

The DIRT Experiment

Litter and Root Influences on Forest Soil Organic Matter Stocks and Function

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“Oh, I’m hoping for a thousand years at least.”

—FRANCIS HOLE’s reply when asked how long his soil experiment
should be maintained

Rationale and Overview

Organic matter is a key component of forest soils. Important properties such as moisture-holding capacity, aeration, and nutrient retention are strongly influenced by, and typically increase with, the amount of soil organic matter present. Organic matter in forests is a major reservoir for nutrients and carbon that fuel microbial processes and support complex communities of soil and forest floor organisms. Because annual inputs of limiting nutrients like nitrogen are low relative to annual demand (see Chapters 3, 10, and 12), plants depend mainly on nutrients released from decomposing organic matter to meet their nutritional requirements. Therefore, the amount and the “quality” (that is, the decomposability and relative amounts of nitrogen and different carbon compounds) of soil organic matter may strongly influence tree growth and forest dynamics. In turn, inputs of fine litter from aboveground (leaves, twigs, seeds, etc.) and belowground (mostly fine roots) determine the amount and quality of organic matter and nutrients in forest soils.

At the Harvard Forest, where the long history of land use has produced changes in soil organic matter and nutrient content, as well as major shifts in forest composition, many questions arise concerning feedbacks between the plants and soils. In particular, we need to understand the rate of incorporation of organic matter into soils, the rate at which soils impoverished in organic matter recover their stocks of carbon and nutrients, and the relative importance of belowground versus aboveground inputs of organics in these processes.

Recognition of the importance of feedbacks from plants in determining soil nutrient dynamics and carbon storage has led to a large number of studies of the rate and byproducts of decomposition of different types of litter. In many such studies, known amounts of plant material are placed in a fine mesh bag in or on the soil surface in forests. The decomposition processes are then typically followed for two to five years, or until 20 to 40 percent of the original litter material remains. Such studies have yielded much information about the roles of litter nutrient content and carbon quality in controlling the relatively rapid cycling of nutrients through the litter layer.

For example, litters with high concentrations of soluble carbohydrates and cellulose, such as leaves of sugar maple or ash, decay faster and both immobilize and mineralize nutrients earlier in the decay sequence than do litters with high concentrations of lignin and other complex polyphenolic compounds, such as leaves of oak or beech. Also, litters with relatively high nutrient concentrations tend to decompose quickly and to release nutrients (which may then be available to plants) more rapidly than do litters with low initial nutrient concentrations. Because individual plant species and different plant tissues often differ in litter chemistry, litter inputs to soils from the various species within a forest ecosystem partially regulate the rates at which nutrients become available to plants. In this way, forest composition may exert a strong influence on soil characteristics, ecosystem processes, and site productivity.

Far less is known about the longer-term fate of aboveground and belowground plant litter and its role in determining soil organic matter content and function over timescales ranging from decades to centuries. Because humus (well-decomposed litter) typically contains most of the nutrients and at least half of the carbon in forest ecosystems, this lack of understanding as to how plant processes influence humus formation represents a critical gap in knowledge about forest ecosystem function. To address this, we established a long-term study of the factors controlling soil organic matter formation: the DIRT (Detritus Input Removal and Transfer) project. The goal of the DIRT project is to assess how rates and sources of plant litter inputs control the accumulation and dynamics of organic matter and nutrients in forest soils over decadal timescales.

Our project is inspired by the work of Professor Francis D. Hole at the University of Wisconsin Arboretum. In contrast to many arboreta, the Wisconsin arboretum is much more than a horticultural collection. Established in the 1930s, its mission has been to re-create and manage a variety of ecosystems representative of those that confronted European settlers on their arrival to the midwestern United States in the nineteenth century. In the early 1950s, plant ecologist and arboretum Director John Curtis challenged a young Dr. Hole to design a long-term study

of soil formation within the arboretum. Curtis's idea was that the restoration of plant communities required much more information about soil-forming processes and plant-soil interactions than was available at the time. Francis Hole, recognizing that the university had made a commitment to sustaining the arboretum as a long-term research site, devised an elegant and powerful experiment to meet this challenge and to address these fundamental but very practical questions about soil processes.

Dr. Hole located his study in native oak forests and prairies. His design called for a series of simple but sustained long-term manipulations of plant inputs to soils, coupled with periodic sampling to assess long-term changes in soil structure and properties. Treatments included altering the inputs of aboveground litter such as leaves and twigs and, in grasslands, the inputs of roots to soils belowground. Experimental treatments at Dr. Hole's plots in the Wisconsin arboretum were started in 1956 in two native forests and a restored prairie. They have been maintained for more than four decades through the ongoing efforts of Dr. Hole, arboretum staff, students, and community volunteers. We were allowed to sample at the Wisconsin forest plots in 1984 and again, in grassland and forest plots, in 1997. Results from the arboretum plots provide us with valuable long-term information against which the effects of the first decade of our experimental treatments at the Harvard Forest can be compared.

