Plant and Soil Community Interactions: Litter Carbon Quality and Functional Microbial Community In New England Forests

Ellen R. Herbert[1], Gaius R. Shaver[2], William C. Longo[3], and Courtney M. Shannon[4]

[1]Department of Biology, Kenyon College, Gambier Ohio, 43022. [2] The Ecosystems Center, Marine Biological Laboratory, Woods Hole, MA O2543. [3] Department of Chemistry, Haverford College, 370 Lancaster Ave. Haverford, PA 19041. [4] Department of Environmental Science, Colorado College, 14 East Cache La Poudre St, Colorado Springs, CO 80903.

Abstract

Changes in aboveground plant community have been shown to alter the rates of carbon mineralization and carbon cycling in soils. There is speculation that changes in the carbon quality of soil organic matter inputs and the subsequent changes in functional microbial community are responsible for altered carbon cycling. We studied the differences in carbon mineralization, carbon quality, and soil enzyme activity under pitch pine (*Pinus rigida*) and beech (*Fagus grandifolia*) canopies. We found higher carbon mineralization rates in hardwood soils and apparent differences in the stage of soil organic matter (SOM) decomposition with pines exhibiting mineralization patterns consistent with SOM in a later stage of decomposition. Between plant type, soils exhibited significant differences in carbon quality, with pines soils having much higher lignin content. We also found significantly different functional microbial communities under each canopy, as determined by enzyme and substrate induced respiration profiles. The results of this study indicate that through the carbon quality and timing of litter inputs, plants can alter carbon cycling and the functional microbial community responsible for this cycling.

Key Words and Phrases: *carbon cycling, enzymes, substrate-induced respiration, community change, carbon quality, soil organic matter, above ground plant community, hardwood forest*

Introduction

It is widely acknowledged that there is an important feedback between aboveground plant community, soil organic matter composition, and nutrient cycling (Beaver *et al.* 1997, Ravit *et al.* 2003, Wolfe & Klironomos 2005). Agren and Bosstta (1996) found that the chemical composition, or quality, of soil organic matter could be correlated with the rate of carbon cycling in soils. Soil organic matter is derived from plant litter-fall, and plant communities affect the composition of soil organic matter through the quantity, quality, and timing of litter inputs (Wolfe & Klioronomos 2005). Arguably the most prominent soil input is leaf litter. The productivity, tissue chemistry, plant morphology, and phenology of a species plays a major role in determining litter inputs to soils (J.G. Ehernfeld 2003).

The species composition of the above ground plant community thus exerts a strong influence on litter quality and quantity, and changes in these plant communities can change the biogeochemical cycling of carbon in an ecosystem (J.G. Ehrenfeld 2001, Shaver *et al.* in press, and Ravit *et al.* 2003). In the context of this experiment, carbon quality refers to the relative liability or recalcitrance of an organic compound—for instance lignin is very recalcitrant and is thus considered to be poor quality carbon. Cromack (1973) found that a high lignin content in leaf matter at the time of leaf-fall inhibits decomposition rates, thus low carbon quality is associated with low rates of carbon mineralization.

The rate of carbon cycling in a soil is not purely an effect of the quality of the carbon in soil organic matter. Soil microbial communities are responsible for the decomposition and remineralization of plant litter and therefore are a major part of the

carbon cycling pathway. Since litter decay is a major component in the recycling of carbon in an ecosystem, decomposition rates directly determine the carbon stocks in soil and the speed at which carbon is mineralized (Melillo *et al.* 1982.) Soil microbial community structure and function are closely associated with aboveground plant community (Wardle *et al.* 2004) and shifts in plant community composition have been found to cause shifts in soil community and in the activity of enzymes involved in the nutrient mineralization processes in soil (Wolfe & Klironomos 2005). Thus, it is predicted that plants create their own soil environment through a complex feedback mechanism whereby litter inputs affect microbial community structure and function which in turn affect nutrient cycling and plant community structure and growth (Wolfe & Klironomos 2005). Although many fragments of this feedback mechanism have been studied, very few studies have attempted to look at this phenomenon holistically. We propose a study of how the species composition of forest communities affects carbon cycling in soils and the functional microbial community responsible for this cycling through the carbon quality of litter inputs.

We have chosen to work in two forest types in Cape Cod, MA. Halfway Pond Island is a relic hardwood forest located on an island in the center of a 232-acre pond in Plymouth County, MA. It is populated by deciduous beech, red maple, and yellow birch forests—representing what biologists speculate was the original forest community in New England before settlement. A pine/scrub oak forest on the edge of the lake represents the current forest type in the area that resulted from the clearing of original forests. These two sites vary drastically in the types of litter inputs they receive. The hardwood forest is dominated by old-growth deciduous trees which drop all their litter

annually, while the pine forest is dominated by younger evergreens, which drop small amounts of litter throughout the season. A dirt road will served as an ecosystem type which receives little organic matter input and is bare mineral soil. Comparisons of soil carbon quality, enzyme activity, and nutrient cycling in these soils will allow us to make inferences about the controls and feedbacks between plant and soil communities that develop through colonization by different species. This study has broader implications for the restoration of forest communities.

Methods

Sites, Ecosystem Characterization, and Soil Sample Collection and Preparation

We collected soils from three different locations in the vicinity of Halfway Pond Island, Plymouth County, MA in October of 2005, shortly after the leaf-fall occurred. At each site we made a visual estimate of the species composition of the vegetation to determine (1) the boundaries of the vegetation type of interest and (2) the possible types of litter falling within this boundary. The hardwood soils were collected under a canopy predominated by beech (*Fagus grandifolia)* and the pine soils were collected under a canopy of approximately 80% pine (*Pinus rigida)* and 20% oak (*Quircus agrifolia*), but the litter in collection areas was almost entirely pine.

Three soil sampling points were chosen at random by throwing a small object. At each of these three sites we used a bulb corer to extract six soil cores, separated each core into an organic horizon and the top 10 cm of the mineral horizon, and measured the thickness of each organic layer. We also collected small litter samples for use in the

substrate induced respiration experiment. In the lab, we homogenized the three organic layers from one sampling point, removed any roots, and loosely packed two inches of soil into PVC respiration core tubes. We froze the remainder of the soil for sub-sample analysis at -20º C. Mineral soils received the same treatment.

Core Incubation and Nutrient Cycling Measurements

We incubated the cores at 20º C for approximately one day before measuring initial CO_2 respiration rates with the LiCor 6200 and then periodically measured respiration for fifteen days. The cores were maintained in the dark at 20ºC throughout the incubation period.

pH and CHN Analysis

A sub-sample of the frozen homogenized soil from each of the three points in each of the ecosystems was thawed for several days prior to analysis. For pH analysis we created a soil slurry with 50 mL DI water and 5-10 g of bulk soil and measured pH according to the methods presented in the SES 2005 Laboratory Manual. A separate sub sample was dried to determine soil moisture. The dried sample was ground and analyzed for C and N on the Perkin-Elmer Elemental Analyzer according to the methods presented in the SES 2005 Laboratory Manual.

