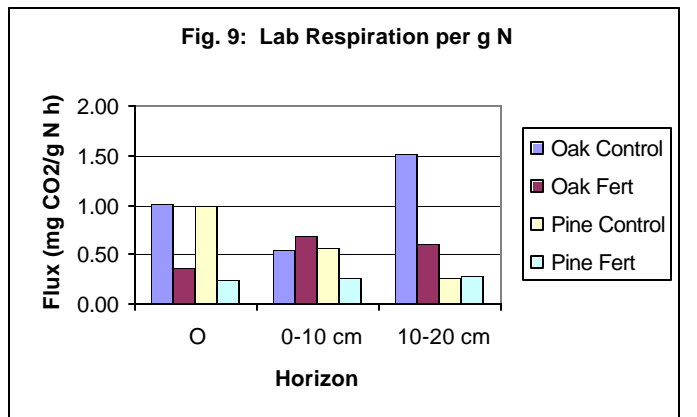


Fig. 9. Incubation respiration per g N

After two weeks of incubation, the soil respiration measurements in the lab show the CO<sub>2</sub> flux to be higher in control than the fertigated plots for both types of forest whether the units were in mg CO<sub>2</sub>-C m<sup>-2</sup> hr<sup>-1</sup>, CO<sub>2</sub>-C/g C h, or mg CO<sub>2</sub>/gN h (Fig. 4, 6 & 8). In addition, oak samples had higher respiration rates than the pine plots (Fig. 3). For all types of soils, the organic horizon had significantly higher respiration rates than the mineral soils. The 0-10cm mineral soils had higher respiration rates than the 10-20 cm horizons (Fig. 4). When respiration rates (mg CO<sub>2</sub>-C m<sup>-2</sup> h<sup>-1</sup>) are considered by horizon, the organic horizon has the highest respiration rate. However, when respiration rates are considered by horizon, there is no clear trend.

Plot	Horizon	%C	%N	C:N molar ratio	C (g m <sup>-2</sup> )	N (g m <sup>-2</sup> )
Oak Control	O	46.90	1.77	31.2	7453	288
Oak Control	0-10 cm	3.07	0.10	34.7	2755	93
Oak Control	10-20 cm	1.14	0.05	28.0	1326	57
<b>Total</b>					<b>11534</b>	<b>437</b>
Oak Fert	O	31.02	1.35	26.9	7473	325
Oak Fert	0-10 cm	1.45	0.07	25.7	1860	84
Oak Fert	10-20 cm	1.23	0.05	28.4	1601	65
<b>Total</b>					<b>10934</b>	<b>474</b>
Pine Control	O	32.64	1.16	33.3	2958	102
Pine Control	0-10 cm	2.96	0.13	28.7	3002	127
Pine Control	10-20 cm	2.08	0.09	26.6	3022	126
<b>Total</b>					<b>8981</b>	<b>355</b>
Pine Fert	O	41.03	1.56	31.1	5839	220
Pine Fert	0-10 cm	2.17	0.17	23.4	3031	238
Pine Fert	10-20 cm	1.21	0.05	26.3	1656	73
<b>Total</b>					<b>10526</b>	<b>531</b>

Soil carbon content (11,534 g C m<sup>-2</sup>) was highest in the oak control plot, while nitrogen content was higher in the fertigated (474 g N m<sup>-2</sup>) than in the control (10934 g N m<sup>-2</sup>). Pine fert (10,526 g C m<sup>-2</sup>) had a higher soil carbon content than pine control (8981 g C m<sup>-2</sup>). (Table II). Soil carbon and nitrogen content tended to decrease with deeper horizons in all plots except for the pine control plot where soil carbon was approximately the same for all horizons (Table II).

