AN ABSTRACT OF THE THESIS OF

Lea I. Wilson for the degree of Honors Baccalaureate of Science in Environmental Science presented on August 14, 2008. Title: Characterization of Soil Carbon and Nitrogen Pools following a Decade of Detritus Manipulation in a Temperate Coniferous Forest

Abstract Approved:_____
Kate Lajtha

Soil organic matter (SOM) is the result of the balance between decomposition and incorporation of detritus from both above and below ground sources into the soil. It composes only a fraction of the soil by mass but it is one of the most critical components. The Detritus Input and Removal Treatment (DIRT) experiment in the HJ Andrews Experimental Forest (Blue River, OR) is a long-term ecological research (LTER) project designed to study the effects of detritus inputs on the accumulation and stabilization of SOM in an old-growth coniferous forest. Our project sought to characterize carbon (C) and nitrogen (N) pools and dynamics on these plots after the first ten years of detrital manipulations. Net N and C mineralization rates were estimated by laboratory incubation methods. C and N pools were characterized by sequential density fractionation and the recalcitrant C pool was estimated by acid hydrolysis. High net N mineralization rates in no root and no input plots complement high dissolved organic nitrogen losses from previous studies on these same plots and suggest that root exudates and root turnover may be critical for effective N immobilization in this pristine old-growth forest. Increased woody detrital inputs lead to a trend for increased C and N content in the light fraction, but we hypothesize that more time is needed to see similar shifts in the heavier fractions. The recalcitrant C pool remains unaffected in all but the no input plots, where the recalcitrant C pool has decreased. Decrease in the C content of the light fraction though double the natural medium-quality litter has been added for a decade in double litter plots provides further evidence of the priming effect.

Key words: SOM, DIRT, LTER, carbon, nitrogen, labile, recalcitrant, priming effect

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Characterization of Soil Carbon and Nitrogen Pools following a Decade of Detritus Manipulation in a Temperate Coniferous Forest

by

Lea I. Wilson

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I understand that my project will become part of the permanent collection of Oregon State University, University Honors College. My signature below authorizes release of my project to any reader upon request.

Lea I. Wilson, Author

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Science was a tough world for me to break into; it seems everyone wants you to have experience, but they would all prefer you get it in someone else's lab. It was Dr. Kate Lajtha who gave me my first job in science, and to her I must extend resounding gratitude. She introduced me to a full and varied gamut of possibilities. Through her and the experience gained in her lab I have had the opportunity to be involved in a number of projects across many fields and specialties. Doubling as an academic advisor, Dr. Lajtha was always available for counsel, support, and even defense through my undergraduate career, which may have been much longer and much less happy without her. She continues to act in a supportive and encouraging manner.

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1 Introduction

"It doesn't matter the question, organic matter is the answer."

– James Cassidy, OSU soils instructor

1.1 *Soil*

What we call soil, and many people think of as "dirt," represents a convergence of time, climate, water, air, and mineral and organic matter. An ecosystem in its own right, the ground beneath our feet provides habitat to a great diversity of organisms, a pathway for important biogeochemical processes, and a reservoir for water and carbon. The functions of soil are essential to life on Earth.

Soil organic matter (SOM) accounts for only 1%-6% of soil by weight on average (Brady and Well 2005), but it is arguably the most important component. SOM is the result of physical, chemical, and microbial processes that decompose and incorporate organic matter into the soil over periods of weeks and centuries (Brady and Well 2005). SOM adds stability to soil aggregates and increases pore volume, nutrient exchange capacity, and water holding capacity. SOM also represents a major source and sink of terrestrial carbon (Sollins *et al.* 1999, Six *et al.* 2002). The source and sink dynamic of carbon in the soil ecosystem has become a hot topic as we struggle with the possible trajectories of global climate change (Sollins *et al.* 1999).

1.2 *DIRT*

In 1956 Dr. Francis D. Hole began the first of a network of plots now called DIRT: Detritus Input and Removal Treatments. The basic experimental design is to manipulate the amount and types of litter on plots, such as doubling the litter, removing the roots, or annually burning. The manipulation is expected to elicit a response in soil nutrient composition and the activities of soil organisms. Since its inception, the DIRT plots have grown to five collaborating sites across the United States, and one in Europe at the Síkfökút Forest in Eger, Hungary (Appendix A). The overriding goal of these plots is to study the accumulation and stabilization of SOM. Because SOM accumulation occurs over decades and centuries it is a long-term study by necessity, but plots are being used to answer short-term questions as well. For example, DIRT revealed inputs from roots are roughly equal to the inputs from litter (Nadelhoffer *et al.* 2004). Studies from plots in Bousson Forest, Pennsylvania and Harvard Forest, Massachusetts, have suggested some trends may be detectable as early as 5 years (see below) (Nadelhoffer *et al.* 2004). A recent look at Dr. Holes' original plots in Wisconsin, now 50 years old, suggest important trends continue to develop (Townsend unpublished data), revealing the art of organic matter dynamics in the soil ecosystem.

It is the hope that research conducted in the early stages of DIRT plots may help identify trends and find parallels across ecosystems. In some cases experiments show soils may be slow to respond to such manipulations. Holub *et al.* (2005) showed that five years was not enough to reflect a change in net or gross nitrogen transformations, nor did five years show change in percent carbon and nitrogen in the mineral soil (Nadelhoffer et. al. 2004). In other cases, responses from detrital manipulations are identified early in the study. Four years were enough to show increased respiration rates in double litter soils and decreased respiration in no root and no input plots during laboratory observations (Nadelhoffer *et al.* 2004). A field respiration study

carried out on the HJ Andrews Experimental Forest plots during the fourth through sixth years after plot establishment, revealed changes in respiration rates as well (Sulzman *et al.* 2005). In addition, Sulzman *et al.* (2005) found evidence to suggest a priming effect, in which some kinds of inputs stimulate metabolism of more stable SOM. Soil priming is identified by an increased release if C-CO_2 from microbial respiration.

1.3 *HJ Andrews: First Decade Project*

Upon death the tissues of living organisms return to the soil. Here organic tissues are broken down and their essential nutrients recycled. Some nutrients are immobilized in new organic tissues immediately. Others may be dissolved in percolating water and washed to new locations while some are released back into the atmosphere. It is the organic materials that are bound tightly to the mineral soil and/or stored in the soil in great volume – specifically nitrogen and carbon – that are of interest in this project.

One of the essential building blocks for life is nitrogen (N). Though found in abundance in the atmosphere, nitrogen in terrestrial systems is often a limiting nutrient. Necessary for protein synthesis, and thus life on this planet, atmospheric N (gaseous N_2) is the most abundant form of N but is unusable by many organisms. Life forms that cannot process N_2 on their own depend on "nitrogen-fixing" bacteria that convert N_2 into more readily available forms, such as nitrate (NO_3^-) and ammonium (NH_4^+). Once fixed, other bacteria act in the recycling process discussed above. These bacteria need both carbon (C) and N for themselves, but it is not often found in proportions ideal for their needs. When C:N ratios are high in detritus C is much more abundant than N and the bacteria hold tight to N; it is only when C:N ratios are low (a relative abundance of N) that microbes will leak N to the soil (Brady and Wells 2005).

N may often be limiting, but not all C sources are created equally. C is essential. It makes "organic" organic and is cycled through the air, water, and soil. C is found in many compounds in living tissues and to a decomposer C sources have differing "quality" (Brady and Wells 2005). Quality may in part describe the C:N ratios discussed above but may also indicate the presence of lignin and polyphenols that are resistant to decomposition (Brady and Wells 2005). The availability of N, in addition to the presence and quality of C dictate the rate of nutrient turnover and may affect the dynamics of biogeochemical processes. For example, an abundant N source coupled with high quality C would cycle rapidly compared to an abundant N source coupled with very low quality C. The rate of cycling is a direct limiter to the amount of C stored in the soil.

The HJ Andrews Experimental Forest (HJA) in Blue River, Oregon (see site description in Methods) is one of twenty-six sites forming the Long Term Ecological Research (LTER) network (LTER 2007). A DIRT plot was established here in 1997 by Dr. Kate Lajtha, Dr. Phil Sollins, and Bruce Caldwell at Oregon State University. This site shares the DIRT goal of studying the accumulation and stabilization of SOM over time. The treatment types on this site are double litter, double wood, control, no litter, no root, no input, and O/A-less (please see Table 1), though for the purposes of the current study, O/A-less plots were not included. As HJA is the only coniferous DIRT site, it may provide unique insights on this subject.