Sequential Extraction for Carbon Fractions

We determined the proportions of water soluble, acid soluble, and acid insoluble carbon fractions using the methods presented by Ricca and Neil (1992.) Water soluble

fractions were analyzed for polyphenols, in tannic acid equivalents, using the Folin-Denis method (Allen *et al.* 1974) and sugars, in dextrose equivalents, using the phenol-sulfuric acid assay (Dubois *et al.* 1956). We assumed that the carbohydrates removed by the acid digestion were cellulose, hemicellulose, and starch, and that the material remaining after digestions was lignin (Effland, 1977).

Microbial community function

We measured soil enzyme activities for three enzymes, which can be are related to the breakdown of glucose (sugars), phenolics and phenolic compounds , and complex organic compounds (lignin): β-glucosidase, phenol oxidase, and peroxidase, using chromogenic substrates according to the methods presented by Sinsabough *et al.* (1999). For each assay, we prepared soil slurry solutions of approximately 10 g bulk soil and 50 mL of a 50 mmol/L acetate buffer (pH 5.0.) The substrate for the β-glucosidase assay was a 5 mmol/L pNitrophenol-β-glucopyranoside solution in a 5 mmol/L acetate buffer. For each sample, an aliquot of 3 mL of soil slurry and 3 mL substrate solution was vortexed, incubated in a mixer for 1.5 hours, and centrifuged. The supernatant was filtered. 0.2 mL of 1 mol/L NaOH was then added to 1.0 mL of the filtrate to develop the color and the absorbance of the filtrate was read on the Shimadzu 1610 spectrophotometer at 410nm.The substrate for the phenol oxidase and peroxidase assay was a 5 mmol/L L-3,4-dihydroxyphenylalanine (L-DOPA) solution. For the phenol oxidase assay, 3 mL of soil slurry and 3 mL substrate solution was vortexed, incubated in a mixer for 1 hour, centrifuged, and filtered. The absorbance of the filtrate was read at 460 nm. For the

peroxidase assay, activities were processed in the same way as phenol oxidase, with L-DOPA substrate and the addition of 3 mL of .3% H_2O_2 at the beginning of the incubation.

Substrate Induced Respiration

To determine the soil respiration response to the temporary addition of different carbon substrates, we supplemented each soil with three different substrates: glucose, hardwood litter, and pine litter according to the methods presented by Kourtev *et al.* (2002). Hardwood litter and pine litter were milled and suspended in a solution of DI water at a concentration of 2 grams ground leaf litter in 100 mL water and the glucose solution was made in a concentration of 0.01 mol/L. All solutions were frozen for 12 hours before their addition to soil samples. 10 g (dry weight) of soil was added to 100mL jars with 2 mL of the substrate and respiration measurements were taken periodically with the LiCor 6200 for 48 hours, with time 0 as the respiration rate before substrate addition.

Yearly Carbon Loss Model

In order to estimate carbon loss (as mineralization) over the course of a year from forest soils, we constructed a simple model based on core incubation respiration rates and several simple assumptions about litter production: (1) since pines drop litter at a relatively constant rate throughout the year, the initial reparation rates initially measured in our cores is representative of the year round respiration rate in pine soils at a constant temperature. (2) Hardwoods are deciduous, thus litter inputs are not constant and the initial respiration rates from the cores represented fall mineralization rates

(decomposition of newly dropped litter), while the final core respiration rates represented spring and summer mineralization rates of litter in a more advanced stage of decomposition. We also assumed decomposition occurred year-round and 24 hours a day.

Results

Field Collected Soils

There was no difference in pH or organic layer depth between pine, hardwood, and dirt road soils (Table 1., $p > 0.05$). Pine organic and mineral soils were higher in carbon than hardwood soils and also had higher C:N and Lignin:N ratios than hardwood soils (Table 2). Dirt road soils had very low carbon content and low C:N ratio and the lowest Lignin:N ratio of any soil analyzed (Table 2).

Core Incubations

Per gram soil, respiration rates in hardwood organic soils were initially similar to pine organic soils, but hardwood respiration decreases linearly while pine organic respiration decreases a great deal within the first 4 days and appears to reach an asymptote on day 9 (Figure 1). Mineral layer soils had respiration rates 85% lower than organic soils, but follow the same general patterns shown by their corresponding organic soils, with hardwood soils decreasing linearly and pine soils decreasing rapidly and leveling out in the later days of incubation (Figure 1). The dirt road soils showed consistently low respiration rates, barley above 0 (Figure 1).

If reparation is expressed on a per unit soil carbon basis, dirt road soils initially show the highest respiration rate per gram soil carbon and this rate rapidly declines over the subsequent days (Figure 2). Hardwood soils exhibit higher rates of respiration, about 30% higher than pine soils, and both pine and hardwood soils maintain their distinct patterns of decrease in respiration rate (Figure 2). By day 15, all soils are converging upon a reparation rate around 0.01 mg C gC^{-1} hr^{-1} (Figure 2).

Carbon Fractions

The five soils differed significantly in the fraction of the proportion and amount of the three carbon fractions extracted from each soil (Figure 3). Pine organic soils had 0.77 g AIS/ g dry soil, almost twice as much as the AIS fraction in hardwood organic soils (0.4 g AIS/ g dry soil, Figure 2). Mineral soils between the two forests were more consistent in terms of carbon fraction composition and had much lower organic matter contents. Per gram organic matter, both had approximately 50% AIS, although pine mineral soils had a much higher proportion of the WS fraction and hardwood soils had a slightly higher proportion of AS material (Figure 3). The acid soluble cellulose and holocellulose fraction is a smaller portion of organic matter in all soils, between 0.17 and 0.04 g AS/ g dry soil. The fraction AS fraction in hardwood organic soils is 21% greater than in pine organic soils. (Figure 3).

Hardwood organic soils contained as much water soluble material as AIS, 0.345 g WS/ g dry soil, 3 times more WS material than pine organic soils. The dirt road showed very low overall organic matter content, about 0.03 g OM/ g dry soil (Figure 2.)

% Lignin was negatively correlated with respiration per unit soil carbon (R^2 = 0.288, Figure 4), but the correlation between high Lignin:N and decreased respiration was much stronger (R^2 = 0.790, Figure 5). There was also a poor negative correlation between % WS material and respiration (Slope = -0.0072, R^2=0.213, data not shown).

Enzyme Activities

Oxidative enzymes generally showed the greatest activity. Phenol oxidase activity was greatest in hardwood organic soils, 4 times higher than in pine soils. Phenol oxides activity showed a consistent pattern of decrease between organic and mineral layers, although this decrease is more prominent tin hardwood soils (Figure 6). Peroxidase activity showed the opposite trend with pine mineral soils having the greatest peroxidase activity at 1.858 µmol substrate h^{-1} g^{-1} dry soil and no activity in hardwood organic soils. The activity of peroxidase increased 91% between pine organic soils and pine mineral soils and from 0 to 1.594 µmol substrate h^{-1} g^{-1} dry soil in hardwood soils. Dirt road soils showed very low overall enzyme activities, with peroxidase showing activities double those other enzymes. β-glucosidase activity was highest in hardwood organic soils, 43% greater than pine organic soils and β-glucosidase activity showed a consistent decrease in activity of 37% between organic and mineral layers (Figure 6).

