

**Long-term leaf and root litter input manipulations influence *Quercus rubra* L. seedling
growth in a mesocosm experiment**

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Abstract

To understand how long-term soil manipulations affect seedling traits, we leveraged a long-term field study at the University of Michigan Biological Station, the Detrital Inputs and Removals Treatment (DIRT) network and conducted a greenhouse experiment to assess the impacts of almost 20 years of above and belowground litter manipulations on *Quercus rubra* seedling growth. We grew *Q. rubra* seedlings from acorns in four distinct treatments, No Litter Input (litter excluded leaf and root litter inputs), No Root Input (barriers in the field to exclude root inputs, but allowed leaf litter inputs), No Leaf Input (aboveground leaf litter excluded with tarps, but allowed root access), and a Control treatment (no litter manipulations either above or belowground) for 6 weeks in 2021. We found a 27% increase in total seedling biomass between seedlings grown in No Root soil compared to No Input soil. Leaf areas increased ~22% in seedlings grown in No Root soils compared to No Input soils. Treatments did not affect green leaf total nitrogen or green leaf C:N. The presence of belowground root litter reduced seedling aboveground biomass; aboveground litter may have a greater effect on seedling biomass and leaf area than belowground litter in isolation. Leaf C, N may be unaffected by shifting inputs of litter either above or belowground over this growth period (6 weeks). As forest growth and litter production respond to a variety of global changes in both positive and negative directions, seedling growth and success will also respond shaping the forests of the future.

Key words: Litter, detritus, plants and soils, plant traits, soil characteristics

Figures and Appendices

Figure 1. Predictions schematic reflects how long-term soil manipulations may influence seedling growth, where (a) depicts greater soil inputs increase seedling biomass, and (b) belowground litter inputs will influence plant responses to a greater extent than aboveground litter inputs.

Figure 2. Project schematic. Soils from Detrital Inputs and Removal Treatments (DIRT) plots used in the seedling mesocosm growth experiment.

Figure 3. *Quercus rubra* seedling biomass. (a) Total seedling biomass (g) of *Q. rubra* plants grown in the No Roots, (+Leaves, -Roots) treatment increased by 27% compared to No Inputs (-Leaves, -Roots) treatments. ANOVA F -value = 2.8201, p -value = 0.0412. (b) +Leaf litter, -Root litter increased aboveground seedling biomass by 32% compared to no input plots, ANOVA F -value = 4.0584, p -value = 0.0086. (c) No treatment effect on belowground seedling biomass, ANOVA F -value = 0.4568, p -value = 0.7129.

Figure 4. (a) Total leaf area was 21.5% greater for seedlings grown in soils with leaf litter present and root litter excluded compared to no input soils. One outlier has been removed, +/- 3 standard deviations from the mean (3NRL). ANOVA F -value = 2.4363, p -value = 0.06791 (b) There was no treatment effect on the number of leaves per seedling per treatment. χ^2 -squared = 6.3661, p -value = 0.0951.

Figure 5. Carbon and nitrogen analysis of plant leaves. (a) There was no significant effect of treatment on leaf %, $Kruskal$ -Wallis $X^2 = 3.3133$, p -value = 0.3458. (b) There was no significant treatment effect on the leaf carbon to nitrogen ratio in seedlings, ANOVA F -value = 1.3429, p -value = 0.2635.

Table 1 (Appendix 1). This experiment used a subset of four DIRT treatments from the University of Michigan Biological Station DIRT plots described below. This treatment order is from the lowest input plots to the highest input plots. Control plots have root and leaf litter inputs, No Input plots have no inputs from either direction, 'No Roots,' and 'No Leaves,' serve as shorthand for no root litter and no leaf litter.

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Appendix 12. Samantha King enzyme activity results 1 (2022). Mean β -glucosidase (BG), cellobiohydrolase (CB), and N-acetylglycosaminidase (NAG) activity across treatments ($n = 3$). White bars indicate enzyme activity per gram soil; black bars represent enzyme activity per gram soil organic matter (SOM). One standard deviation is shown by the length of each error bar. Treatments with the same letter are not significantly different. * indicates statistically significant differences in means based on ANOVA testing ($\alpha = 0.05$).