Controls on organic matter accumulation in soils has been a core theme of the National Science Foundation's LTER program since its inception. As part of this large program, we modified Dr. Hole's experimental design and established the DIRT project as a long-term intersite experiment, comparing the Harvard Forest with a nutrient-rich maple forest in Pennsylvania (Allegheny College Bousson Environmental Research Reserve) and a temperate rain forest in Oregon (H. J. Andrews Experimental Forest, U.S. Forest Service). Our hope is to develop additional linkages to similar experiments located across climate, vegetation, and soil texture gradients. This will allow an assessment of the importance of a range of physical and biological factors in controlling the accumulation of soil organic matter.

Experimental Design

Treatments in the DIRT experiment consist of chronically altered aboveground and belowground inputs of plant materials to permanent plots in a mid-successional oak-maple-birch forest in the Tom Swamp tract in close proximity to the experimental hurricane (see Figure 2.8). The manipulations, which are modified from Francis Hole's design (Figure 15.1) were started in the fall of 1990 and are as follows:

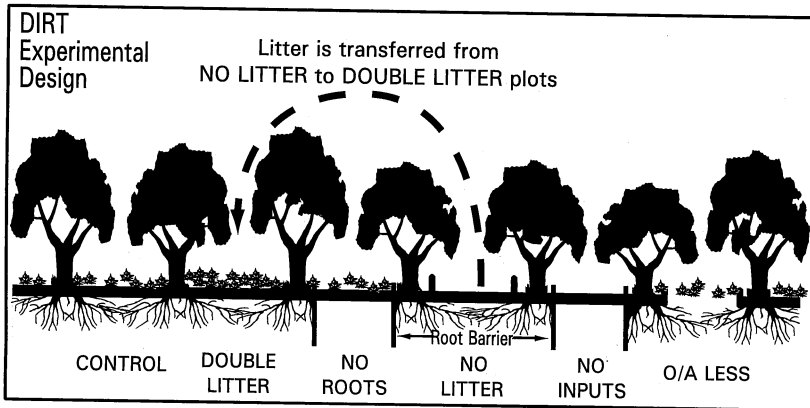


Figure 15.1. A conceptual diagram of the long-term Detritus Input Removal and Transfer (DIRT) experiment on the Tom Swamp tract of the Harvard Forest. Through a simple set of manipulations that can be carried out relatively easily over many decades, the project can assess many fundamental processes involved in the incorporation and dynamics of organic matter in soils. The surface organic soil horizon (Oea) is shown in black.

Treatment

CONTROL

DOUBLE LITTER

NO ROOTS

NO LITTER

NO INPUTS

IMPOVERISHED SOIL (O/A-LESS)

Manipulation

Normal litter inputs

Twice aboveground litter inputs

Roots excluded from plots by lined trenches

Aboveground litter excluded from plots

No aboveground litter and no roots

Organic and A horizons replaced with B horizon soil, normal inputs thereafter

Each of the 3-by-3-meter plots is located beneath an intact forest canopy and is replicated three times (Figure 15.2). The plots are placed between trees so that no stems are rooted in them, and the ground vegetation is removed as needed by clipping and occasional herbicide applications. Aboveground litter of leaves, twigs, and other fallen material is collected and excluded from NO LITTER plots with a thin mesh fabric. This collected material is then added to the DOUBLE LITTER plots in order to augment normal inputs. Root growth is prevented in the NO ROOTS treatment by excavating 1-meter-deep trenches around the plots, lining them with plastic barriers, and then back-filling the trenches with soil. The NO INPUTS treatment is a combination of the NO LITTER and NO ROOTS treatments. In the IMPOVERISHED or O/A-LESS treatment, soils were experimentally impoverished of organic matter by removing the forest floor and the upper 15 centimeters of mineral soil and replacing these with deeper, less organic-rich B horizon soil from an adjacent pit. The O/A-



Figure 15.2. A NO-LITTER plot in the DIRT experiment. In these plots, all aboveground litter (leaves, twigs, fruits, etc.) is removed and placed on an adjoining DOUBLE-LITTER plot. Low, coarse fencing keeps leaves from blowing onto the plot in fall. Instrumentation, including the lines for automated temperature probes, CO₂ flux measuring ring, and lysimeters, emerge through the soil surface. Photograph by J. Gipe.

LESS treatment does not involve ongoing manipulations beyond this initial treatment. This experimental impoverishment is intended to allow us to estimate (1) the fraction of total litter inputs (aboveground plus belowground) that is eventually transferred from litter to soil organic matter and (2) the amount of time that is required for organic-poor soils to recover to predisturbance conditions.