SIR

The largest increase in respiration was seen after the addition of glucose—a 9-fold increase in respiration for organic soils and a 2-fold increase in mineral soils. Respiration after the initial spike 1.5 hours after glucose addition decreased to initial fluxes by hour

24. There was no difference in the response to glucose addition between pine and hardwood soils (Figure 7a).

Pine organic and pine mineral soils showed a large response to the addition of hardwood litter, pine organic soils showed a 7 fold increase in respiration while pine mineral showed a 4 fold increase in respiration. Hardwood soils showed a diminished response to the addition of hardwood litter, organic an mineral soils showed only a 3 fold increase in respiration (Figure 7b). Organic soils, both pine and hardwood, returned to initial respiration rates by hour 24. However, mineral soils appeared to return to initial respiration rates more slowly and did not return to initial respiration rates until hour 48 (Figure 7b).

Mineral soils showed the greatest response to pine litter addition. Respiration increased more than 3 fold in pine mineral soils and more than doubled in hardwood mineral soils. Respiration rates in these soils remained elevated until hour 48. Pine and hardwood organic soils showed respiration increases 2.5 hours after substrate addition of 67% and 62% respectively (Figure 7c). Hardwood organic soils returned to initial respiration rates by 24 hours while pine respiration rates remained elevated through the experiment (Figure 7c). Dirt road mineral soils had very small responses to substrate addition and in all cases dirt road respiration increased from a small negative value to 0 after the substrate addition and returned to negative values by hour 24 (Figure 7a, b, & c.)

Carbon Loss Model

Soils differed significantly in the amount of carbon lost through respiration over the course of the year. Pine organic soils lost about 10 % of their initial carbon content

while hardwoods lost about 15 % (Figure 8). Hardwood mineral soils lost twice as much of their initial carbon as pine mineral soils, and the dirt road soils lost over 50% of their initial carbon over the course of the modeled year (Figure 8).

Discussion

Carbon Mineralization and Carbon Quality

Hardwood and pine soil showed differences in both the magnitude the and pattern of carbon mineralization rates. The fact that each plant cover showed a consistent pattern between organic and mineral soils indicates that there is indeed some difference in carbon mineralization resulting from the difference in soil metabolism between the two forest soils. When carbon loss through mineralization was modeled for one year, the magnitude of loss in organic soils between 5 % and 20 % of initial carbon stocks was similar to that found by Shaver *et al.* (in press) in Alaskan Tundra soils. We also found a negative correlation between carbon loss and % lignin similar to that found by Shaver *et al.* (in press) and Hobbie (1996). The observed differences in mineralization rates were not determined by the size of the initial carbon pool but by the quality of the carbon present.

This difference in soil organic matter carbon quality appears to be a function of both the quality and timing of litter inputs. Hobbie *et al.* (1992) and Shaver *et al.* (in press) found that carbon quality of organic matter was the largest determining factor of decomposition rates in Alaskan tundra soils and that differences in vegetation led to the differences in the chemistry of plant litter which becomes soil organic matter. Pine and other evergreen species have consistently been found to have higher fractions of

recalcitrant AS and AIS carbon fractions than deciduous litters (Versfeld *et al.* 1968 & Berg 2000). Although we did not determine the actual carbon fraction contents of litter inputs at each site, sites were chosen to directly reflect two very different litter types—evergreen and deciduous. Therefore we assume that since our soil carbon quality data reflects the differences in litter carbon quality found by Versfield (1968) and Berg (2000) our carbon quality data is consistent with the above observations concerning the quality of litter inputs.

Carbon Quality

The linear decrease carbon mineralization rates seen in hardwood soils, which initially had the largest fraction of WS sugars and polyphenols, indicates that these labile fractions are steadily decreasing in abundance. These soils also had high respiration per unit carbon indicating that carbon mineralization in these soils is accelerated by the respiration of large amounts of their easily labile carbon pool by microbial communities This is especially apparent in the dirt road mineral soils which contain only labile carbon and show the greatest modeled loss of carbon over a year. This is consistent with the observation made by Berg and Tam (1991) that initial concentrations of WS carbon fractions are positively correlated with initial soil carbon mass-loss rates.

Lignin:N Ratio

We also found the same retarding effect of lignin on respiration rates as Cromack (1973) and Hobbie (1996). However, we found a stronger correlation between decreased respiration rates and high lignin:N ratios. Our values for lignin:N ratio were comparable

to Mellilo *et al.* (1980), though slightly higher indicating that perhaps we over estimated the amount of lignin in our soils. However, the negative correlation between this ratio and respiration is opposite of the positive correlation observed by Mellilo *et al.* (1980) and Berg (2000). They attribute this trend to (1) the tendency of low molecular weight N to bind with lignin and create extremely recalcitrant humic compounds and (2) the inhibition of peroxidase by N (Mellilo *et al*. 1980 and Berg 2000). However, this trend is observed only in soils in the final stages of decomposition that contain very little material other than lignin, and our soils were not in this final stage of decomposition. In fact, Berg (2000) notes a positive correlation between N content and decomposition on early and middle stages of decomposition because nutrients are often limiting to decomposition in these stages.

Timing of Litter Inputs

Since our collection of soils occurred shortly after the leaf-fall, we were able to observe some effects of the timing of litter inputs on soil metabolism. The timing of litter inputs have a synergistic effect with carbon quality on soil organic matter mineralization. Timing of litter inputs to soils is a function of plant phenology and morphology, both of which vary greatly between evergreen and hardwood species. Pines drop a small amount of litter over the course of the year and rarely turn over the entirety of their leafy biomass annually. Furthermore, Berg (2000) suggests that evergreen leaves may die well before they drop and undergo substantial decomposition before they reach the ground. Our soil collection was made after the annual senescence of leaves in the hardwood site and therefore the hardwood soils contained fresh litter input with high WS and AS fractions.

Berg (2000) describes the "litter decay continuum" as having two distinct stages: the first in which the availability of labile carbon substrates is high and decomposition is linear and the second in which labile substrate is limiting and decomposition is described by an asymptotic function where decomposition rates approach zero. Although Berg (2000) measured decomposition in units of mass lost, our measurement of carbon loss as remineralized material is comparable. Thus the asymptotic nature of the decrease in carbon mineralization in pine organic soils over the course of the core incubation may indicate that the organic matter in pine soils is in a later stage of decay, where SOM has become enriched with lignin and decomposition rates are suppressed. We also conclude from this that carbon mineralization rates in hardwood soils are much more variable seasonally than in pine soils.

Furthermore, we observed that all soils appeared to be converging upon a similar respiration rate per gram soil carbon by the 15th day, an observation congruent with Berg's (2000) observation that as labile substrates are preferential degraded by microbial communities, mineralization of organic matter approaches a "limit value" controlled by the decomposition of lignin. Further investigation of the mass-loss of specific of carbon fractions is needed to determine the validity of this assertion for mass-loss in incubations of such a short duration.