Table 1: Detritus Manipulation Treatments at the HJ Andrews Experimental Forest

Treatment	Abbreviation	Description
Double Litter	DL	Litter removed from no litter plots is partitioned by area and spread over double litter plots
Double Wood	DW	Additional wood is added to these plots
Control	CT	No manipulation
No Litter	NL	Plots are covered with screens, which are swept annually
No Roots	NR	Plots are trenched to remove influence from roots
No Inputs	NI	Plots are both screened and trenched
O/A-Less	OA	The Organic and A horizons have been removed, leaving the B horizon exposed

While ten years is short in terms of soil, it is somewhat significant in terms of humans and should not be disregarded. The purpose of the research described in this paper was to quantify the distribution of C and N after ten years of detrital manipulation, and characterize any changes or trends. We did this by:

- *Quantifying N movement and immobilization by measuring net mineralization rates;*
- *Identifying trends in the spread of C and N pools in relation to the mineral soil using sequential density fractionation;*
- *Quantifying the labile C pool by short-term laboratory respiration; and*
- *Quantifying the recalcitrant C pool by acid hydrolysis.*

Identifying trends early is critical for the establishment of a timeline and comparing trends seen in coniferous HJA to those seen in other plots.

2 Methods

2.1 *Study Site*

The HJ Andrews Experimental Forest (44°15'N, 122°10' W) is located in the central Cascades near Blue River, Oregon USA. The DIRT site is in old-growth coniferous forest at 531 m elevation (Sulzman *et al.* 2005). Dominant overstory tree species are *Pseudotsuga menziesii* (Douglas-fir) and *Tsuga heterophylla* (western hemlock). *Thuja plicata* (western red cedar) and *Taxus brevifolia* (Pacific yew) are also found in the stand. Dominant understory species include *Acer circinatum* (vine maple), *Cornus nuttallii* (Pacific dogwood), *Vaccinium* spp. (huckleberry), and *Polystichum munitum* (sword fern) (Sollins *et al.* 2006). *Rhododendron spp.*, and *Oxalis spp.* are also present (Fig. 1). The climate is Mediterranean, characterized by cool, wet winters and warm, dry summers (Sollins 2006) with an average annual temperature of 8.7°C during the period 1973-2002 measured at the HJA headquarters (Sulzman *et al.*2005). During the same period average annual precipitation was 2,370 mm, mostly as rain (Sulzman *et al.* 2005).

Fig. 1 A "No Litter" plot at the HJ Andrews Experimental Forest. Visible are Douglas-fir, western red cedar, and vine maple, as well as sword fern and oxalis.

Soil samples were collected July of 2007 by first removing the O-horizon and taking cores from the 0-10 cm zone of the A horizon. Each treatment is replicated three times in the study site so n=3 for each treatment (Appendix B). We took four cores from randomly selected locations on each treatment plot (18 plots total) and homogenized them, yielding one representative sample for each treatment plot. Moist soil was kept chilled until return to the lab, where it was sieved to 2 mm. Gravimetric water content for each homogenized sample was determined by oven-drying the soil at 105^{o}C (Jarrell *et al.* 1999).

2.2 *Short-term Potential Nitrogen Mineralization (Net)*

Experimental Design

To determine the potential nitrogen mineralization we used a variation of the short-term (28 day) static incubations described in Robertson *et al.* (1999). We added water to bring the newly-collected, sieved soil to field capacity. From each sample, we took four sub-samples of 15-20 g each and divided them into two sets of two: two for immediate termination, and two for incubation. The sub-samples do not provide true replication, but rather are for quality control in later analysis. In addition, we oven-dried a small amount of soil from each plot at 100^{o}C in order to correct for soil moisture in later calculations.

In order to terminate microbial activity, the first set was immediately submerged in 100 mL of 1M KCl and left on a shaker table set to high for 1 h then allowed to equilibrate for 16 h. The next day samples were filtered with GF/F glass filters, which had been pre-combusted at 500^{o}C for 1 h in a Barnstead Thermolyne 1500 furnace to eradicate any organic residues. Following filtering samples were frozen until later analysis.

The second set of samples was incubated in the dark for 28 days. These samples were covered with semi-permeable plastic wrap to allow for air exchange without excessive moisture loss. Moisture loss was monitored periodically by sample weight. We added 0.5 mL of DDI water uniformly on day 8 to bring samples back to field capacity. After 28 days microbial production was terminated and the solutions filtered using the method outlined above.

Analysis

Net NH_4^+ and NO_3^- production was determined using an Orion autoanalyzer. We used standards with concentrations of 0.025ppm, 0.05ppm, 0.075ppm, 0.15ppm, 0.2ppm, 0.6ppm for NH_4^+ and 0.1ppm, 0.25ppm, 0.05ppm, 1.0ppm, 2.0ppm, and 4.0ppm for NO_3^-. Peaks were interpreted using a linear regression equation for the standards (correlating each peak height to a known concentration) where y was the estimated PPM based on x, the peak height.

Our rates are reported on a gravimetric basis, μg $NH4^+$/$NO3^-$ per g dry soil (this is the same as mg nutrient per kg dry soil), as given in Robertson *et al.* (1999):

$$\mu g \text{ element/g soil} = (I*V)/W$$

where I is the concentration of ion in extract in mg/L (mg/L = PPM); V is the volume of extract in mL, plus soil water; and W is the dry mass of soil. It is important to note soil moisture as well as water added to bring the samples to field capacity must be included in the volume of extract (*ie*, 100mL 1M KCl + mL additional water + mL soil moisture).

We averaged the duplicates for each plot, and then averaged the plots for both initial and incubated concentrations of $NH4^+$ and $NO3^-$. Our rates of ammonification and nitrification are

expressed in µg $NH4^+/NO3^-$ per g dry soil per day. Net mineralization is the difference between nitrogen and ammonium over the course of the incubation, so that

$$\text{Net N Mineralized} = [(NH4^+_{final} + NO3^-_{final}) - (NH4^+_{initial} + NO3^-_{initial})]/ 28 \text{ days}$$

(Robertson *et al.* 1999)

It is appropriate to show such results with standard error (SE). In this experiment n=3 for all values.

2.3 *Short-term Potential Respiration (C Mineralization)*

Experimental Design

Respiration potentials were determined using a variation of the method described by Robertson *et al.* (1999). Two sub-samples of each treatment were taken of 25mL or approximately 15-18 g and each was transferred to its own 50 mL Erlenmeyer flask. The consistent volume was necessary for determining headspace in analysis (Fig. 2).

Fig. 2 Short-term respiration setup, shown with septum caps

Samples were kept with open dishes of water to maintain humidity and prevent soils from drying. Samples were incubated in the dark and respiration rates were recorded every other day over the course of a month. We used an injection volume of 1 mL in a Hach Carle™ series 100 gas chromatograph. We ran standards of 500 µL, 400 µL, 300 µL, 200 µL, 100 µL, and 50 µL of 1% CO_2 from Scott Gas™ at the beginning and end of each run to establish correction peaks. For the first two runs we capped our samples at time of first respiration measurement and left them for approximately two hours between samples, keeping a record of the exact number of minutes. Because of low sensitivity in the gas chromatograph, samples were later capped the night before they were to be run. The low concentrations of CO_2 belayed concerns over anaerobic conditions.

Analysis

We used the integrated peak areas given by the integrator for known concentrations from the standards to interpret CO_2-C concentrations in the samples. We used a linear regression model for the standards, where y is the estimated µL of CO_2-C per mL sample based on x, the peak area. Final peak area subtracted from initial area yields the CO_2-C respired by the sample between the two times. When reporting the rate of respiration, the mass of dry soil being measured, the headspace in the flask, and the number of minutes (which can be converted to days) allowed between sampling events must be accounted for. In addition, because this method reported CO_2-C in µL/mL instead of PPM, we had to perform a conversion to mass units. We used an equation adapted from Introduction to Soils (2006), where:

Respiration (µg CO_2-C/day/g soil or g C) = Net Respiration (CO_2-C µL/mL) * 32 (mL headspace) * [1/22,414(mol CO_2/mL CO_2)] * 44.01 (g CO_2/mol) * [12.01/44.01 (g C/g CO_2) * 100,000 (µg/g) * [1/ #min] * 1440 * [1/g dry soil or 1/g C in sample]

We compared our treatments both on a per g soil basis, and on a per g C in soil basis. Grams of C per g soil were found using percent carbon analysis from the bulk soils.

2.4 *Sequential Density Fractionation*

Experimental Design

The process of fractionating soil was carried out using sodium polytunstate (SPT or NaPT) after Sollins *et al.* (2006). We recycled SPT for fractionating using a variation of the technique described by Six *et al.* (1999) whereby used SPT was filtered through GF/F filters and dripped through sulfonated Lewatit Monoplus™ 2-Propentrile resin. The filtrate was condensed at 100^{o}C and filtered again through GF/F filters.