Our field measurements allow us to link quantitatively the changes in soil properties and processes to the amounts of carbon (energy) and nutrients entering the soils in organic matter. The value of this information will increase greatly as the manipulations continue over the next decades or centuries. In the field, we measure a number of parameters, including CO₂ fluxes from the forest floor, soil moisture, and soil temperature. In addition, we collect soil solutions (water and leachate) from beneath the forest floor using zero tension lysimeters and, at a depth of 30 to 40 centimeters, suction lysimeters. These water solutions are analyzed for ammonium, nitrate, phosphate, dissolved organic carbon (DOC), and dissolved organic nitrogen (DON). We also collect samples periodically from the forest floor and mineral soil (0 to 10 centimeters

and 10 to 15 centimeters deep) in order to track changes in organic matter and nutrient contents. Soils were sampled from the plots at the beginning of the experiment in 1990 and again after one (1991), five (1995), and ten (2000) years. In the future we plan to sample once per decade. Forest floor and soil samples are assayed for total soil organic matter, carbon, nitrogen, and nutrient contents and for standard soil properties (acidity, cation exchange capacity, base saturation, texture). We have also measured CO₂ release and net nitrogen mineralization and nitrification under constant temperature and moisture conditions and gross nitrogen fluxes using ¹⁵N pool dilution methods on the samples collected in Years 1 and 5. As with the field measures, laboratory results are used to quantify the effects of plant litter inputs on biological processes and carbon and nitrogen dynamics.

The biotic communities of the soils were analyzed in subsamples from our Year 5 collections. Microbes (bacteria and fungi) and microfauna (protozoa and nematodes) were counted and classified into functional categories (protozoa as flagellates, ciliates, and amoebae; nematodes by feeding type) rather than species to assess the effects of litter and root inputs on forest floor and soil biota.

Initial Results

Although the DIRT project addresses long-term questions about soil organic matter formation, plant-soil interactions, and nutrient cycling, results from the first few years of the experiment proved useful for addressing unanswered questions about important ecosystem processes. Processes investigated in the initial years of the study were fine root production, temperature sensitivities of rhizosphere (fine roots and closely associated microbes) respiration versus bulk soil respiration, and shifts in belowground community structure.

Partitioning Soil CO₂ Flux

As discussed in preceding chapters, measuring fine root production, decomposition, and respiration are among the most problematic issues in ecosystem studies. Because of the nature of our experimental design, in which some plots have roots whereas others are trenched to exclude roots, and some have new litter and others do not, we can use field measures of soil respiration during the first year after the start of manipulations along with mass balances to estimate these processes. Our mass balance approach indicated that live root respiration, production of aboveground fine litter (leaf, twig, and other fine litter), and fine root detritus each constituted about one-third of carbon inputs to soil in this stand. This suggests that fine root and leaf litter

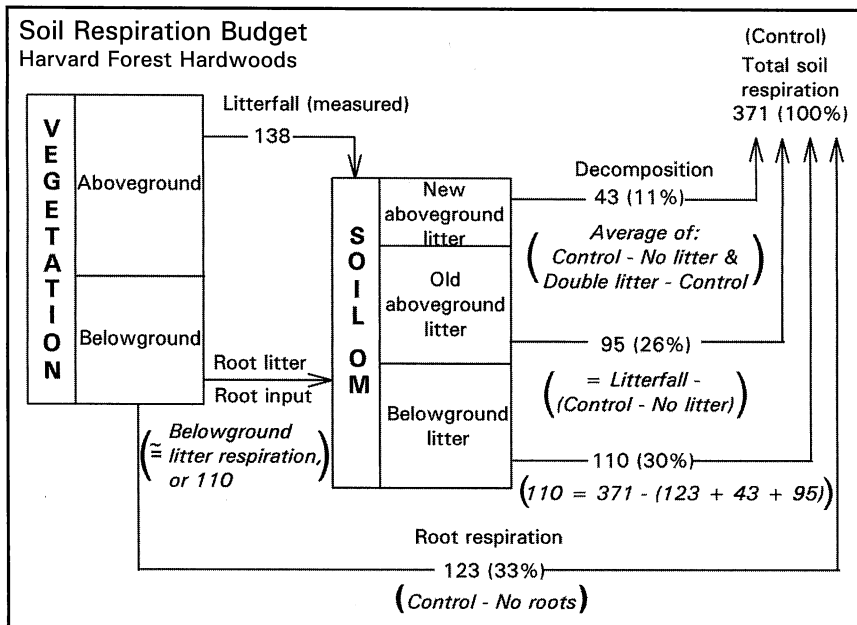


Figure 15.3. Soil respiration budget for a hardwood forest (based on the DIRT experiment) at the Harvard Forest. Italics show how live root respiration, decomposition of new (previous fall) and old aboveground litter, and decomposition of belowground (mostly fine root) litter were estimated from seasonal CO₂ fluxes occurring under different treatments. Respiration from the decomposition of aboveground and belowground litter are assumed equal to annual inputs. Flux measurements were made during the first full year after the start of treatments (see text). Numbers are fluxes (grams carbon per square meter per year) and percentages of total soil respiration for each component. OM, organic matter. Modified from Bowden, Nadelhoffer et al. 1993, with permission from NRC Research Press.

production are approximately equal in this forest type. Importantly, this finding narrows the range of uncertainty in estimating fine root production and suggests a method that can be applied elsewhere.