Table III:

<b>Comparison of Soil Horizon pH for Pine and Oak Control Plots</b>				
Horizon	PC 1	PC 2	OC 1	OC2
O	5	4.74	3.7	4.32
A	N/A	4.34	4.3	5.37
A1	4.6	N/A	N/A	N/A
B1	5.3	5.05	5	N/A
B2	5	5.18	4.9	N/A
E	4.8	N/A	4	N/A

Table IV:

<b>Comparison of Soil Horizon pH for Pine and Oak Fertigated Plots.</b>				
Horizon	PF 1	PF2	OF1	OF2
Top O	4.6	5.29	3.7	4.756
Buried O	N/A	5.19	N/A	N/A
A	4.7	5.25	4.1	6.75
B1	6.1	5.54	5.4	N/A
B2	5.6	N/A	4.8	N/A
E	N/A	5.19	4.7	N/A

Tables III & IV. The pH from SES 1999 Forest Plant and Soil C and N Balances Lab.

The pH of the soil horizons is higher in the pine than in the oak, with only insignificant variation (Tables III & IV). The pH of the fertigated plots is generally higher than the control plots (Table III & IV).

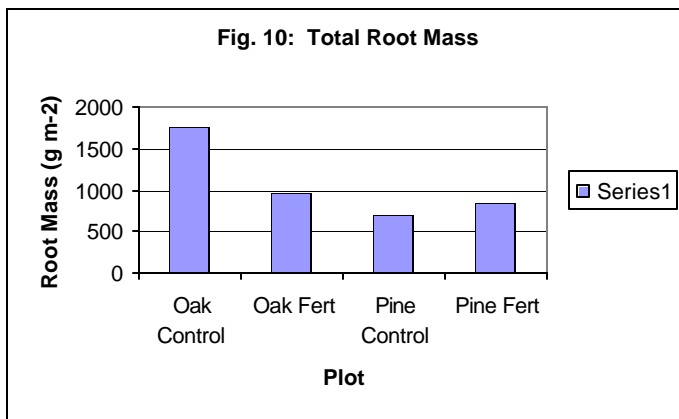


Fig. 10: Average total root mass for each plot.

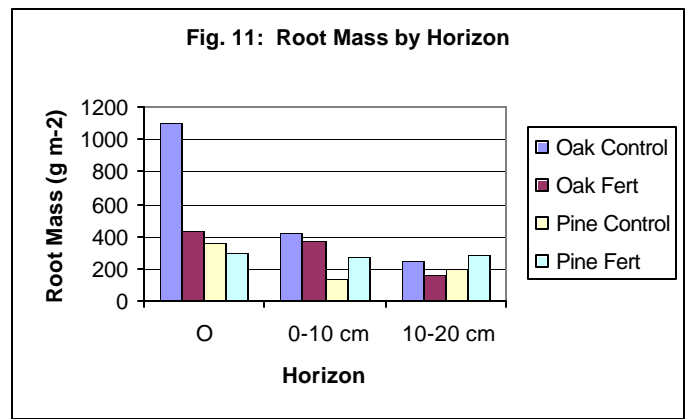


Fig. 11. Average root mass measured by horizon.

Varying results for the root mass was found. Total root mass was found to be higher in the oak control plot (1768 g roots m<sup>2</sup>) than in the oak fert plot (956 g roots m<sup>-2</sup>). In the pine plots, root mass was higher in the fert (843 g roots m<sup>-2</sup>) than in the control plot (691 g roots m<sup>-2</sup>).

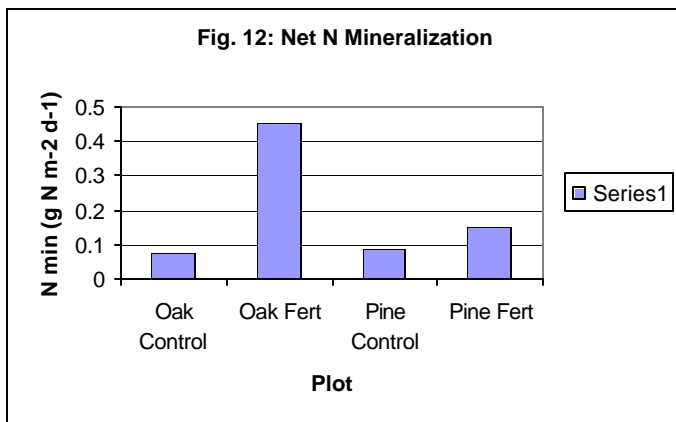


Fig. 12. Net mineralization after 2 week incubation.