A sub-sample of each soil was oven dried to 50^{o}C so that the percent moisture in the bulk soil could be accounted for when comparing oven-dried fractions later. We used 150 mL centrifuge vials for 50 g of soil. Solutions of SPT were prepared at 1.65 g/cm^3, 1.80 g/cm^3, 2.00 g/cm^3, 2.40 g/cm^3, and 2.65 g/cm^3. We used a pipette method to determine density by measuring 1000 µL of SPT solution at room temperature. Samples were shaken in 1.65 g/cm^3 SPT solution for 2 hours on a shaker table at a low setting. They were spun at 3,000 rpm in an Avanti J-HC centrifuge for 10 min. The floaters were aspirated and the pellet preserved for the next density fractionation. Any materials to float at a given density were filtered on GF/F filters with at least one L of water or until bubbles caused by SPT ceased to appear. The density fraction was transferred to a pre-weighed aluminum tin, and dried in the oven at only 50^{o}C to preserve soil structure. This process was repeated for every density except for densities $\geq$ 1.80 g/cm^3 samples were only shaken for 1 hour in the SPT solution. At higher densities, samples were rinsed in the centrifuge until the density of the rinse solution was at or below 1.0 g/cm^3. Once dry, soil fractions were weighed, ground in a Certiprep Mixer/Mill 8000 and transferred to scintillation vials. They were sent out to be analyzed for C and N using a CHN detector. As the process is quantitative – theoretically all fractions would add to 100% of initial soil mass – it was important to conserve soil in all steps.

Analysis

The mg of C/g soil was determined using the following equation:

[g dried soil in fraction*%C as determined by analysis*10]/dry g soil fractioned

The same equation was used to determine mg N/g soil, but with %N as determined by analysis instead.

The mg C in fraction/g C in bulk soil was determined using:

[g dried soil in fraction*%C as determined by analysis*10]/[dry g soil fractioned*%C bulk sample as determined by analysis/100]

The same equation was used to determine mg N/g N in bulk soil but with %N as determined by analysis instead.

Because of missing plot samples n=3, 2, and 1 for treatments throughout the data.

2.5 *Acid Hydrolysis*

Experimental Design

Approximately 50g of bulk soil were measured into 50mL plastic centrifuge tubes. Soil was density fractionated at 1.65g/mL using SPT (as outlined above) to remove the dominant organic influence from the light fraction. Samples were dried in an oven at 50^{o}C for 48 h to remove moisture without damaging soil structure. Two sub-samples of 2 g for each sample were transferred into glass hydrolysis tubes. We added 25 mL of 6 M HCl and incubated samples on a block digester for 18 hours at 100^{o}C. Following incubation, samples were rinsed with at least 300 mL DDI water that was filtered over samples on baked GF/F filters (as described above).

Samples were dried at 50°C, ground in our Mixer/Mill 8000, and stored in scintillation vials for C analysis using a CHN detector.

Analysis

The non-hydrolysable (stable) C pool was simply the C remaining after hydrolysis but the %C returned by the CHN detector were corrected for by the %C for the initial sample. To report the percent of the C pool that is recalcitrant:

$$\% C_R = (M_B * \% C_B)/(M_H * \% C_H) * 100$$

Where C_R is the recalcitrant C; M_B is the mass of the bulk soil in g; C_B is the bulk C; M_H is the mass hydrolyzed soil recovered; and C_H is the hydrolyzed C.

This is the percent C that is recalcitrant or stable. We averaged plots to analyze by treatment last. For NI only, n=2 because of inadequate sample size.

3 Results

3.1 Short-term Potential Nitrogen Mineralization (Net Mineralization)

N turnover rates in soil extracts varied greatly across treatments. NH_4^+-N was the most prevalent form of N across all plots (Fig. 3). CT, DL, and DW plots tended to produce higher net levels of NH_4^+ than other treatments (Fig. 3*a*). An inverse relationship was apparent between ammonification and nitrification on all plots except NL (Fig. 3*b*). Trenched plots (NI and NR) produced more NO_3^--N/kg dry soil/day than any other plot (all other values hover around 0). The highest rates of net mineralization and nitrification and the lowest rates of ammonification were measured in the extracts from the NI and NR plots. The differences between other plots appear insignificant, though the CT may have a slightly higher mineralization rate than the DL, DW, and NL plots.

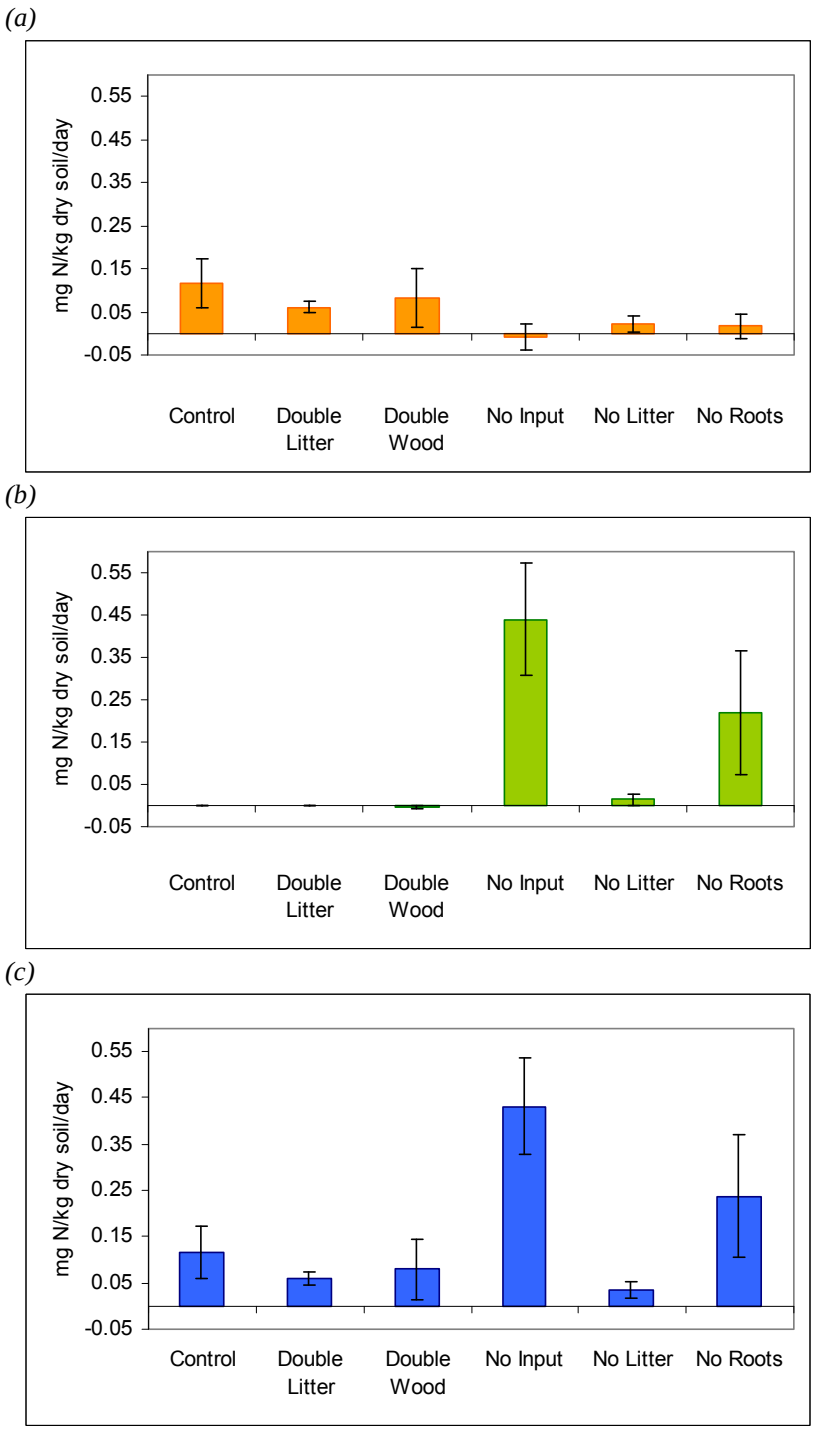


Fig. 3 Rates of net ammonification (a), nitrification (b), and mineralization (net production). 0-10 cm, A-horizon. Bars indicate ±1 SE.

3.2 *Short-term Potential Respiration (C Mineralization)*

Respiration results were of questionable use. Although calibration curves were consistently good with R^2-values on most days around 0.98 (Fig. 4), much of the headspace data are nonsensical. We recorded extreme differences between sampling events; for example, on day 3 (July 27) sample 1B had no peak for measurements taken at either 10:58 or 11:45. At 14:18, approximately 2.5 h from its last sampling, the area under the curve was 1,686 units. We took a final measurement at 14:46 and graphed an area of 8,478 units – a difference of what works out to be just over 3 μL CO_2 in approximately 0.5 h following at least 3 h with no measurable respiration. In addition, respiration chronographs of the two sub-sets (with identical plot representation), were inconsistent (Fig 5).