These conclusions can be drawn directly from differences in soil CO₂ efflux in Year 1 of the treatments (Figure 15.3). Soil respiration on the unaltered control plots is 371 grams of carbon as CO₂ per square meter per year (g C·m⁻²·yr⁻¹). We assume that total respiration from the decomposition of leaf litter is equal to the annual contribution of leaf litter carbon (measured as 138 g C·m⁻²·yr⁻¹). Of this, the amount decayed in the first year is equal to the mean of the differences in soil respiration between the CONTROL and NO LITTER plots and the CONTROL and DOUBLE LITTER plots (43 g C·m⁻²·yr⁻¹). This leaves 95 g C·m⁻²·yr⁻¹ as the amount of CO₂ generated by the decay of older aboveground litter. Live root respiration was estimated as the difference between CO₂ flux in the

CONTROL plot and the NO ROOTS plot ($123 \text{ g C}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$). The remaining soil respiration ($371 - 138 - 123 = 110$) is assumed to come from the decomposition of root litter, which is assumed equal to fine root production.

Temperature Regulation of Rhizosphere versus Bulk Soil Respiration

Soil respiration is a critical process in global as well as local biogeochemical cycles. Models of the global carbon cycle used to predict ecosystem-atmosphere interactions under global warming are sensitive to variations in the relationship between soil respiration and temperature. Broadscale simulation models, however, typically use a single exponential function (Q_{10}) to predict releases of CO_2 to the atmosphere from soil respiration (see Chapters 12 and 13). In the soil-warming experiment we learned that this Q_{10} function actually varied with changes in carbon quality and nitrogen availability. Analogous results emerge from the DIRT plots. Comparisons of soil respiration on treated plots in Year 4 showed that respiration by fine roots and associated rhizosphere organisms responds more to temperature than does bulk soil respiration (Figure 15.4). The Q_{10} value (increase in respiration for each 10°C increase in temperature) for the roots and rhizosphere (4.6) was significantly greater ($P < .05$) than the Q_{10} values for both the untreated controls (3.5) and the treatments without roots (NO ROOTS = 2.5, NO INPUTS = 2.3). Q_{10} values changed little with either addition or exclusion of leaf

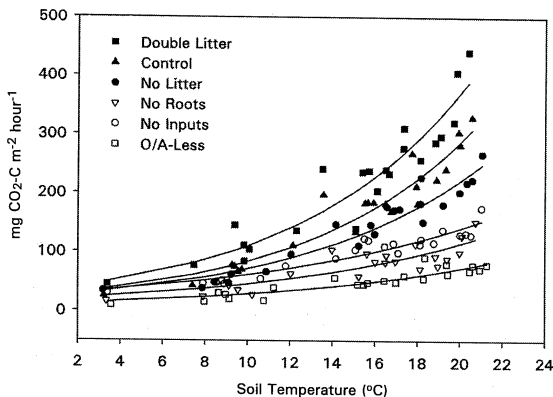


Figure 15.4. Relationship by treatment between mean daily soil CO_2 flux and soil temperature (5 centimeters soil depth) from 16 June 1994 through 14 June 1995. An exponential function of the form $y = \beta_0 e^{\beta_1 T}$ was fitted to the data, where y = flux, β_0 and β_1 are fitted constants, and T = temperature. Modified by permission from *Nature* (Boone, Nadelhoffer et al. 1998), copyright 1998, Macmillan Publishers Ltd.

litter. The findings suggest that soil respiration should be most sensitive to temperature in systems in which roots contribute a large portion of total soil CO₂ efflux. This finding has important implications for global carbon cycling models (compare with Chapter 13).

Litter Effects on Dissolved Organic Carbon

Soil solutions were collected after each rain event during the growing season of Years 4 (1994) and 7 (1997). In Year 4, there were no significant differences in DOC concentration between treatments, and within-treatment variance was large (Figure 15.5). However, by Year 7, DOC concentrations were significantly higher in the solutions collected below the forest floor from DOUBLE LITTER plots and were significantly lower in O/A-LESS plots. Overall results for DOC concentrations were DOUBLE LITTER > CONTROL = NO LITTER = NO ROOTS > NO INPUTS > O/A-LESS. There were no significant differences in DOC concentrations between treatments in the soil solution collected from the mineral horizon in either year. Results from the forest floor lysimeters suggest that changes in organic matter availability cause changes in the organic chemistry of forest soil solutions within less than a decade. In contrast, we infer from the mineral soil data that DOC losses from the mineral soil to groundwater are relatively insensitive to changes in forest floor organic matter dynamics. However, differences in DOC inputs to mineral soils as controlled by amounts of litter inputs are likely to influence carbon accumulation in mineral horizons.