Appendix 13. Samantha King enzyme activity results 2 (2022). Mean phenol oxidase and peroxidase activity across treatments ($n=3$). White bars indicate enzyme activity per gram soil; black bars represent enzyme activity per gram soil organic matter (SOM). One standard deviation is shown by the length of each error bar. Treatments with the same letter are not significantly different. * indicates statistically significant differences in means based on ANOVA testing ($\alpha = 0.05$).

Introduction

Forests are responding to global change in ways that have altered their composition and function (Morin et al., 2018; Zhang et al., 2018). However, predicting how forests will be structured and how they will function in the future is difficult as many environmental filters – such as soil nutrient availability, existing ecosystem biodiversity, and rising [CO₂] concentrations—will alter seedling growth and impact future forests (Yuan et al., 2019). Filters that may impact seedling growth – such as soil texture, soil moisture, litter input, and ion exchange capacity (Bunemann et al., 2018) – are changing with shifts in forest composition and inputs over long periods of time. In fact, trees influence soil and soils influence trees in complex ways that over time impact soil fertility, plant productivity, plant community composition, and forest succession (Bever, 1994; Van Der Putten et al., 2013). Plant leaf and root inputs directly influence soil organic matter (SOM) content, nutrient availability, and microbial community structure (Hassan et al., 2022). In turn, soil properties, including the soil microbial community, can positively or negatively impact seedling success in forests (Mangan et al., 2010). As forests change in their composition over time as well as the quality and amount of litter entering forests ecosystems via leaves and roots also changes (Norby, 2011) emerging soil properties plausibly will shape seedling success (Mangan et al., 2010).

While it is clear from experiments that global change such as elevated [CO₂] can change the relative inputs of leaves and roots into ecosystems, very few experimental studies have run long enough to explore how changing the relative inputs of leaf and root litter impact seedling success. However, there are experiments that have explicitly manipulated forest leaf litter and root inputs for periods of time long enough to change soil nutrient mineralization. Leaf litter additions can increase mineral soil C content in forest soils (Tanner et al., 2016), increase soil organic carbon (SOC) mineralization (Wang et al., 2017), and increase fungal biomass (Rinnan et al., 2008). However, this stimulation of carbon and other nutrients is not observed in all ecosystems in which litter inputs are manipulated (Hassan et al., 2022). For example, one 14-

year manipulation in a temperate deciduous forest found no change in total soil carbon (C), nitrogen (N), phosphorus (P) and fine root biomass with increasing litter inputs (Huang & Spohn, 2015). Even doubling litter additions did not affect mineral soil C in a temperate forest in the eastern USA (Crow, Lajtha, Bowden, et al., 2009) (Crow, Lajtha, Filley, et al., 2009). Similarly, the effect of leaf litter removal can have a variety of impacts on SOC and nutrient availability (Sayer, 2006). Excluding leaf litter from soil can impact soil pH, total N, total P, and microbial enzymatic activity in a positive, neutral, or negative way (Liu et al., 2021).

While less work has been conducted on root litter relative to leaf litter, root inputs are important for SOC and nutrient dynamics (Gross & Harrison, 2019). In fact, root litter and exudate inputs to soil C can exceed those of aboveground litter inputs in temperate ecosystems (Berhongaray et al., 2019; Keller et al., 2021; Rasse et al., 2005). In a field experiment where researchers manipulated leaf and root litter inputs in a mixed hardwood, temperate forest in Connecticut, USA, soils in the root only (leaf litter was excluded) treatment had more mineral associated SOC than soils in the leaf litter only plots after three years of manipulations. Perhaps surprisingly, when root litter and leaf litter are both included in plots, the impact on nutrients and soil C is not always additive (Feng et al., 2022; Jing et al., 2021; Man et al., 2022).