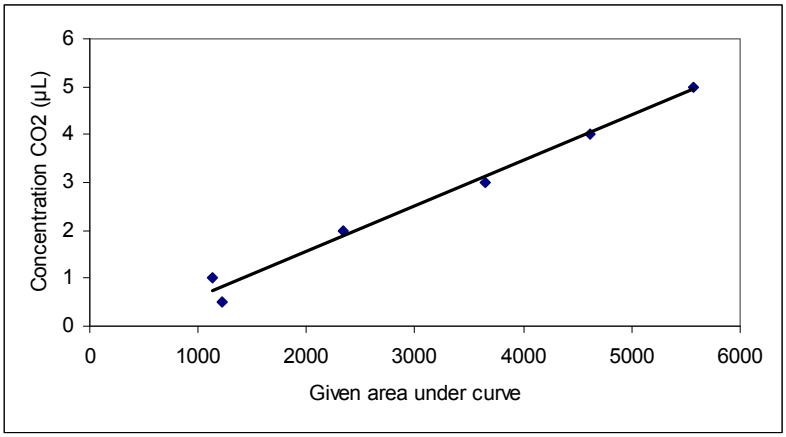
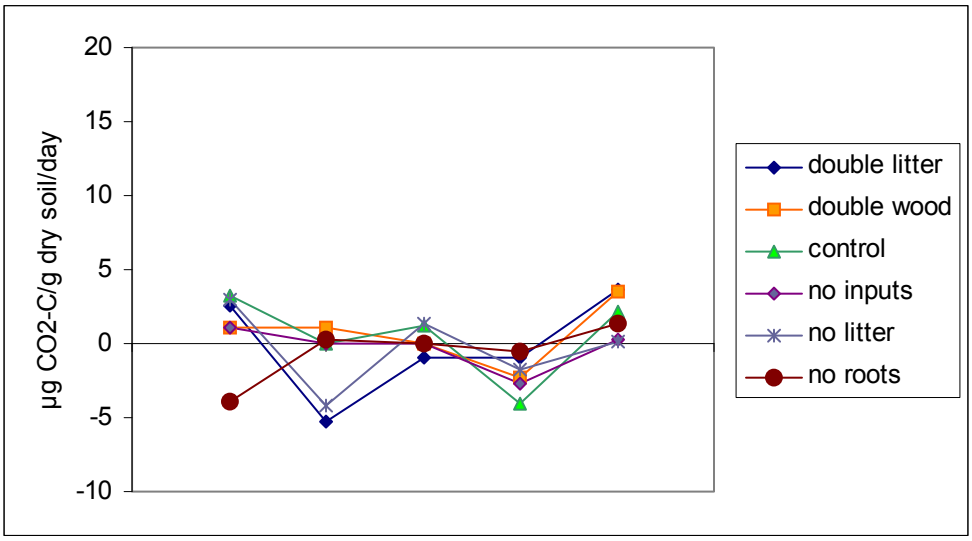


Fig. 4 Example calibration curve for CO_2 concentration. Shown for set 1, day 19 (Aug. 7). $Y = 0.0009x - 0.3427$, $R^2 = 0.9865$.

The most obvious issue with the results was negative respiration (Fig. 6). Respiration on a per g C scale did not change the relationship between treatments (Fig. 6). Some possible causes for error follow in the discussion.

(a)



(b)

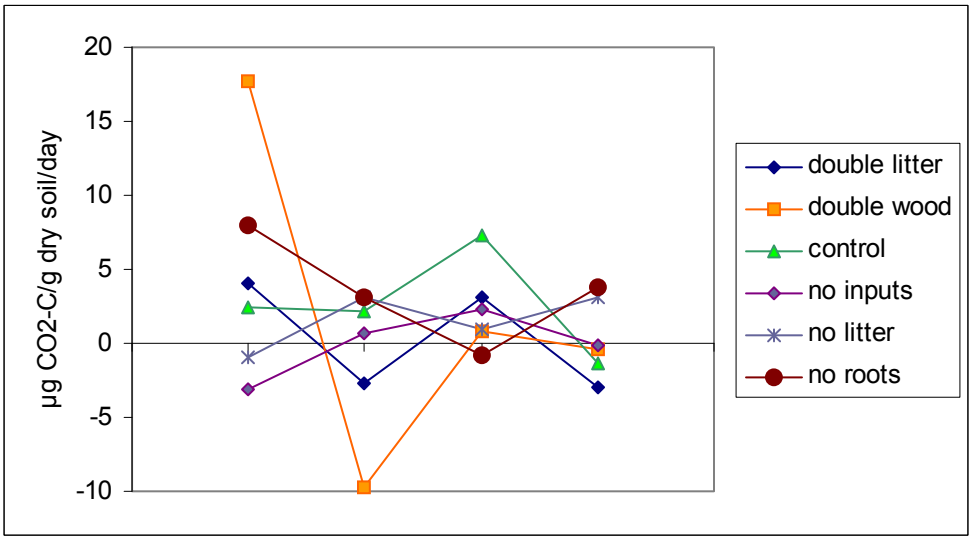
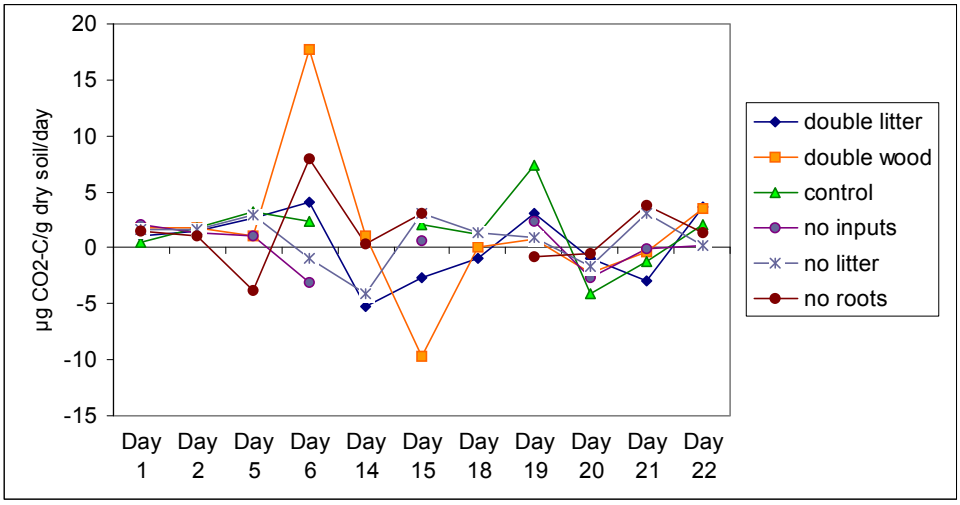


Fig. 5 Comparison of chronographs for respiration rates of set 1 (a) and set 2 (b). Sets contained one each of all treatments.

(a)



(b)

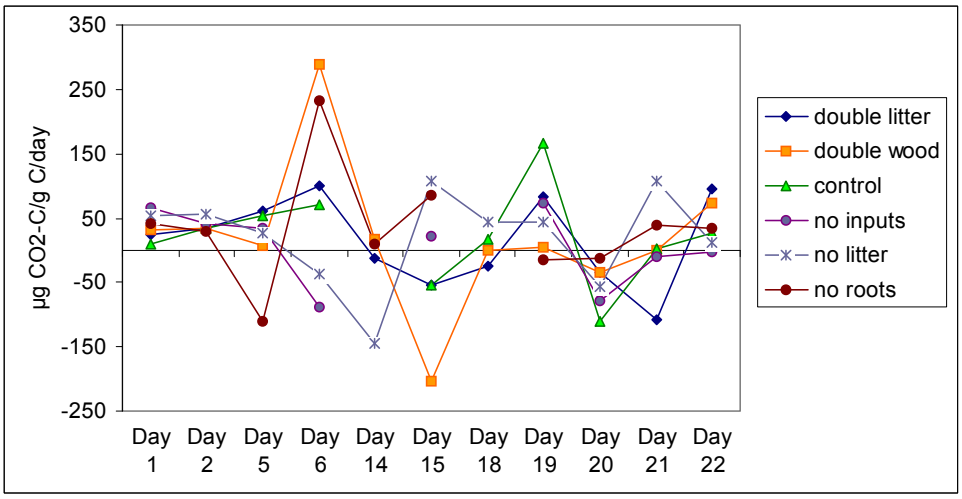
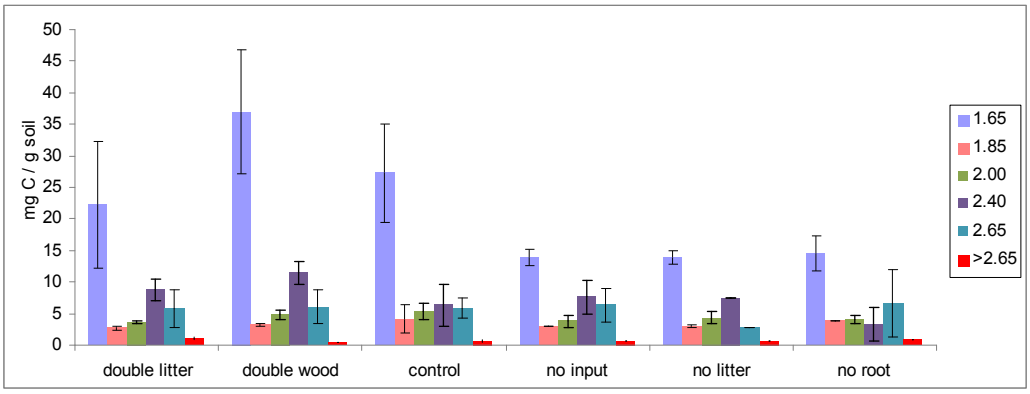


Fig. 6 Short-term respiration per g dry soil (a) and per g C in soil (b). 0-10 cm, A-horizon. Note change in scale.