Functional Microbial Community

Soils under different plant cover types showed differences in functional microbial communities through differing responses to substrate induced respiration and different enzyme profiles. These differences, especially in substrate induced respiration response

were much greater than those found by Kourtev *et al.* (2002), albeit our selection of plant species for comparison were much different than the mixed deciduous community studied by Kourtev. Differences in enzyme activity predominate in organic soils and enzyme activity did not always correlate with substrate availability or substrate liability.

Kourtev *et. al.* (2002) found that measures of microbial community function—enzyme profiles and substrate induced respiration responses—were well correlated with microbial community structure. We saw that the activities of the two enzymes responsible for the decomposition of the WS fractions (glucose and phenols) β-glucosidase and phenol oxidase, are highest in hardwood organic soils. The response of hardwood organic soil respiration to substrate addition also indicates a microbial community geared toward the decomposition of labile carbon fractions. The large response to glucose represents the maximum respiration of the soil microbial community when provided a saturating concentration of the most labile of carbon fractions.

The response of the hardwood organic layer to hardwood litter did not show an increase of this magnitude, indicating that the soil community respired the small amount of labile carbon. We did not measure the concentration of polar solutes in the hardwood litter solution, but the response to the addition of this substrate might indicate that it was about one third half that of the glucose solution. The large response to hardwood litter seen in pine organic soil further supports our conclusion that hardwood organic soils cultivate functional microbial communities geared toward the breakdown of labile carbon substrates while pine soils contain a more functionally diverse community.

We assumed that the majority of the respired carbon in hardwood soils represents the WS as AS fraction as indicated by the enzyme profile. In the glucose SIR experiment,

both soils respond equally to sugar addition after 1.5 hours, so the increment between the hardwood respiration peak and the pine respiration peak after the addition of hardwood litter must be due to the respiration of other substrates—most likely the AS fraction in the hardwood litter. This assertion is corroborated by the activity of peroxidase in pine organic soils not seen in hardwood organic soils. This is indicative of a functional microbial community in hardwood organic soils wholly different from the community present in pine organic soils. Our findings are consistent with Berg's (2000) hypothesis that there is a succession of the soil biota community based on their ability to compete for substrates of differing chemical compositions.

This difference in community may not be purely temporal, either. For instance, all mineral soils have large portions of WS and AIS fractions but show the highest activity of peroxidase. Soils were collected shortly after a heavy rain and we suspect that these fractions were temporarily leached into mineral soils where microbial communities were geared for the decomposition of lignin and had not yet produced large amount of other enzymes. We suspect, then, that microbial community composition responds less to the temporary availability of substrates and the microbial community present in soil is indeed determined by the long term availability of specific carbon fractions.

Conclusions

Plant leaf litter chemistry and the timing of leaf inputs have a very complex interaction with soil communities which control the biogeochemical cycling of carbon. This intricate relationship appears to be long-standing and the effects of plant community displacement on soil microbial communities and vice versa are unknown. This interaction

has important implications for the restoration of native vegetation and invasive species management in areas like Cape Cod and further research efforts should be directed towards this facet of plant-soil interactions.

Acknowledgements

I would like to thank my advisor Gus Shaver for his help designing and interpreting my experiment. I would like to thank Rich McHorney, Clara Funk, Allison Burce, and Laura Wittman for their help in the lab and in field and their addition input on the design of my experiment. I would like to thank my collaborators Will Longo and Courtney Shannon, and my fellow students Sabrina Moreau, Laura Nagel, Adam Akullian, and Cara Machacek for their help in the lab.

Works Cited

Allen, S.E., H.M Grimshaw, J. Parkinson, and C. Quarmby. 1974. Chemical analysis of ecological materials. Blackwell Scientific, Oxford, United Kingdom.

Beaver, JD, YS Rawat, SP Singh. 1997. Incorporating the soil community into plant population dynamics: the utility of the feedback approach. *Journal of Ecology* **85**: 561-73.

Berg, Bjorn. 2000. Litter decomposition and organic matter turnover in northern forest soils. *Forest Ecology Management* **133**: 13-22.

Cromack, K., Jr. 1973. Litter production and litter decomposition in a mixed hardwood watershed and a white pine watershed at Coweeta Hydrologic Station, North Carolina. Dissertation. University of Georgia, Athens, Georgia, USA.

Dubois, M., K.A. Gilles, L.K. Hamilton, P.A. Rebers, and F. Smith. 1956. Colormetric methods for determination of sugars and related substances. *Analytical Chemistry* **28**: 350-356.

Effland, M. J. 1997. Modified procedure to determine acid-insoluble lignin in wood and pulp. TAPPI **60**: 143-144.

Ehrenfeld, Joan G. 2003. Effects of Exotic Plant Invasion on Soil Nutrient Cycling Processes. *Ecosystems* **6**: 503-523.

Harmon, Mark E. and Kate Lajtha. 1999. Analysis of detritus and organic horizons for mineral and organic constituents. Pages 143-166 *in* G.P. Robertson, D.C. Coleman, C.S. Bledsoe, and P. Sollins, editors. Standard soil methods for long-term ecological research. Oxford University Press, New York, New York, USA.

Hobbie, Sarah E. 1996. Temperature and plant species control over litter decomposition in Alaskan tundra. *Ecological Monographs* **66**: 503-522.

Kourtev, Peter S., Joan G. Ehrenfeld, and Max Hagglom. 2002. Exotic Plant Species Alter the MicrobialCommunity Structure and Function in the Soil Community. *Ecology* **83**: 3152-3166.

Melillo, J.M., J.D. Aber, and J.F. Muratore. 1982. Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. *Ecology* **63**: 621-626.

Ravit, Beth, Joan G. Ehrenfeld, and Max M. Haggblom. 2003. A Comparison of Sediment Microbial Communities Associated with *Phragmintes australis* and *Spartina alternaflora* in Two Brackish Wetlands in New Jersey. *Estuaries* **26**: 465-474.

Ricca, Andrea and Chris Niell. 1992. Sequential Extraction of Carbon Fractions. MBL.1-7

SES Lab Manual. 2005. MBL.

Shaver, Gaius R., A.E. Giblin, K.J. Nadelhoffer, K.K. Thieler, M.R. Downs, J.A. Laundre, and E.B. Rastetter. In press. Carbon Turnover in Alaskan Tundra Soils: Effects of Organic Matter Quality, Temperature, Moisture, and Fertlaizer.

Sinsabaugh, R.L., M.J. Klug, H.P. Collins, P.E. Yeager, and S.O. Peterson. 1999. Characterizing soil microbial communities. Pages 318- 341 *in* G.P. Robertson, D.C. Coleman, C.S. Bledsoe, and P. Sollins, editors. Standard soil methods for long-term ecological research. Oxford University Press, New York, New York, USA.

Versfeld, D.B. and B.S. van Wilgren. 1986. Impacts of woody aliens on ecosystem properties. Pages 293-246 *in* Macdonal, Kruger, and Ferrar, Eds. The ecology and management of biological invasions in southern Africa. Oxford University Press, Cape Town, South Africa.