Here, we leveraged the Detrital Inputs and Removals Treatment Experiment (DIRT) experiment in northern Michigan to create a mesocosm experiment exploring how soils with long-term inputs of leaf litter, root litter, and both root and leaf litter might impact seedling growth and traits relative to a treatment in which root and leaf litter had been excluded. The DIRT experiment in northern MI has excluded leaf litter inputs (hereon indicated as No Leaves/NL), excluded root litter inputs (No Roots/NR), excluded both leaf and root litter inputs (No Inputs/NI) and allowed leaf and root litter (Control) into plots ($n = 3$) since 2004 leading to changes in SOM biomarkers (vandenEnden et al., 2018). We grew *Quercus rubra* L., an important deciduous forest species in northern MI, in each of the soil treatments for 6 weeks and measured aboveground biomass, belowground biomass, and total plant biomass, leaf nitrogen content,

and leaf C to N ratio. We had two predictions: 1) Seedlings grown in soils in which both leaf and root litter were added would accrue higher biomass than in where either leaf or root litter were excluded because soils enriched with both root and leaf litter would have higher C content and greater nutrient availability. 2) Seedlings grown in soils with root litter inputs, but no leaf litter inputs, would have more biomass than seedlings grown in soils with leaf litter and no root litter. We expected that soils with root litter would have higher soil C and N content as root litter (and exudates) were interspersed in the soil matrix and retained. In sum, seedling biomass, leaf, and root area would be highest in soils with both litter and roots, followed by plus root soils, plus leaf soils, and then by the no inputs treatment, where root and leaf litter were both excluded.

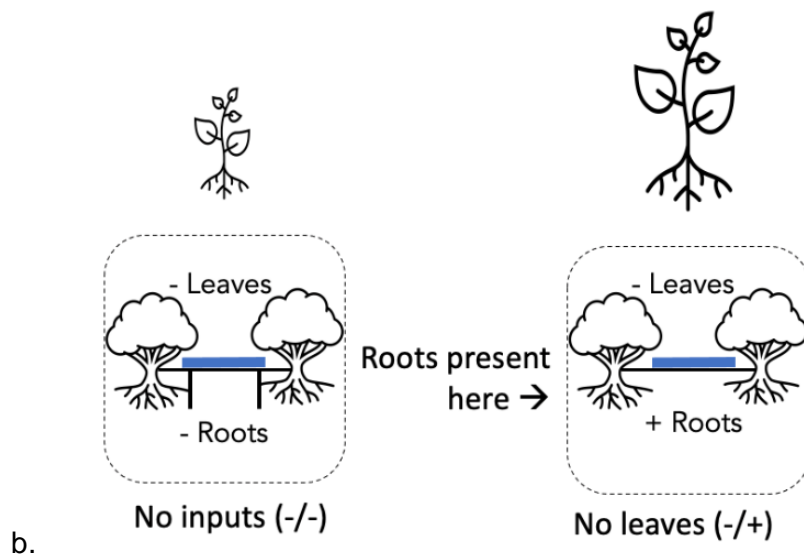
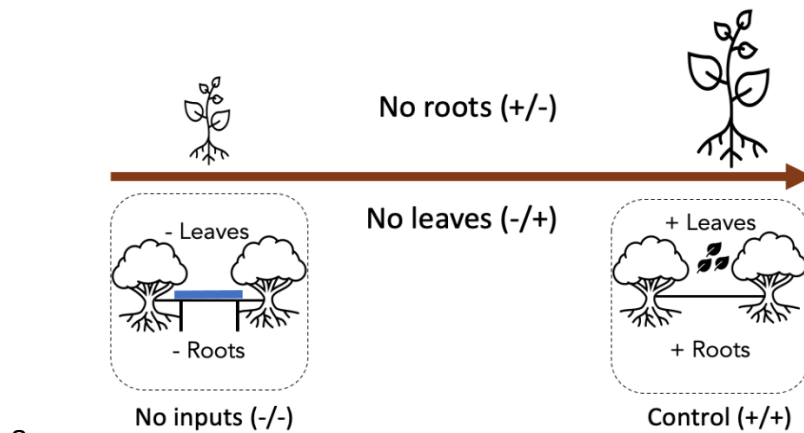


Figure 1. Predictions schematic reflects how long-term soil manipulations may influence seedling growth, where (a) depicts greater soil inputs increase seedling biomass, and (b) belowground litter inputs will influence plant responses to a greater extent than aboveground litter inputs.