3.3 *Sequential Density Fractionation*

DW appears to be the most influential treatment at this time. A trend may be developing in DW towards an increased C pool in the 1.65 g/cm^3 and the 2.40 g/cm^3 fraction when compared to CT (Fig. 7). This trend is less apparent when C is normalized by the C in the bulk soil (Fig 7).

(a)



(b)

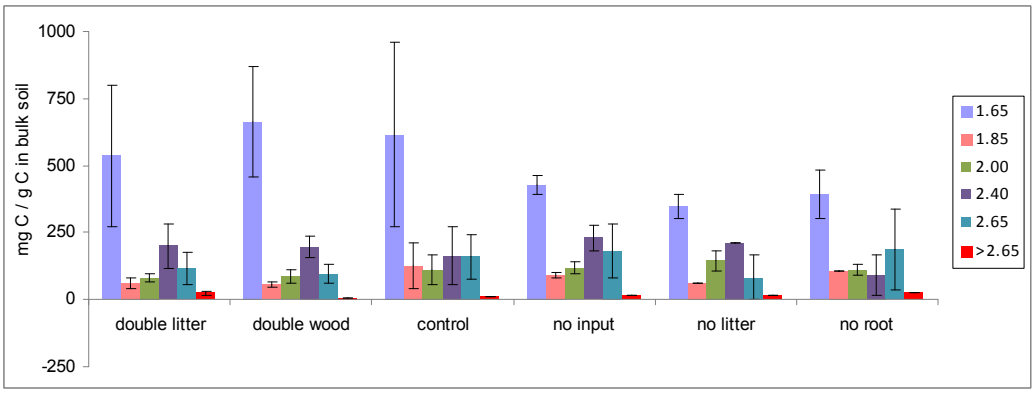
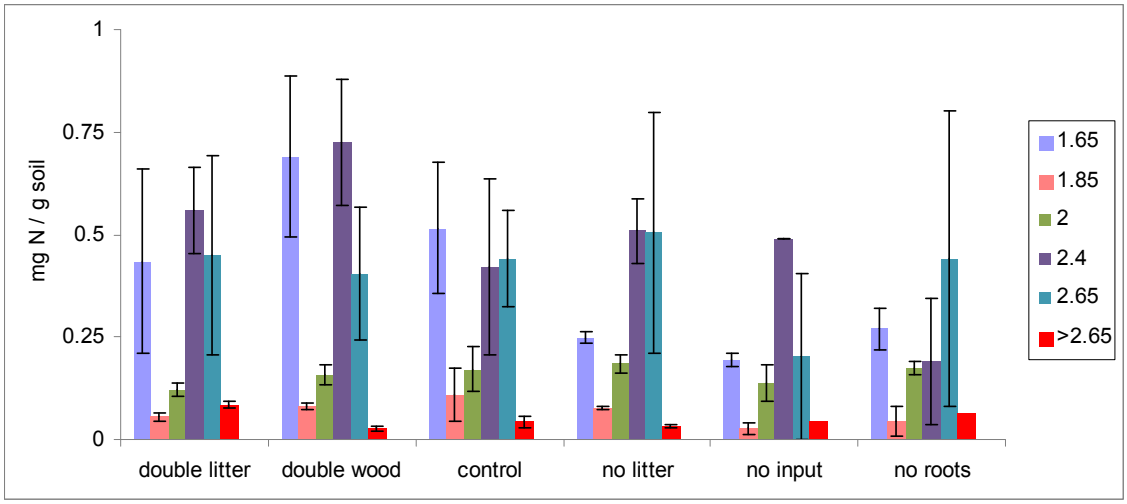


Fig. 7 Comparison of C standardized by g soil (a) and by g C in bulk soil (b) by fraction across all treatments. Bars indicate ±1 SE.

The N pool was also largest in the 1.65 g/cm^3 and 2.40 g/cm^3 fraction of the DW and DL treatment when N is normalized by g of soil (Fig. 8). Per g soil, N in the 1.65 g/cm^3 fraction of NI, NR, and NL is lower than in the CT but does not appear to be so in any other density cut, except perhaps NR at 2.40 g/cm^3. In fact, NI and NR appear slightly higher than CT in the 2.40 g/cm^3 fraction.

(a)



(b)

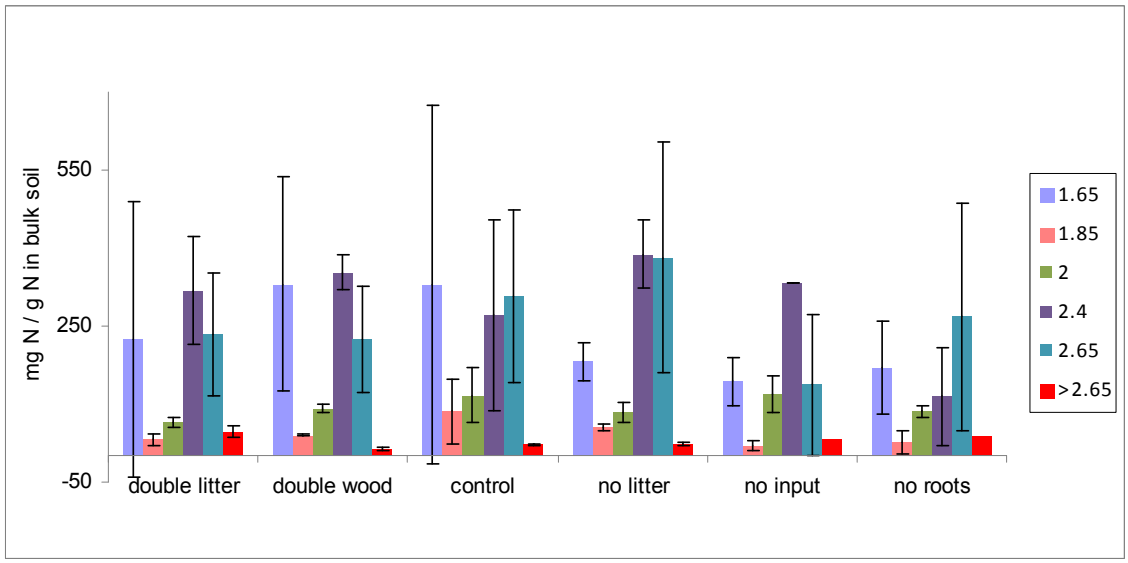


Fig. 8 Comparison of N standardized by g soil (a) and by g N in bulk soil (b) by fraction across all treatments. Bars indicate ±1 SE.

N pools across treatments are much less uniform than C pools in size and location within fractions across treatments (which may be visualized as the size of the pools per fraction as a percent of the total C and N in a treatment [Fig 9]). A trend in declining C:N ratios is uniform across all treatments as fraction densities increase to about 2.40 g/cm^3 (Fig. 10). Bulk NL showed the greatest reduction in C:N (Fig. 10).

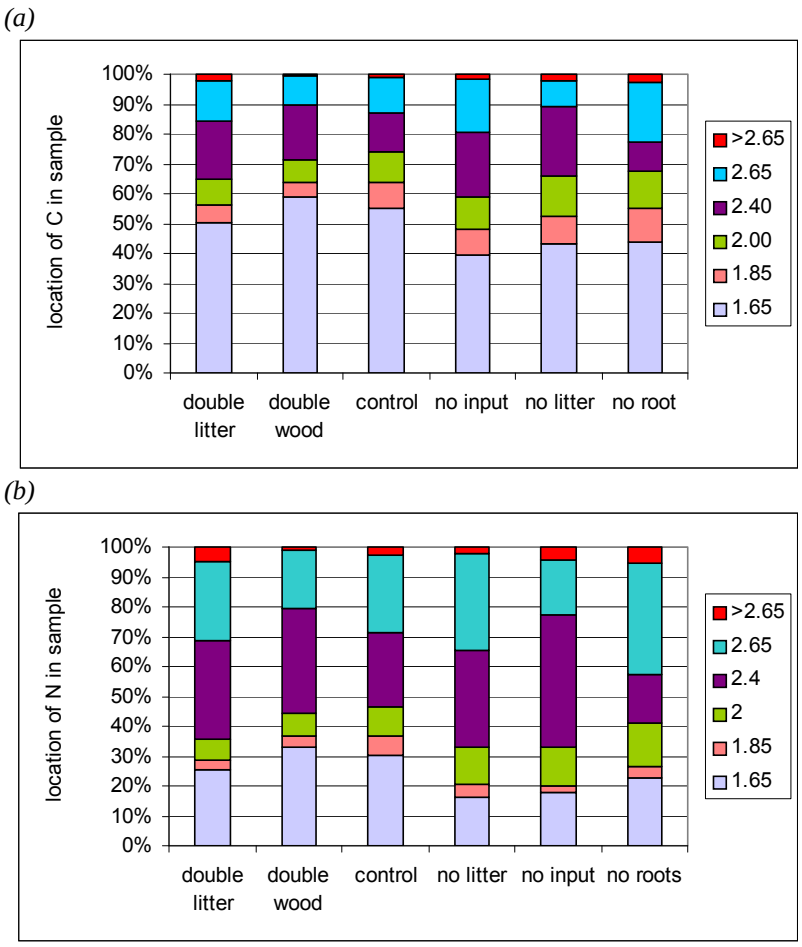


Fig. 9 Location of C (a) and N (b) pools as a percent of the total C and N in the treatments.