Wolfe, Benjamin E. and John Klironomos. 2005. Breaking New Ground: Soil Communities and Exotic Plant Invasion. *BioScience* **55**: 477-487.

Warde, D.A., R.D. Bardgett., J.N. Klironomos, H. Stala, W.H. van der Putten, and D.H. Wall. 2004. Ecolgical linkages between aboveground and belowground biota. *Science* **304**: 1629-1633.

Figure 1. Soil respiration per gram soil for forest organic and mineral soils. Respiration rates were measured periodically for 15 days using a LiCor 6200 CO_2 analyzer. Data points represent the average of 3 cores from each soil type at each time point. Error Bars = SE C.I.

Figure 2. Soil respiration per gram carbon for forest organic and mineral soils. Respiration rates were measured periodically for 15 days using a LiCor 62oo. Data points represent the average of 3 cores form each soil type.

Figure 3. Organic matter fractions extracted from forest organic and mineral soils. Data represents and average of the mass of each fraction in sub-samples from each site with the mineral portion of the soil not represented.

Figure 4. Linear regression of % Lignin vs. respiration rate per gram carbon. Data points represent the average of 3 cores from each soil type. Error Bars = SE

Figure 5. Linear regression of Lignin:N ratio vs. respiration rate per gram carbon. Data points represent the average of 3 cores from reach soil type. Error Bars = SE

Figure 6. β-glucosidase, phenol oxidase, and peroxidase enzyme activates in forest organic and mineral layer soils. Data represents the average of 12 sub-samples from each site.

Figure 7a. Glucose substrate induced respiration in forest organic and mineral soils. Data represents the average of 3 sub-samples from each soil type.

Figure 7b. Hardwood litter substrate induced respiration in forest organic and mineral soils. Data represents the average of 3 sub-samples from each soil type.

Figure 7c. Pine litter substrate induced respiration in forest organic and mineral soils. Data represents the average of 3 sub-samples from each soil type.

Figure 8. Soil carbon loss over 365 days based on carbon loss model. Error bars = SE.

Table 1. Field-collected soil organic layer thickness and pH.

		Pine Forest		Hardwood Forest		Dirt Road
		Organic	**Mineral**	**Organic**	**Mineral**	**Mineral**
Organic Layer Thickness	cm	5 ± *0.76*	*n/a*	5.45 ± 0.34	*n/a*	*n/a*
pH		4.12 ± *0 .16*	4.17 ± *0.23*	4.33 ± *0 .16*	4.62 ±*0 .25*	4.24 ± *0.11*

Table 2. Field-collected soil carbon and nitrogen content and Lignin:N ratio.

	Pine Forest			Hardwood Forest			Dirt Road
	Litter	**Organic**	**Mineral**	**Litter**	**Organic**	**Mineral**	**Mineral**
% Carbon	52.524%	36.694%	5.797%	50.563	25.645%	5.167%	.169%
C:N Ratio	55	29	33	56	22	26	24
Lignin:N	34.4*	57	66	26.8*	39	76	7

* Lignin:N ratios taken from Mellilo *et al*. (1982).

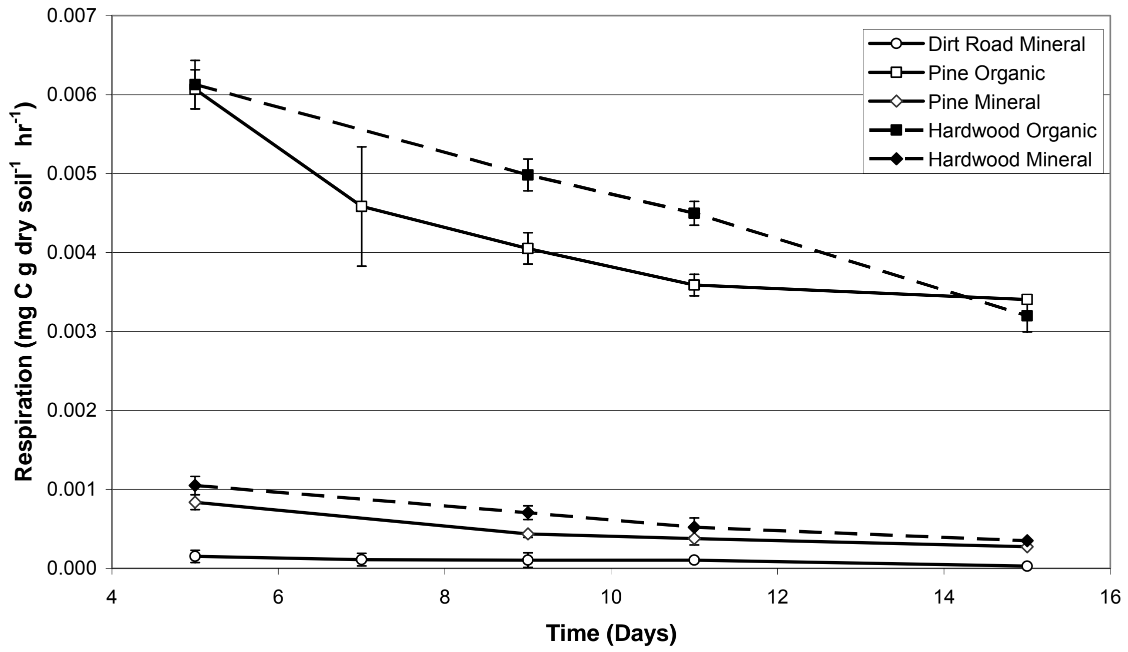

Figure 1. Soil respiration per gram soil for forest organic and mineral soils. Respiration rates were measured periodically for 15 days using a LiCor 6200 CO2 analyzer. Data points represent the average of 3 cores from each soil type at each time point. Error Bars = SE.