Methods

Site description

To address our questions, we conducted an experiment with soils collected from the University of Michigan Biological Station Detrital Inputs and Removals Treatment (DIRT) experiment, located in northern Lower Michigan, USA (45°56'N 84°71'W) (Nadelhoffer et al., 2004; Lucas E. Nave et al., 2013). The DIRT site receives an average of 817mm of precipitation each year including 294 cm of snowfall (Nave et al., 2013). The soils are classified as entic Haplothods of the Runicon series and are loamy sands (Percent sand/silt/clay: 93/6/1, pH ~4.8) (Soil Survey Staff, 1991, UMBS data, AmeriFlux site US-UMB (Lucas E. Nave et al., 2019)). The entire treatment is located on an outwash plain, and as such, soil fertility is low (Lucas E. Nave et al., 2019).

Detrital Inputs and Removals Treatment Network (DIRT)

The Northern Michigan DIRT experiment was established in 2004 to explore the plant input controls on SOM content and processes over decadal time scales (Nadelhoffer et al., 2004). Northern hardwood forest species dominate the DIRT site. The forest is 100 years old and historically it was harvested and regularly burned in the 1800s and 1900s (Michigan DNR, Whitney, 1987). Historic fire-dependent species have given way to bigtooth aspen (*Populus grandidentata*), red maple (*Acer rubrum*), red oak (*Quercus rubra*), and white pine (*Pinus strobus*) (Hardiman et al., 2011; Ring et al., 1995). Nine experimental input treatments were established in 3 x 3 m plots for 27 plots in total (n = 3). Treatments are maintained twice each year (see Nadelhoffer et al., 2004 for details) in the following array: (1) Reference plots, with no disturbance, (2) Control plots, where seedlings, vegetation, and large woody biomass are

removed twice per year, (3) No input plots, where both above and belowground litter is excluded from entering the plots, (4) No Leaf litter plots, where tarps prevent leaf and other aboveground litter from entering the plots, (5) No Root plots, where tarps extend along the perimeter of the plots to 140 cm depth to prevent root ingrowth into the plots, (6) Fertilized plots, where 30 kg ha⁻¹ year⁻¹ nitrogen is added to the surface of the plots, (7) Double Litter plots, where above ground leaf litter is allowed to enter the plots and the aboveground litter that falls on No Leaf and No Inputs plots is added, and (8) Double Litter + Fertilizer, where plots are fertilized in addition to receiving aboveground litter from no leaf and no input plots (same quantity of nitrogen as the fertilizer treatment), and (9) Wood chips (Bowden et al., 2014; Lajtha et al., 2018; Nadelhoffer et al., 2004; Santos et al., 2016). For this experiment, we collected soils from a subset of four treatments – the No Input plots, the No Root plots, the No Leaf input plots, and the Control plots (see table 1 for detailed treatment descriptions). In June of 2021, we collected soils from each of the three replicate plots of the four selected treatments to use in a mesocosm experiment. We removed two soil ‘bricks’ (20 cm wide × 30 cm long × 10 cm deep) from random edge locations in the plots to minimize disturbance of the DIRT experiment. We homogenized soils into one large bulk sample per treatment and kept them cool (4°C) until we established the mesocosm experiment.

Table 1. Subset DIRT network treatments: this experiment subset 4 unique DIRT treatments from the UMBS plots, they are described below. Throughout this paper, I will maintain this treatment order as a sort of continuum depicting fewest to most inputs into the plots - the no inputs treatments have the fewest, and the control plots have the greatest. ‘No Roots,’ and ‘No Leaf,’ serve as short hand for no belowground litter and no aboveground litter.