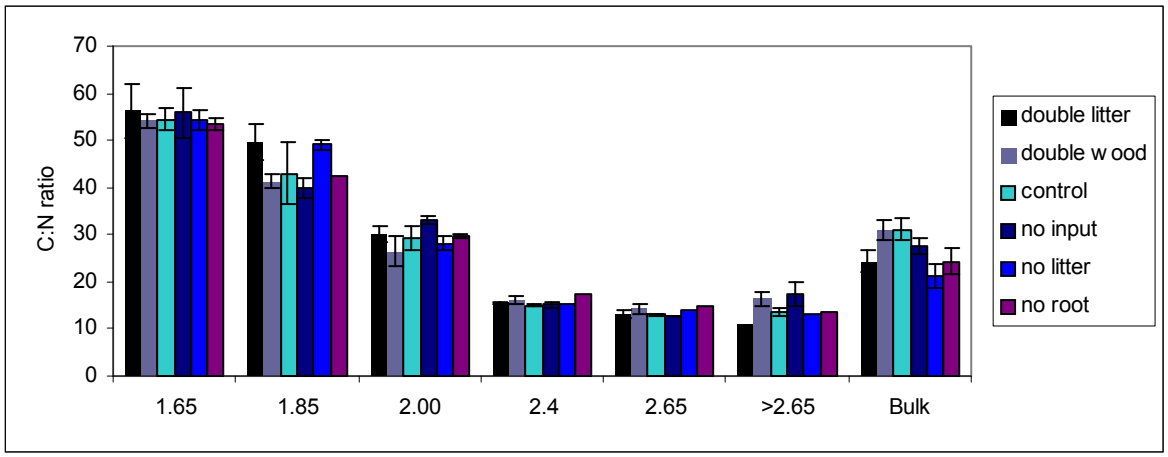


Fig. 10 C:N ratios of all treatments across densities fractions. Bars indicate ± 1 SE.

3.4 *Acid Hydrolysis*

The percent recalcitrant C was similar to other forest soils (see discussion) and did not vary much, except for between NI and CT (Fig. 11). Variation between g C in the pseudo-replicates was similar to variation in g C in hydrolysable and non-hydrolysable soil (Fig. 12).

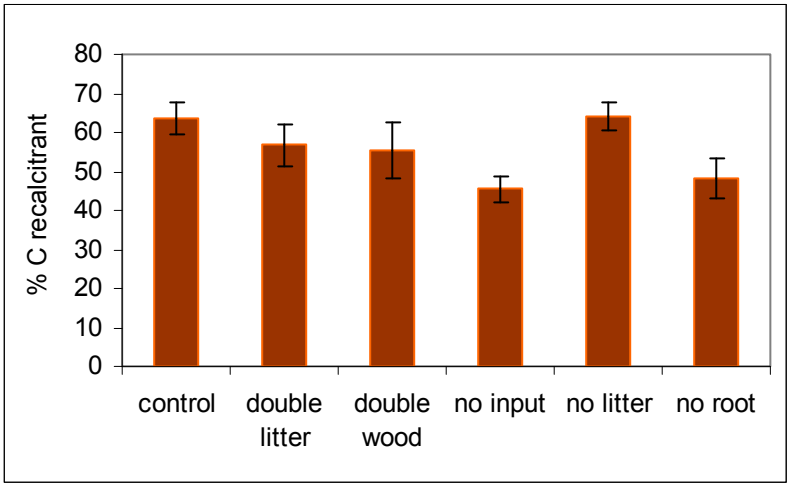


Fig. 11 Recalcitrant C as a % of initial sample C (light fraction removed). Bars indicate ±1 SE.

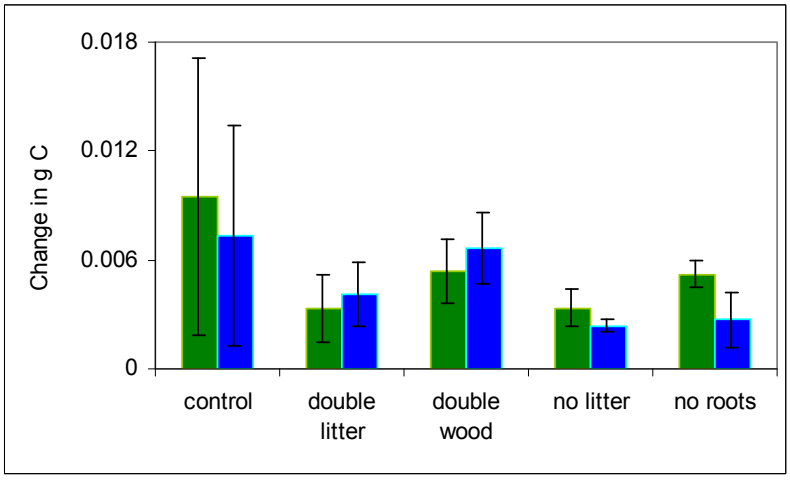
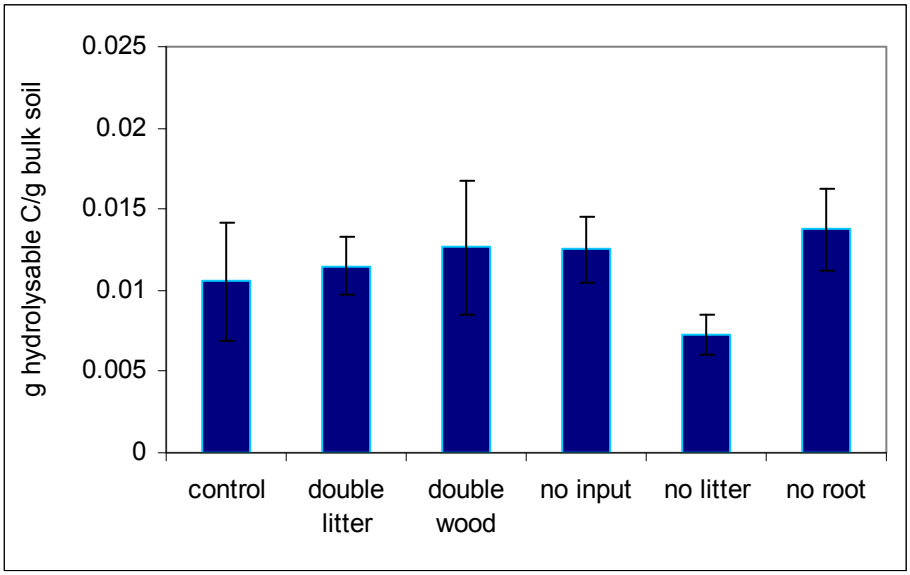


Fig. 12 Absolute value of the difference in g C between pseudo-replicates (green) and between pre- and post- hydrolysis soils (blue). No Input (NI) omitted due to lack of pseudo-replicates. Bars indicate ±1 SE.

With the light fraction removed, there is slightly much more recalcitrant C in the mineral soil than hydrolysable (Fig 13).

(a)



(b)

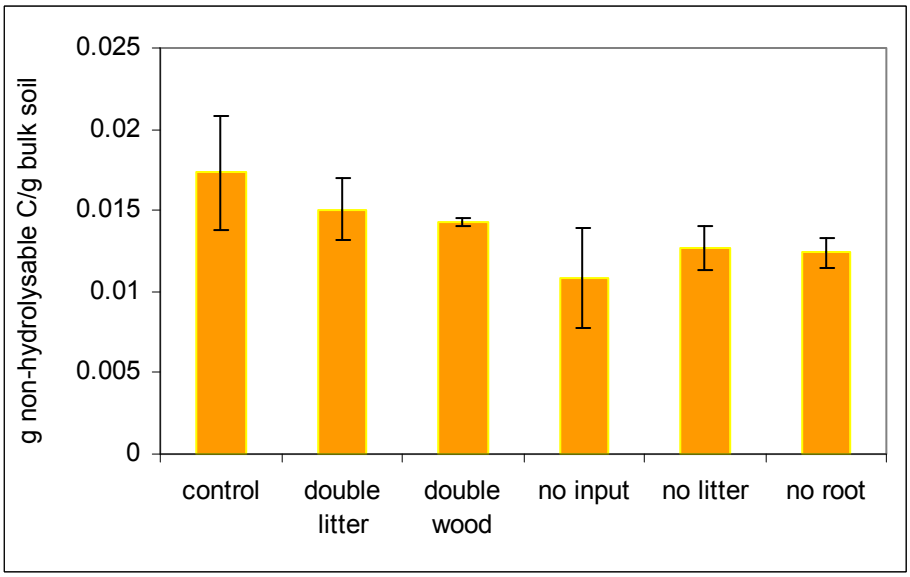


Fig. 13 Mass of hydrolysable C (a) and non-hydrolysable C as a function of the mass of bulk soil (a). Bars indicate ±1 SE.