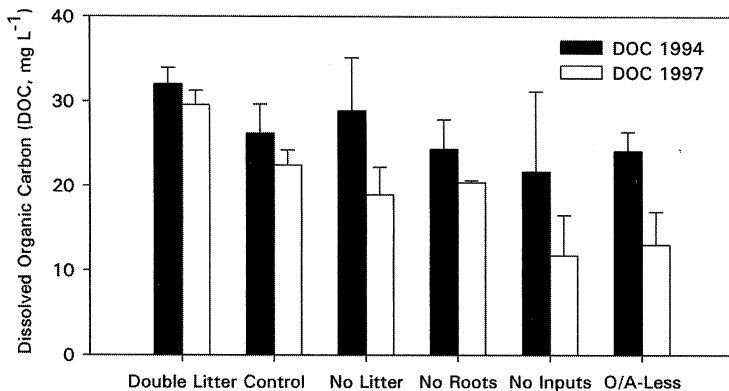


Figure 15.5. Mean concentrations of dissolved organic carbon (DOC) (± 1 standard error) in solutions collected beneath the forest floor in 1994 and 1997. From J. A. Aitkenhead and W. H. McDowell, unpublished data.

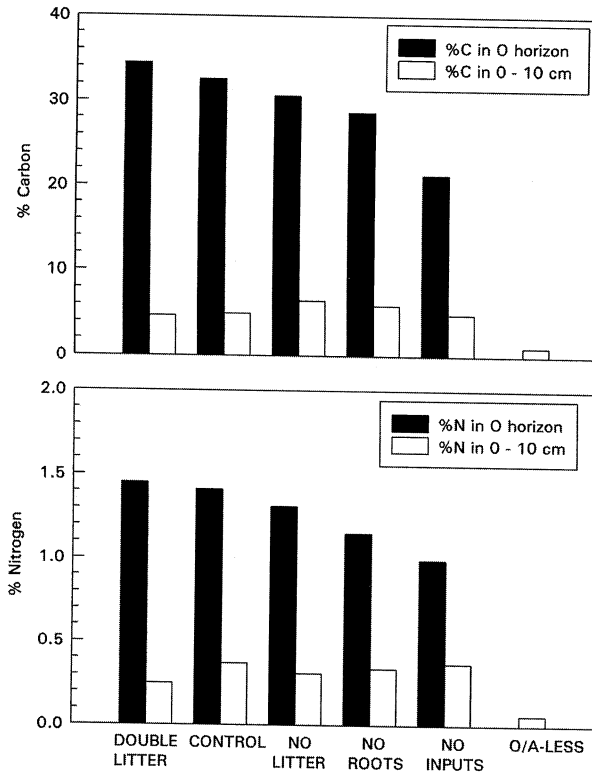


Figure 15.6. Percents of carbon and nitrogen in the forest floor (O horizons) and upper 10 centimeters of mineral soil after five years of litter and root manipulations on the DIRT plots. Bars show means ($n = 9$).

Cumulative Effects on Soil Properties

OVERVIEW

Changes in processes observed in the field during the initial years of manipulations were consistent with changes in both properties and processes after five years of treatment. The concentrations of carbon and nitrogen in the forest floor increased or decreased with increases or decreases in aboveground litter and root inputs (Figure 15.6). Mineral soils, however, did not show similar trends. Mineral soils respond less and/or more slowly to manipulations of plant inputs because most inputs occur directly to the forest floors. Also, organic matter in the mineral soil is likely more stable than is the organic material in the forest floor because of increasing structural complexity and physical protection by association with mineral particles.

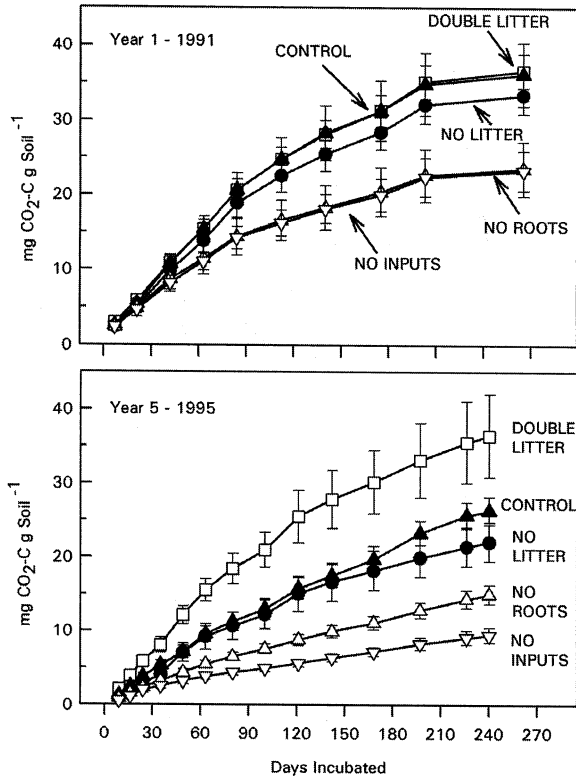


Figure 15.7. Cumulative respiration of forest floor materials (Oea horizons) collected from the DIRT experiment one year (top) and five years (bottom) after the start of manipulations in 1990. Samples were incubated at 22°C and -66 kPa moisture. Symbols show means and standard errors ($n = 9$).