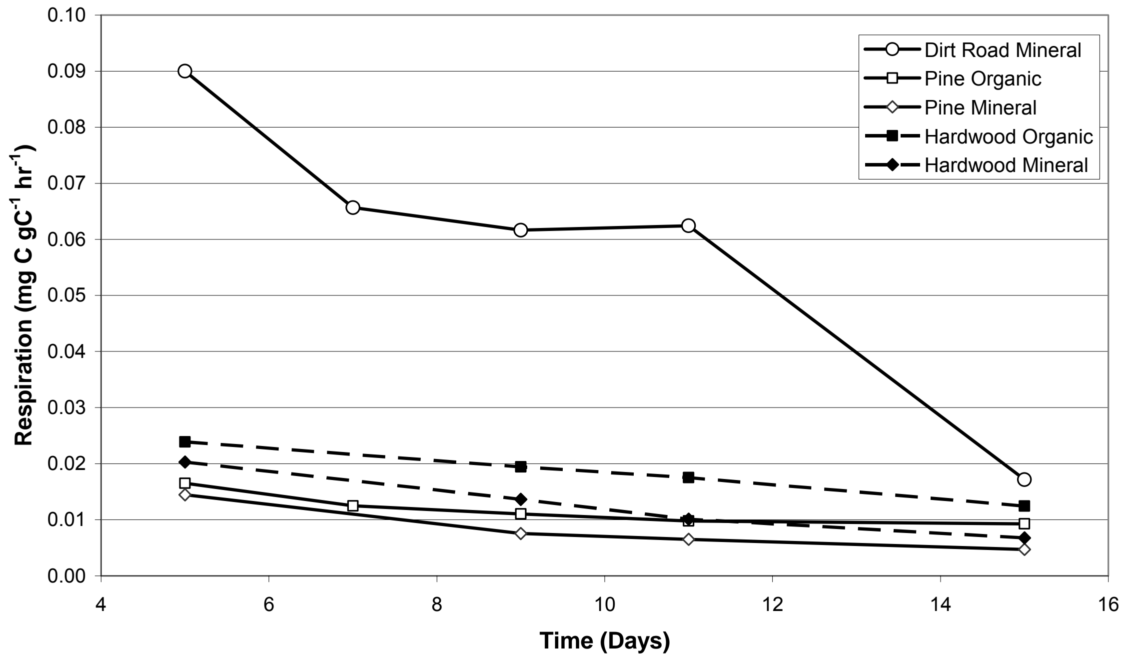

Figure 2. Soil respiration per gram carbon for forest organic and mineral soils. Respiration rates were measured periodically for 15 days using a LiCor 62oo. Data points represent the average of 3 cores form each soil type.

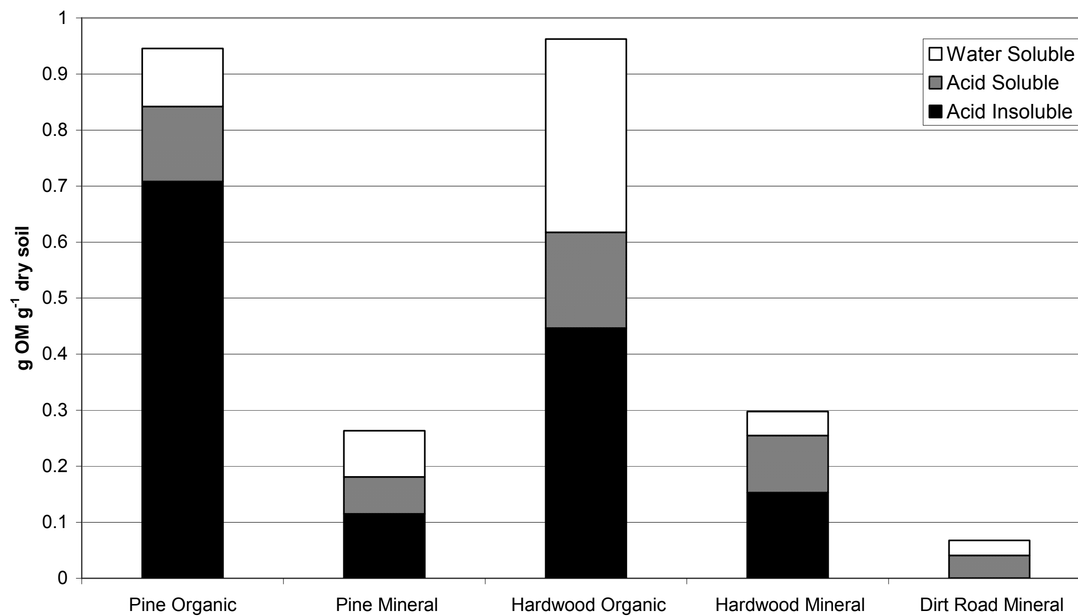

Figure 3. Organic matter fractions extracted from forest organic and mineral soils. Data represents and average of the mass of each fraction in sub-samples from each site with the mineral portion of the soil not represented.

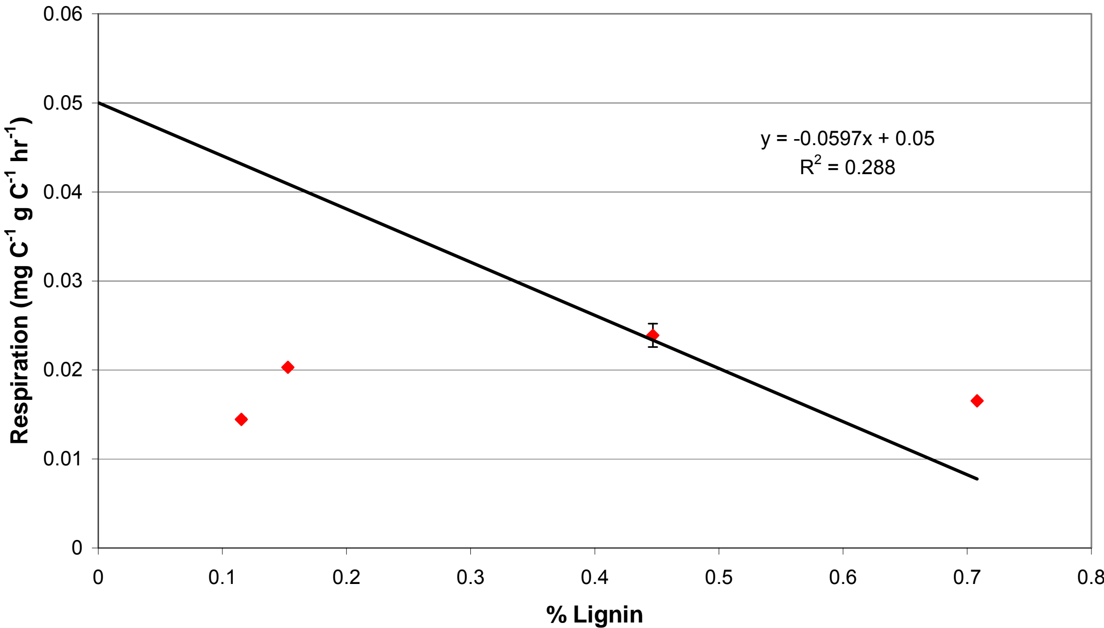

Figure 4. Linear regression of % Lignin vs. respiration rate per gram carbon. Data points represent the average of 3 cores from each soil type. R2 = 0.288. Error Bars = SE.