4 Discussion

In this study we measured mineral soils in a laboratory setting. Any differences in N-related immobilization would mean treatments are affecting the N pools in mineral soil. A laboratory observation also indicates that all observations are independent of active contributions from the rhizosphere, whether that is the intention of the treatment or not. (The rhizosphere is the area surrounding the roots and is an extremely active zone both chemically and biologically [Brady and Well 2005]). In addition, comments from these observations are limited in scope without gross N transformation rates.

Net N transformations are a measure of the difference between gross N transformations and immobilization of N in organic tissues. A small difference therefore is indicative of balance between the two, whereas a large difference indicates imbalance. In our case, the highest nitrification and mineralization rates were observed on the NR and NI plots, those plots without roots. Previous study at this site showed an increase in dissolved organic N (DON) on the plots without roots (NR and NI) and a decrease in dissolved organic carbon (DOC) (Lajtha *et al.* 2005). It was suggested by Lajtha *et al.* (2005) that the labile C from the rhizosphere, high quality C from both root and mycorrhizal activity (Lajtha *et al.* 2005), is of greater importance in immobilization of N than is the bulk SOM. The high net N transformation on these plots combined with previous findings of high rates of DON loss implies it is the immobilization of N and not the gross transformation of N that is lowered by the elimination of the rhizosphere.

Net mineralization in the DL and DW plots is not yet significantly lower than in CT but may be showing a trend towards lower mineralization for the inverse reason: high immobilization rates from an increase in C. Indeed, Lajtha *et al.* (2005) found an increase in DOC on the DW plots at this site but no increase in DON. It seems plausible the DW plots would be moving towards

higher rates of N immobilization. The most notable result of net-N observations was that there were differences among plots. Holub *et al.* (2005) found no difference existed after five years or less on either the HJA or Síkfökút plots. At ten years, however, it is possible that soils are beginning to show a response to treatments at the coniferous site.

We hypothesized that the DL and DW plots would have higher soil respiration rates due to a more active C pool than the CT and that those plots without high quality inputs (NR, NI, and NL) would have much less labile C and thus lower respiration rates. Field observations in year six of the HJA DIRT site showed the DL, DW, and NI plots were diverging in a statistically significant way, however NR and NL had yet to truely respond (Sulzman *et al.* 2005). Unusable data from soils collected in year ten prevent us from commenting on any further development in these trends. An incomplete picture of this soil C pool is also unfortunate as evidence for priming was observed by Sulzman *et al.* in 2005. The "priming effect" (Bingeman *et al*. 1953) is the stimulation of increased SOM decomposition by addition of fresh organic matter (Fontaine *et al*. 2003). Our density fractions show possible evidence for this phenomena and so the subject will be revisited below.

We are unsure about what caused the trouble with measuring consistent values for respiration treatments as shown in the results; because of consistently good standard curves it is hard to blame the gas chromatograph alone. We conjecture the negative respiration was a result of inadequate intervals between sampling events, indicative of very low respiration overall. Low respiration rates however further minimize the concern of anaerobic conditions damaging the viability of soil organisms. The soil was kept cool and moist for approximately a week prior to the commencement of these observations; this may explain low respiration rates, however microbial activity should have been revived when brought to room temperature and field capacity.

What has not been discussed yet is the potential for C saturation, a theoretical maximum for stabilized C in any given system. Multiple SOM stabilization models have been explored over the years; Sollins *et al.* (1996) proposed three mechanisms for the control of stabilization and destabilization of SOM: *recalcitrance*, a chemical resistance inherent in some organic molecules to microbial decay; *interactions*, the chemical reactions between organic-organic and organic-inorganic molecules that change their decomposability; and *accessibility*, which refers to the physical location of the SOM relative to microbes. In a similar nature Six *et al.* (2002) propose three ways in which soil stabilizes C based on an extensive review of the subject: chemical stabilization, which are chemical associations with clay and silt complexes that bind SOM to mineral surfaces; biochemical stabilization, a combination of the inherent and acquired resistances of organic matter to decomposition; and again, physical protection. Physical location may influence both the presence of bacteria (as reviewed in Six *et al*. 2002), and local environmental conditions; for example anaerobic pockets occur in the interior of soil aggregates (Sextone *et al.* 1985). In addition to properties of the soil and organic matter, disturbance is a major factor in increasing the rate of SOM mineralization (Six *et al*. 2002), something agriculturalists have been aware of for some time (Bingeman *et al*. 1953). The ability of a soil to stabilize C might therefore depend on the amount and quality of inputs (such as the DIRT plot is experimentally manipulating), but also on the texture and clay content of the soil, presence of organomineral complexes, and its history of disturbance (Six *et al.* 2002).

The HJA plots are in old-growth coniferous forest with little disturbance and high inputs of varying quality under historical conditions. By altering the inputs over the last decade we know we have altered some outputs from the soil and soil microorganisms (respiration levels [Sulzman *et al*. 2005], DOM [Lajtha *et al*. 2005], mineralization rates [current study]) but have we caused an alteration in SOM stability? Or, have we altered the C:N ratio, shifting the parameters of a

biologically meaningful pool (Sollins 1999)? Sollins *et al.* (2006) showed that much of the difference between density fractions is due to a decrease in the C content of fractions. The decrease in C:N is directly related to a decreasing thickness of the organic layer in addition to change in mineralogy (Sollins *et al.* 2006). The idea of a sheath of organic matter is part of the proposed "onion" model, whereby an organic and highly stable, likely proteinaceous layer on the surface of minerals creates a more active surface, further increasing the layering of organic material, and consequently, decreasing particle density (Sollins *et al.* 2006). By fractionating and hydrolyzing the soil we are hoping to define the current SOM pool parameters and the level of SOM saturation on the site so we can measure any changes that occur. Shifts in the features of density layers, such as SOM content, its influence on further SOM stabilization, and the association of microbial communities with the SOM pools at different densities, could help us estimate the potential C sequestration of old-growth coniferous forests. Further, by altering chemical and biological interactions at the aggregate level, we may see how altering the inputs changes (or does not change) this stabilization capacity.

The usefulness of hydrolysis lies in its ability to isolate the biochemically stable fraction of C. The hydrochloric acid digests organic compounds thought of as relatively labile, such as proteins and polysaccharides (Six *et al.* 2002). Its short-coming is that the acid does not digest the aromatic compounds in young C sources, such as those found in lignins (Sollins *et al.* 1999. We removed the light fraction (1.65 g/cm^3 or all visible plant material) in order to remove the bulk of young, undecomposed C from the soil before we attempted to isolate the recalcitrant pool from the truly mineral-bound organic pool, which is common practice (Bruun *et al.* 2008; Helfrich *et al.* 2007; Six *et al.* 2002).

The method of isolating the recalcitrant C pool through acid hydrolysis is actually somewhat contentious. Opponents make the claim that the non-hydrolyzed pool is not always old, nor does

hydrolyzing preferentially remove new C (Bruun *et al.* 2008; Balesdent 2006). In the case of Bruun *et al.* (2008) however, the light fraction was not removed prior to acid treatment, and as they themselves point out, this affected their results. Advocates of acid hydrolysis argue that ^{14}C dating in multiple studies show the non-hydrolysable fraction contains very old C and since the acid digests labile C it is suitable to the purpose at hand (Six *et al.* 2002). Both Bruun *et al.* (2008) and Balesdent (2006) stated that physical fractionation is a more accurate assessor of stable SOM than any chemical methods. Bruun *et al.* (2008), however, who tested both chemical and physical fractionation methods, concluded none of the methods they tested were especially accurate in isolating the stable SOM pool. Helfrich *et al.* (2007) tested five chemical methods for isolating stable SOM pools, including acid hydrolysis. They reported all methods isolated some stable pool but none were fully suitable. Helfrich *et al.* (2007) however did remove the light fraction prior to acid hydrolysis and were a bit more optimistic about the results (Helfrich *et al.* 2007). Isolation of the stable SOM pool is clearly a work in progress. With no strong leaders, we chose to continue with the acid hydrolysis technique.

We saw high internal variation in our pseudo-replicates, almost equal to the variation seen between hydrolysable and non-hydrolysable percentages C. Our non-hydrolysable C pools are roughly equivalent to those reported for other forest soils (55% in Paul *et al.* [2006]; 53% in Tan *et al.* [2004]) except for a slight but notable increase in the CT and NL plots and decrease in the NI plots compared to these numbers. C in the NI plot is the least recalcitrant; compared to the CT it would seem to be decreasing, perhaps metabolizing C sources that were previously less favorable. At this point, data still appears contrary to suggestions by Crow and Lajtha (2004) that rates of decomposition may be slowing and consequently recalcitrant pools increasing in trenched plots. If these results are accurate, the next question might be is this a reflection of high levels of saturation? Will treatments affect the saturation with more time?