LABORATORY INCUBATIONS

A comparison of laboratory incubations of forest floor samples collected during the first and fifth years of treatment was consistent with field measures. These trials, in which samples are held at constant temperature and humidity, show large effects on organic matter quality and microbial processes (Figure 15.7). Doubling the aboveground litter inputs increased six-month laboratory respiration by about 40 percent relative to respiration of samples from plots with normal (CONTROL) litter inputs. Preventing the ingrowth of roots (NO ROOTS) on plots decreased respiration by 43 percent relative to controls. Exclusion of aboveground litter and root inputs for five years (NO INPUTS) decreased cumulative respiration by almost two-thirds relative to controls. These patterns are consistent with field results (see Figure 15.3) indicating that litter inputs from aboveground and from roots are approximately equal. Respiration of

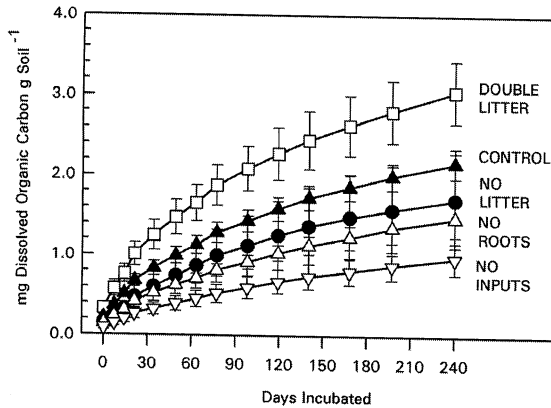


Figure 15.8. Cumulative dissolved organic carbon (DOC) release from forest floor materials (Oea horizons) collected from the DIRT plots five years after the start of manipulations in 1990. Samples were incubated at 22°C and -66 kPa moisture. Symbols show means and standard errors ($n = 3$ to 4).

samples from the NO LITTER plots, however, was not reduced as much as would be expected given the large increase in respiration after doubling litter inputs. Curiously, doubling litter inputs increased respiration much more than exclusion of litter inputs decreased respiration. This suggests that additional litter inputs might stimulate or enhance decomposition of existing more recalcitrant organic matter. Patterns of DOC release from incubations in response to five years of treatment were similar to those of respiration (Figure 15.8). Moreover, cumulative DOC release was about one-tenth of the release of CO_2 and showed overall patterns similar to those obtained in the field from lysimeters (see Figure 15.5).

Net nitrogen mineralization (release of plant-available nitrogen as measured in sequential leaching from incubated soils) under laboratory conditions was also influenced by five years of plot manipulations (Figure 15.9, top). However, differences in mineralization among incubations of samples from treated plots were not as consistent or as pronounced as were differences in respiration. Cumulative nitrogen release from DOUBLE LITTER, CONTROL, and NO LITTER incubations was similar. This could indicate that the source of most mineralized nitrogen is from leaf litter more than five years old. However, excluding root ingrowth from the plots, whether alone (NO ROOTS) or in combination with litter exclusions (NO INPUTS), decreased laboratory nitrogen mineralization. This suggests that root turnover, root exudation, or both processes contribute strongly over short timescales to mineralization.

The absence of roots, while decreasing net nitrogen mineralization

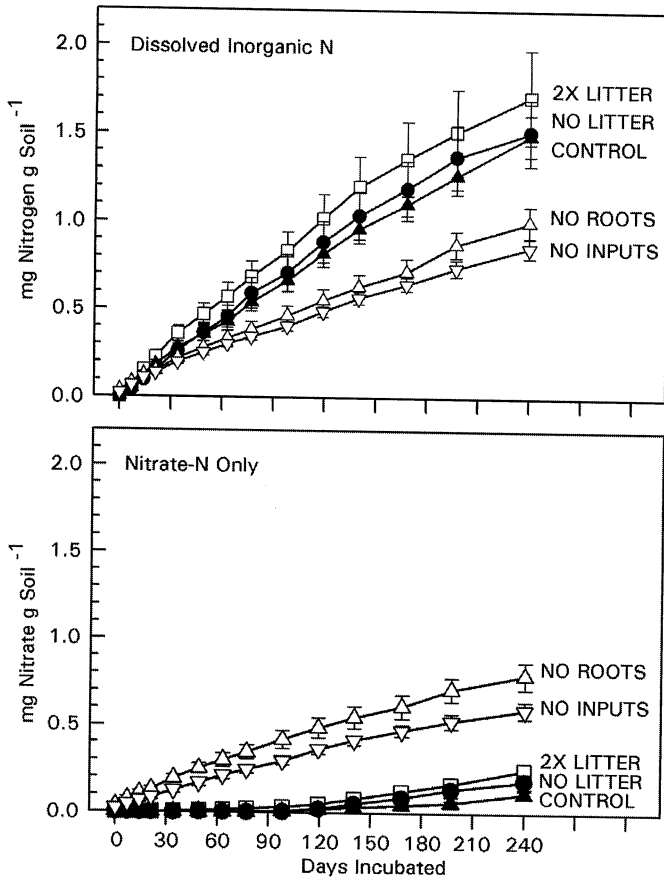


Figure 15.9. Cumulative dissolved inorganic nitrogen ($\text{NH}_4 + \text{NO}_3$) (top) and nitrate-nitrogen leached from incubations (bottom) (22°C , -66 kPa moisture tension) of forest floor samples (Oea horizons) collected from DIRT plots after five years of manipulations. Symbols show means and standard errors ($n = 9$).