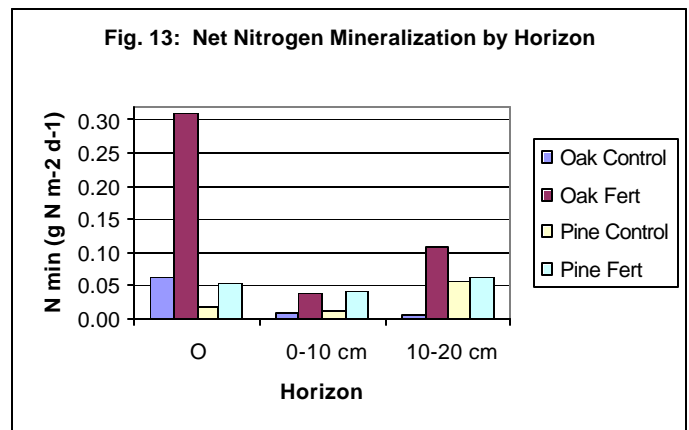


Fig 13. Net mineralization broken down by horizon.

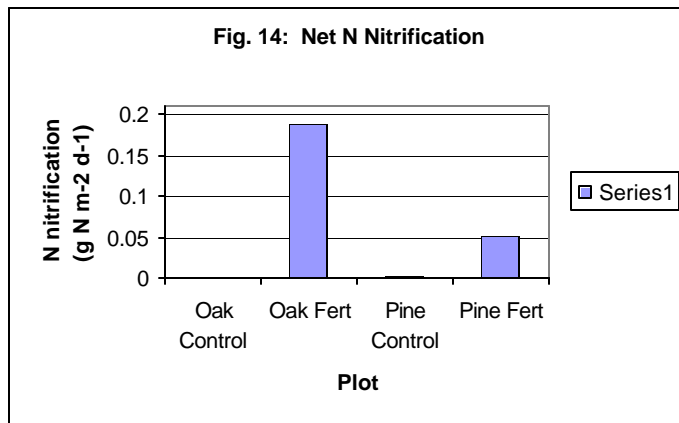


Fig 14. Net nitrification after 2-week incubation.

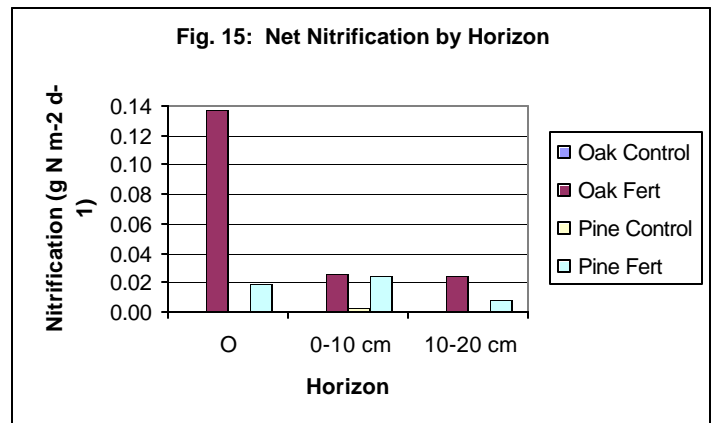


Fig. 15 Net nitrification broken down by horizon.