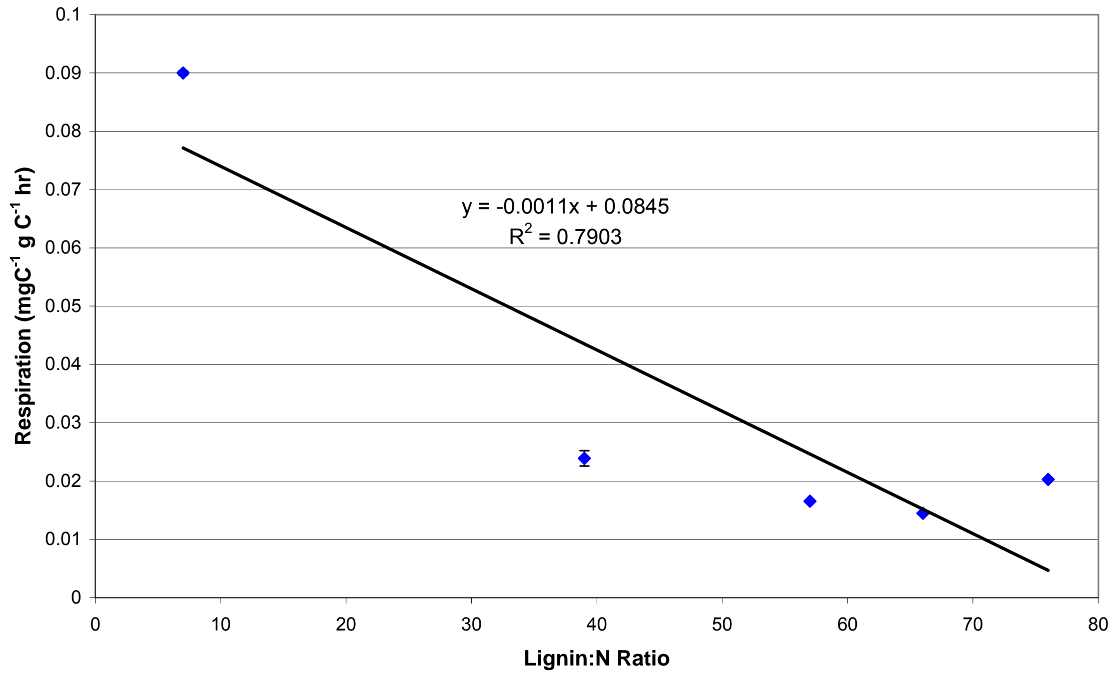

Figure 5. Linear regression of Lignin:N ratio vs. respiration rate per gram carbon. Data points represent the average of 3 cores from reach soil type. R^2 = .7903. Error Bars = SE.

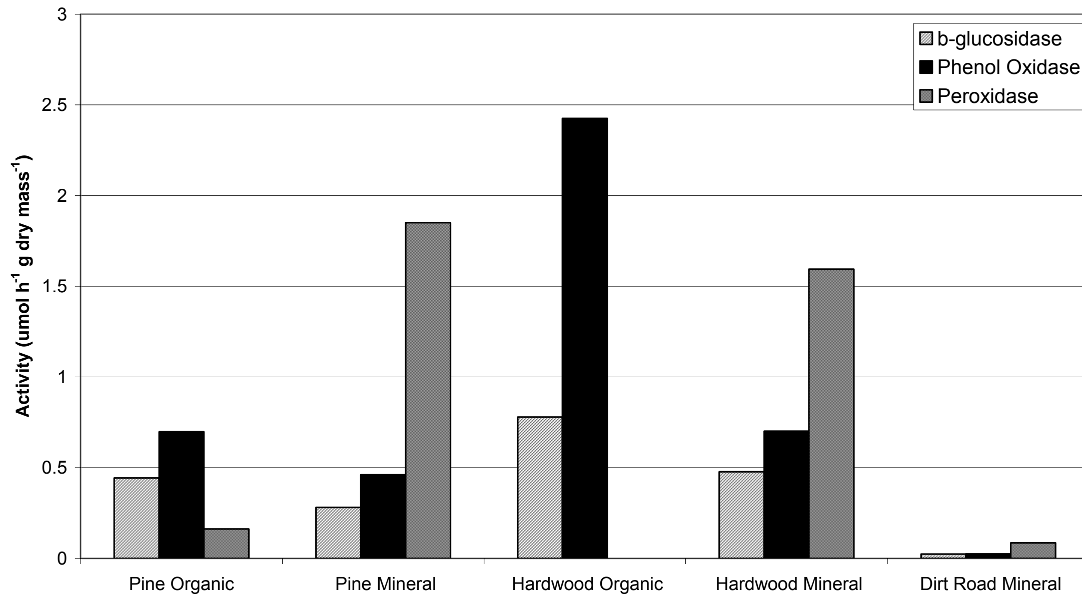

Figure 6. β-glucosidase, phenol oxidase, and peroxidase enzyme activates in forest organic and mineral layer soils. Data represents the average of 12 sub-samples from each site.

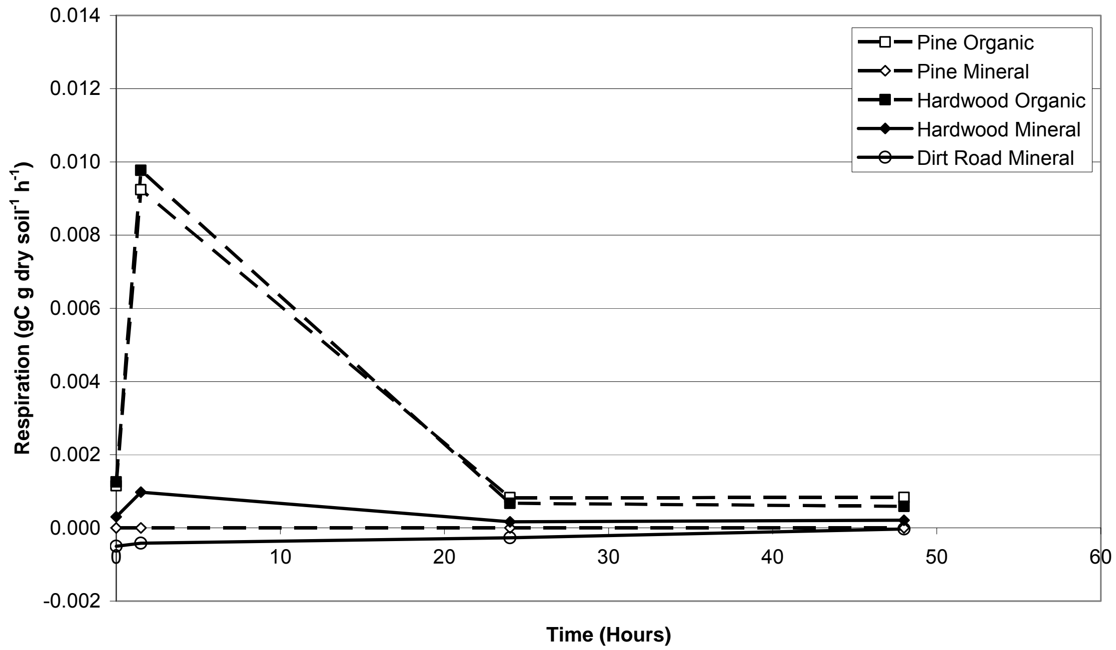

Figure 7a. Glucose substrate induced respiration in forest organic and mineral soils. Data represents the average of 3 sub-samples from each soil type.