overall (Figure 15.9, top), increased net nitrification (Figure 15.9, bottom); nitrate-nitrogen constituted more than half of the nitrogen released from NO ROOTS and NO INPUTS soils whereas nitrate-nitrogen release from soils collected from plots with roots was essentially zero until after three months of incubation. We speculate that the absence of roots and competition from mycorrhizal hyphae has allowed free-living microbes, including nitrifiers, to increase in the forest floor and that this activity carried over to laboratory incubations. The lack of response in net nitrogen mineralization to variations in aboveground litter suggests that microbial immobilization exerts strong control over soil nitrogen dynamics.

Effects on Soil Communities

Forest soils are generally dominated by fungi rather than bacteria. This is true in the Harvard Forest as well, where total fungal-to-bacterial biomass ratios averaged 200 across all treatments and horizons. Mean ratios for mineral soils (114) were significantly lower than for organic soils (305). The lowest ratios were found in the O/A-LESS mineral soils, where total fungal-to-bacterial biomass ratios averaged 20. This suggests that the fungal-to-bacterial ratios decline with increasing recalcitrance of soil carbon or total accumulation of soil organic matter.

Total fungal biomass was much greater than total bacterial biomass in forest floors (Figure 15.10) under all treatments. Total fungal biomass varied with leaf litter input (highest values in DOUBLE LITTER and the lowest in NO LITTER and NO INPUTS plots), but not in the absence of roots (NO ROOTS). Total bacterial biomass varied inversely with fungal biomass except in DOUBLE LITTER plots, where both fungal and bacterial biomasses were high. Active biomass of both fungi and bacteria were remarkably similar across treatments in forest floors. Given the strong effects of manipulations on mineralization and respiration, neither total nor active bacterial population size is a good predictor of soil processes. Active fungal biomass did not differ among treatments in forest floors.

Fungal biomass was also greater than bacterial biomass in mineral

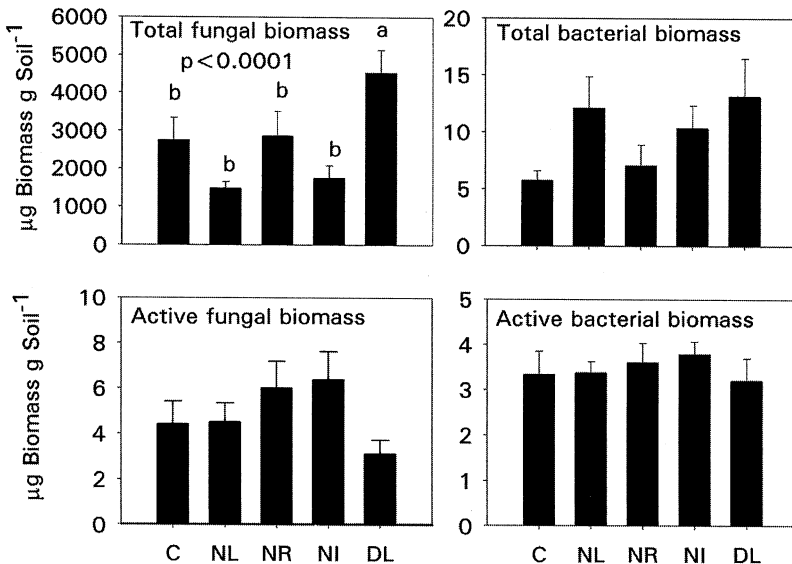


Figure 15.10. Fungal and bacterial biomass in forest floors (O horizons) in Year 5 of the DIRT manipulations. C, CONTROL; NL, NO LITTER; NR, NO ROOTS; NI, NO INPUTS; and DL, DOUBLE LITTER.