Soil nitrogen, nitrogen mineralization and nitrification were higher in the fertiligated plots than in the control plots (Fig. 9, 11, 13), and usually higher in the organic layer than the mineral layers (Fig. 10, 12, 14). Net mineralization and nitrification were highest in the oak fert ( $0.455 \text{ g N m}^{-1} \text{ d}^{-1}$  mineralized and  $0.190 \text{ g N m}^{-1} \text{ d}^{-1}$  nitrified) compared to a range of  $0.076\text{-}0.154 \text{ g N m}^{-1} \text{ d}^{-1}$  for mineralization in other plots and  $0.0002 - 0.052 \text{ g N m}^{-1} \text{ d}^{-1}$  for nitrification in other plots.

## DISCUSSION

During the past three years, oak and pine forests have shown different soil respiration responses to fertiligation. The field soil respiration measurements for the 2<sup>nd</sup> 1999 data set continued to show the same trends as those found in the past. Oak control plots had higher respiration than the oak fert plots while pine fert plots had slightly higher respiration than pine control plots when the units were expressed in  $\text{mg CO}_2\text{-C m}^{-2} \text{ h}^{-1}$ . The respiration rates for all plots were lower for the 2<sup>d</sup> 1999 data set than previous measurements probably due to the fact that the measurements were taken later in the year, when temperatures were lower. Unlike the measurements taken during the previous experiments, pine respiration rates are much higher than oaks than pines. However, pine respiration rates may be higher because they were taken the day after the oak rates when the weather was warmer (approximately  $8^\circ\text{C}$  on the 1<sup>st</sup> day and  $13^\circ\text{C}$  on the 2<sup>nd</sup>). In addition, the workers the second day were more experienced and used heavier weights to keep the respiration chambers in place which may have lead to less  $\text{CO}_2$  escaping from around the edges.

Field respiration data and incubation respiration calculated per g C, and especially per g N, show that when the availability of carbon or nitrogen increases, respiration does not increase proportionally. More carbon was found to be available in the oak control and the pine fert plots (Table II). However, higher carbon content in the pine fert plot may not be statistically significant since in the SES lab, it was found to be lower. In both oak and pine plots, more nitrogen is available in the fertiligated plots. If nitrogen were limiting to decomposition and had no negative effects on respiration, and increase in N would predict an equally high increase in respiration. Similar respiration per g N and C

in every horizon indicates that lower respiration rates in the mineral layers are due to limited nutrients.

The lab incubation data is likely to be more reliable since all the measurements were taken under similar conditions and the jar used for the measurements could be tightly sealed to prevent CO<sub>2</sub> escape. This may indicate that respiration is higher in both the pine and the oak control plots than in the fertigated. However, laboratory soils have been disturbed, homogenized, and subject to thermal shock (Thierron, 1996). They no longer contain living roots, and do not fully represent conditions in the field.

Different dynamics in pine and oak forests may lead to different respiration rates between forest types and different responses to fertigation. Every year except the 2<sup>nd</sup> 1999 measurements show higher field respiration rates in the oaks. Oaks soils have also been discovered to have higher respiration rates after incubation. In addition to having differences in litter quality and soil carbon and nitrogen content, oak and pine plots may have different responses to nitrogen loading. For example, in one experiment at Harvard forest, the pine plot experienced larger changes in extractable nitrogen, foliar N, nitrification, and N leaching (Aber et al., 1993). In this study, oak forest soils appear to be more affected by nitrogen additions than pine soils. The control and fertigated oak plots have a larger difference in respiration rates, root mass, net N mineralization and nitrification, than do the pine plots.