Sequential density fractions showed a distribution of C and N pools that were consistent in the light fraction with the treatments (that is, large C pools in DL, DW, and CT and smaller C pools in NI, NR, and NL) but otherwise only slightly altered in the "heavy" or mineral-associated states. C:N ratios decrease uniformly by fraction across treatments to about 2.40 g/cm^3 or 2.65 g/cm^3. This is consistent with a trend reported by Sollins *et al*. (2006), though our C:N appears to be stabilizing at a heavier density then theirs. C and N expressed by mass do not decrease uniformly; rather they decrease severely in the 1.85 g/cm^3 and 2.00 g/cm^3 fractions and then spike again at 2.40 g/cm^3, which is especially true of N. Though the high standard error in our results keeps these relationships from being significant, they remain a curiosity. Perhaps they are a reflection of increased N immobilization in microbial community associated with the chemical bonds developing at this density? The N spike is also roughly equivalent to the one reported by Sollins *et al.* (2006), however at a heavier density; while their peak appeared in a 2.28 g/cm^3 fraction, ours is not until the 2.40 g/cm^3 fraction. It would seem that something significant is developing in the 2.40 g/cm^3 fraction that should be explored further.

A decreased C pool in the 1.65 g/cm^3 fraction of the DL plot relative to both DW and CT plots seems to be forming. Though the trend was still weak, it is possible that this is further evidence of a priming effect. A decrease in C content though double the natural litter has been added to these plots implies elevated microbial activity. This is somewhat reflected by low net mineralization rates. It also agrees with other findings that it is C sources of a lower quality that stimulate priming, rather than those of higher quality (Fontaine *et al.* 2003). This begs the question, at what point (if ever) will the DW plots also show a priming effect? We found large pools of N in the DW plots, especially in the 1.65 g/cm^3 and 2.4 g/cm^3 fractions which are somewhat puzzling, as theoretically high C inputs would immobilize much of the available N.

A major concern in all our analysis using a CHN detector was the source of error. Whether the source is natural variability, mechanical error, or sample contamination, it would be an important piece of knowledge to more appropriately measure shifting C and N pools as well as this phenomenon in the future.

5 Concluding Thoughts

We have sought to characterize the C and N pools in an old-growth coniferous forest after ten years of detrital manipulation by measuring the rates of net C and N mineralization and by quantifying the C and N pools through mineral fraction and hydrolysis. Though we failed to identify an active C pool, we were able to successfully link current trends in net nitrogen mineralization rates to previously observed DON losses. We successfully quantified C and N pools by density fractionation that have not yet shifted significantly beyond the light fraction. These pools also may have provided further evidence in the ongoing effort to understand the priming effect. With the removal of the light fraction, we identified a high percentage of C that is recalcitrant and does not vary much by treatment. Experience with questionable data over the course of this research has invited us to think more critically about causes for error so we can provide clean, quality data to fellow and future researchers on this long-term ecological research plot.

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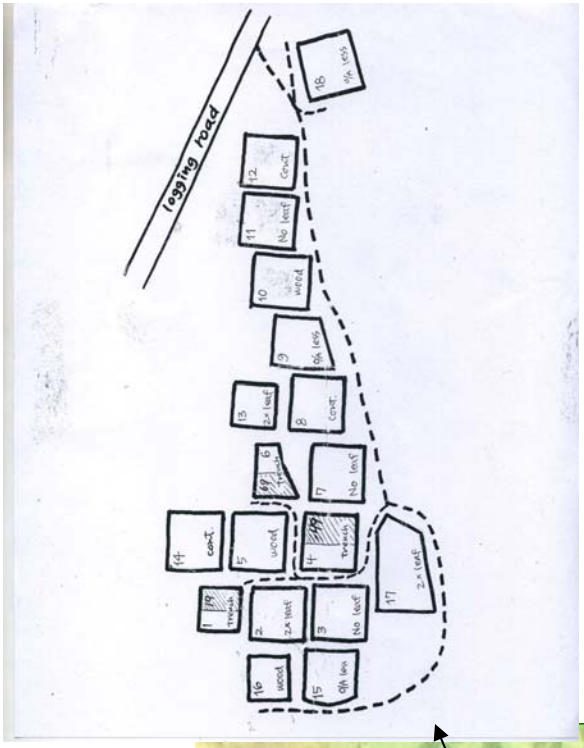
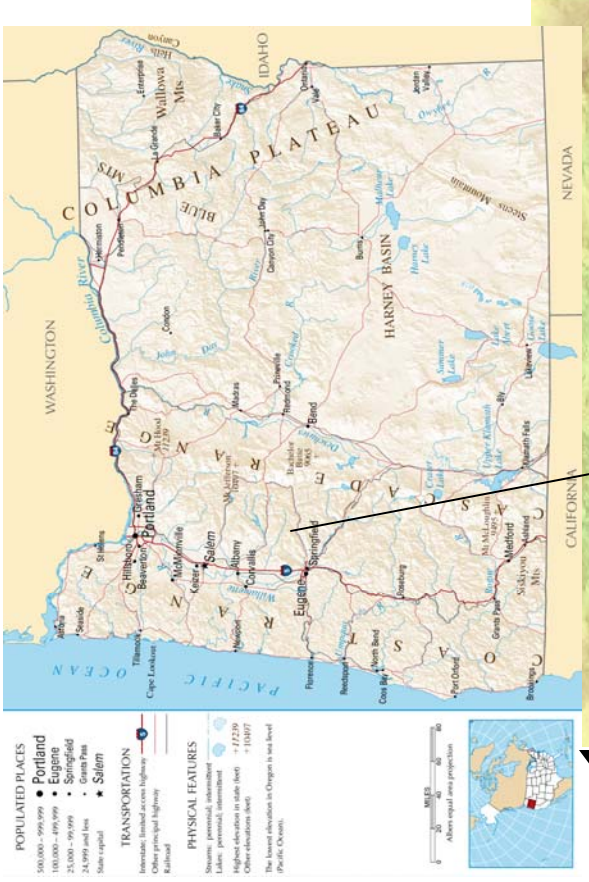
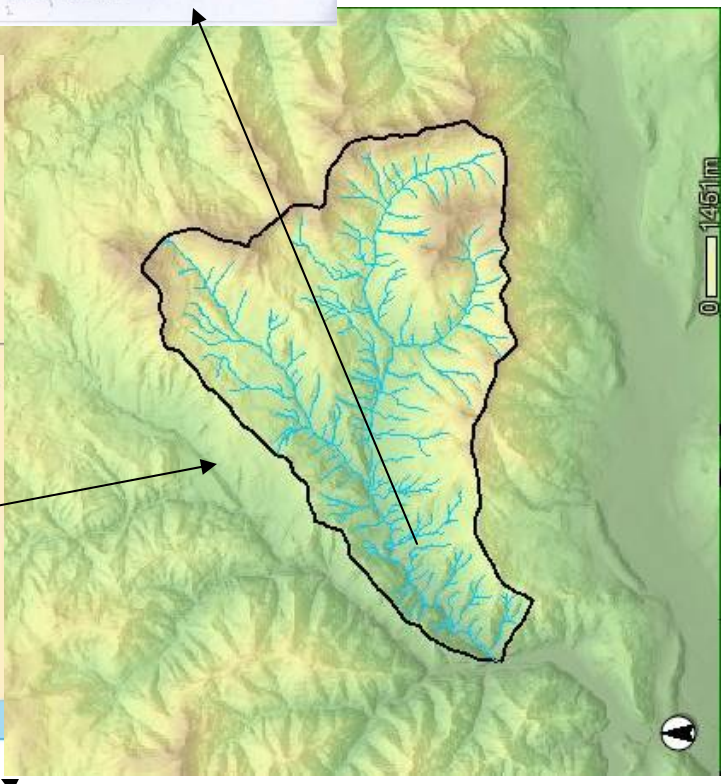
Appendix A: The DIRT Network

Site Location	Initiated	Ecosystem Type
University of Wisconsin Arboretum	1956	sugar maple forest, oak forest, and prairie
Harvard Forest, Massachusetts	1990	oak forest
Bousson Experimental Forest, Pennsylvania	1991	black cherry-sugar maple forest
HJ Andrews Experimental Forest, Oregon	1997	old-growth coniferous
Michigan Biological Laboratory at Pellston	2004	oak forest
Síkfökút Forest, Eger, Hungary	2000	oak forest

Appendix B: Site Location and Plot Map







Map of Oregon, public domain. Accessible from: http://en.wikipedia.org/wiki/Image:Map_of_Oregon_NA.png

HJ Andrews Experimental Forest, interactive map program provided at: http://wwwgis.forestry.oregonstate.edu/website/hja1/viewer.htm

Plot maps, unknown illustrator