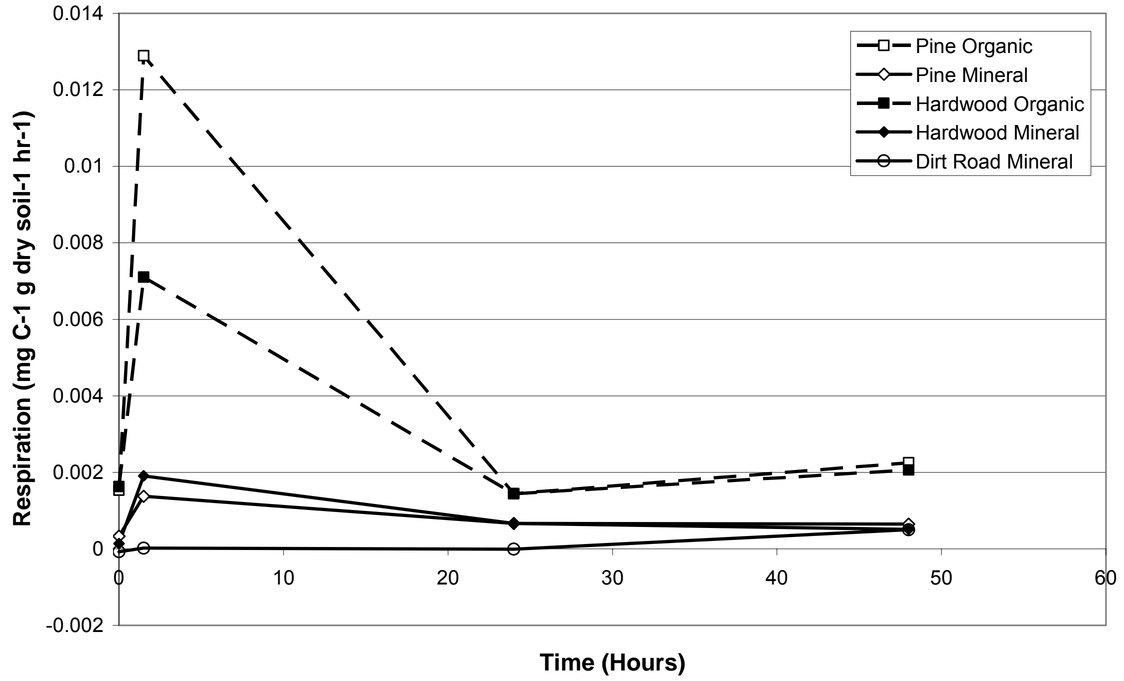

Figure 7b. Hardwood litter substrate induced respiration in forest organic and mineral soils. Data represents the average of 3 sub-samples from each soil type.

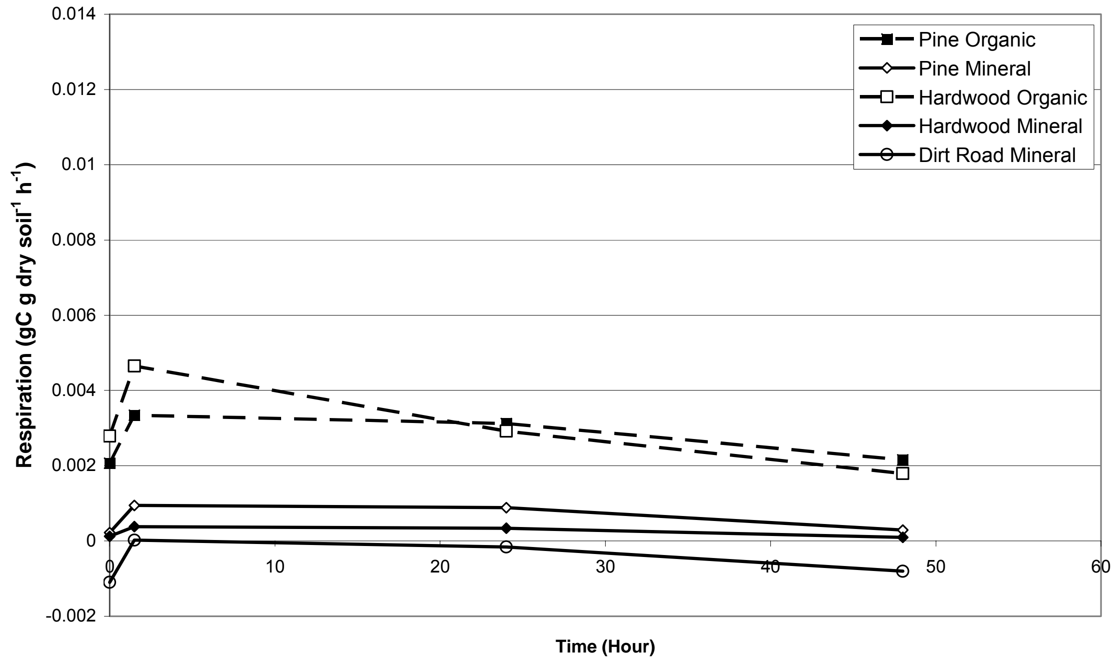

Figure 7c. Pine litter substrate induced respiration in forest organic and mineral soils. Data represents the average of 3 sub-samples from each soil type.

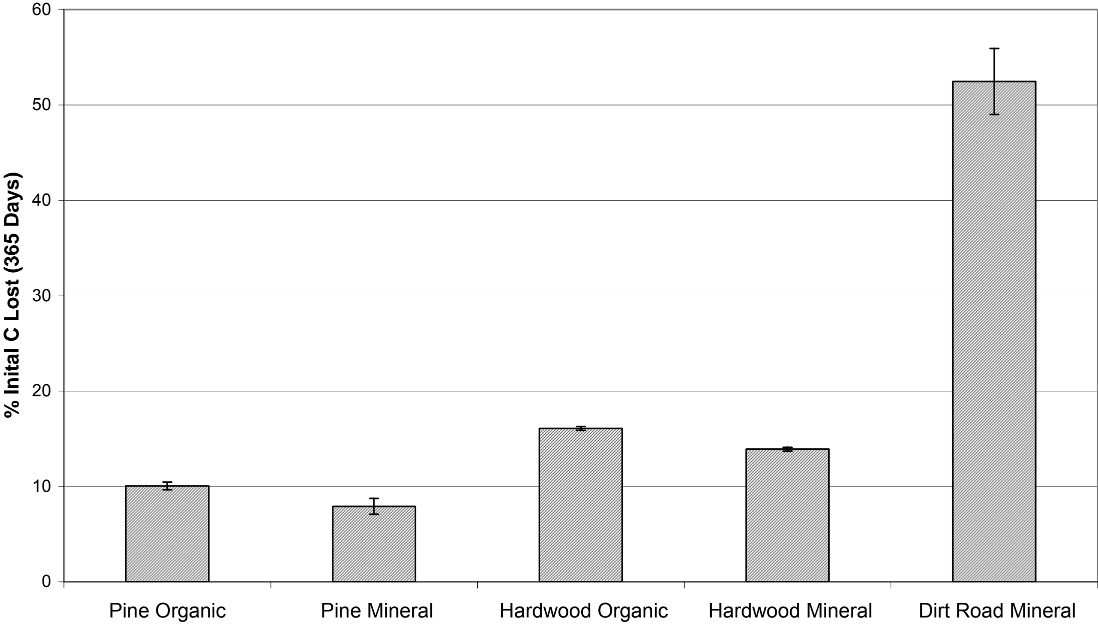

Figure 8. Soil carbon loss over 365 days based on carbon loss model. Error bars = SE.