One of the difficulties in determining the reason soil respiration is higher at one site than another is the difficulty in determining the root and microbial contribution to respiration (Bowden et al., 1993). However, studies have shown a large contribution to soil respiration comes from roots. At Harvard Forest, Massachusetts, approximately two thirds of the soil respiration is due to root activity (both living and decomposing roots). The Harvard Forest data are comparable to previous study suggesting that root respiration, and decomposition is responsible for 70-80% of the total soil respiration in a wide range of forest types (Bowden et al., 1993). Since root biomass appears to have such an influence on respiration, this may lead to the higher rates in control plots. Root biomass is expected to be higher in the control plots. When nutrients are limiting, the plant must produce more roots to obtain needed nutrients. As nutrients become more available, plants allocate less primary production to root biomass and more to above ground production. Although oak control plots have more roots than oak fert, pine fert plots appear to have more roots than pine control. While this could possibly explain the reason field respiration is higher in the fertigated pine plots and control oak plots, I am not at all confident in the measurement. Both oak and pine control plots qualitatively appeared to have more roots than fertigated plots. The difference may be from the sorting technique that was used. Picking roots out of soil is not necessarily an accurate way to measure their biomass. Many fine roots were not counted. Therefore, it is possible that both oak control and pine control have greater root biomass than the fertigated plots.

In addition to the higher respiration rates from roots in control plots, the microbes may also be contributing to higher respiration rates in the control plots. While the fert plots have higher mineralization and nitrification rates suggesting microbial activity, they also have the lowest respiration rates (Fig. 4, 12, 14). Microbial respiration has also been found to decrease with fertilization (Soderstrom et al., 1983). Microbial biomass and soil respiration decrease was found after 150 kg NH<sub>4</sub>NO<sub>3</sub> N ha<sup>-1</sup> to different coniferous

forest podzols in Sweden. After only three months of fertilization, a respiration decrease was evident which persisted even after 3-5 years (Soderstrom et al., 1983). Decrease in respiration has also been found to be proportional to the N application (Baath, 1981). While studies have shown decreased microbial activities after fertilization (Soderstrom et al., 1983) and urea additions, others have shown an increase (Soderstrom et al., 1983).

It is significant to consider that laboratory experiments have shown a decreasing respiration rate even when no plants were present (Soderstrom et al. 1983). The data from 2<sup>nd</sup> 1999 experiment support this conclusion. The respiration rates from the incubated samples, which showed the strongest correlation between fertilization and low respiration rates, were measurements only of microbial activity. No live root respiration was present since the roots were killed when samples were removed from the ground.

Several proposed hypotheses proposed by Soderstrom et al. (1983) provide possible explanations for decreasing microbial respiration rates with N additions: nitrogenous compounds may directly inhibit microbial metabolism, carbon may be less available due to nitrogen compound condensation, nitrogen might increase carbon retention, the osmotic potential in the soil may have a partial sterilization effect, decreasing pH may inhibit microbes. Another hypothesis is that N does not control litter decomposition (Prescott, 1995).

Although direct studies on microbes were not performed in this experiment, there are several hypotheses that appear to be less likely. Microbial activity does not appear to be inhibited in fertigated plots since the net mineralization and nitrification rates were highest in the fertigated plots. Both mineralization and nitrification show microbial activity in the soils. Decreases in pH should lead to a decrease in microbial respiration. The decrease in pH did not appear to cause a decrease in microbial respiration since pH was lower in the control plots while respiration was higher.

Possible explanations could be that microbes do not have to expend as much energy to obtain the easily available nitrogen and carbon, and that N content does not determine litter decomposition (Prescott, 1995). Prescott (1995) suggests that studies have not been conclusive whether litter decomposition is faster slower or the same and differences may be due to microclimates. N accumulation in decomposing litter may be due to "luxury uptake"(Prescott, 1995). However, since a direct study on microbes at the treatment plant was not done, more research is needed to determine the effects of nitrogen on the soil microbes.

In summary, the field soil respiration rates showed higher rates in the oak control and pine fert plots ( $\text{mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$ ). The incubation measurements revealed higher respiration rates in the control plots for both oak and pine plots. N mineralization, nitrification, soil N content were all found to be higher in the fertigated plots. Soil carbon content was higher in the oak control and pine fertigated plots. Because of the problems encountered measuring field soil respiration measurements, more measurements should be done to obtain conclusive results. Likely explanations for higher respiration rates in the control plots include higher root and microbial respiration rates. If soil respiration is lower in the fertigated plots, the STP may be retaining more carbon than expected.

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