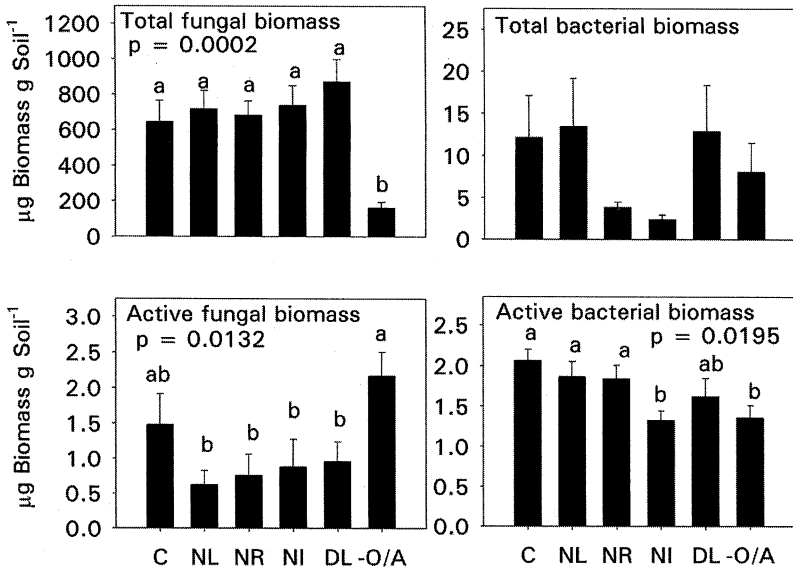


Figure 15.11. Fungal and bacterial biomass in mineral soils (0 to 10 centimeters) in Year 5 of the DIRT manipulations. C, CONTROL; NL, NO LITTER; NR, NO ROOTS; NI, NO INPUTS; DL, DOUBLE LITTER; and -O/A, O/A-LESS.

soils (Figure 15.11) and was lowest in O/A-LESS plots, while active fungal biomass was greatest in this same treatment. In contrast, patterns of active bacterial biomass in mineral soils followed patterns of easily degradable organic matter: the O/A-LESS plots had the lowest carbon and nitrogen contents, followed by the NO INPUTS soils (Figure 15.6), both of which had low active bacterial biomass (Figure 15.10).

Protozoan densities were extremely variable, with few significant differences among treatments or relationships to microbial abundance (Figure 15.12). Although protozoa are grazers of both bacteria and fungi, patterns of protozoa numbers appeared to follow trends in total fungal biomass rather than trends in bacterial biomass. This might be expected in these soils where fungal biomass dominates the microbial community. In organic horizons, fungal biomass was greatest in the DOUBLE LITTER and lowest in the NO LITTER treatments; total protozoan numbers were higher in the DOUBLE LITTER treatment than in either the NO LITTER or NO INPUTS treatments, but were greatest in the NO ROOTS treatment. This did not correspond to patterns of microbial abundance, and none of these trends was statistically significant. In mineral soils, protozoan numbers were low in the O/A-LESS treatment, corresponding to low total fungi and low carbon content, but other patterns in protozoa abundances did not match microbial abundance across treatments. Nematode abundance was variable in organic soils but greatest in DOUBLE LIT-

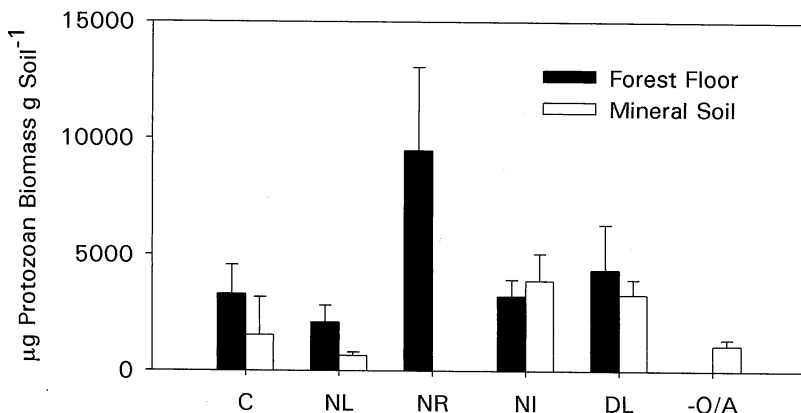


Figure 15.12. Protozoan populations after five years of manipulations in the DIRT experiment. C, CONTROL; NL, NO LITTER; NR, NO ROOTS; NI, NO INPUTS; DL, DOUBLE LITTER; and -O/A, O/A-LESS.

TER and NO ROOTS treatments, closely matching patterns of protozoa. In mineral soils, numbers of nematodes per gram of soil were very low but were lowest in the O/A-LESS and in NO ROOTS treatments. Again, patterns matched those of total protozoa abundance. Nematodes graze on fungi and bacteria as do protozoa, and they can also graze on protozoa.

Summary

Our manipulations of litter and root inputs to forest soils are designed to (1) quantify the proportions of aboveground litter and root inputs that become stored as organic matter with long residence times; (2) quantify how organic matter formation influences soil properties such as nutrient and water retention; and (3) characterize how the nutrient-supplying capacities of soils are influenced by plant litter and root inputs. These goals will require decades of manipulations to be achieved. We have, however, used results from the first years of the experiment to address important questions about forest ecosystem function. Thus, although the overarching goals are long term, there are important short-term benefits as well. This is a key to sustaining the interest necessary for justifying the continued maintenance of the plots. Another important feature of long-term experiments is that the manipulations themselves be simple and require a minimum of effort to maintain. This is the case for the DIRT plots, which require only several days of activity to remove and add litter annually to subsets of the plots. More effort is required to establish the plots and to retrench plots from which roots are excluded (every eight to twelve years). Once established, however, these plots are soon on their way to achieving Professor Hole's goal.