Treatment	Description
No Inputs, (-/-)	Aboveground and belowground litter inputs were excluded from the plots (aboveground litter excluded with 1-mm mesh cloth covering and polyethylene tarp, belowground litter inputs excluded up 140 cm depth).
No Roots, (+,-)	Roots are excluded with a plastic, water-permeable tarp-like_barrier (akin to an in-ground garden barrier) that extends from the soil surface to 140 cm depth.

No Leaf (-,+)	An 80% sunblock shade cover overlaid with a regular outdoor poly tarp prevents aboveground litter inputs from entering the plots. The cover always remains on the plots outside of seasonal sampling (2× annually).
Control, (+,+)	Natural above and belowground litter inputs can enter the plots. Seedlings and herbaceous material are removed from the plots 2×/year, in the autumn and spring.

Mesocosm Experimental Design

For the mesocosm experiment, we filled 200, 5cm × 10 cm tree growth cone-shaped mesocosms ($n = 75$) with sieved soils (2-cm sieve). Prior to sieving the soil, we removed any large organic matter from the soil surface like large pieces of leaves, sticks, and acorns. After randomizing each mesocosm by treatment, we inserted one *Q. rubra* acorn approximately 2 cm into the top of the soil. Seeds were sourced from Sheffield’s Seed Company (Locke, New York, USA). *Q. rubra* is a common successional species in northern Michigan and at the DIRT site, making it an ideal species to study in the context of altered soil conditions. Prior to planting, we surface sterilized seeds using 70% ethanol and stratified all seeds for 60 days according to germination instructions from Sheffield’s Seed Company and according to established methods (Mccarthy-Neumann & Ibañez, 2013).

The greenhouse at UMBS is heated passively, and we watered plants twice daily to account for high ambient temperatures. To avoid burning and mimic forest conditions, we shaded all plants with a 40% shade cloth. Rodents predated approximately 50% of the seeds over the course of the 6-week experiment. We watered mesocosms 2x a day (to soils’ saturation) and moved mesocosm trays around on the benches to account for variation in sunlight and greenhouse orientation over time. We noted seedling emergence (defined as the cumulative number of seedlings or sown seeds to grow out of the soil surface out of the total number of planted seeds) and seedling mortality (defined as the number of dead seedlings per germinated seeds) at the conclusion of each day, (Milbau et al., 2017)).

After 6 weeks, we harvested the seedlings to analyze biomass and plant traits. First, we removed the aboveground biomass by clipping each plant stem at the soil surface. We immediately placed all clipped seedlings in paper bags and in the refrigerator for the remainder of the harvest. Next, we separated the roots from the soil by gently shaking soil from root clumps and then submerging roots in deionized water and using tweezers to remove as much soil particulate matter as possible from the roots. We stored all cleaned roots individually in plastic bags and kept them cool for less than 2 weeks until we scanned them to estimate root area and subsequently dried them at 60°C for 3 days to estimate biomass. While we harvested plants, we sieved soils from each mesocosm individually using a 2mm sieve and immediately stored approximately 100g of each sample in the freezer for future nutrient or enzyme analyses.

To count leaves and calculate leaf area, we scanned all the fresh leaves from each of the harvested plants (Epson Perfection V19 Model J3714 flat-bed scanner). We included a known area in each scan to avoid incorrectly scaling the images to one another. Using ImageJ, we hand traced leaf outlines and calculated leaf area by setting a scale that converts pixels to cm², a common method for leaf area analysis (Maloof et al., 2013). We tallied each full or partial leaf per seedling and counted all leaves across all treatment seedlings. To calculate root area, we scanned 15 roots per treatment and analyzed the scans for root area using WinRhizo. We ground dried leaves + stems and roots to a fine powder with a ballmill grinder and then analyzed them for total C:N (LECO CN628).

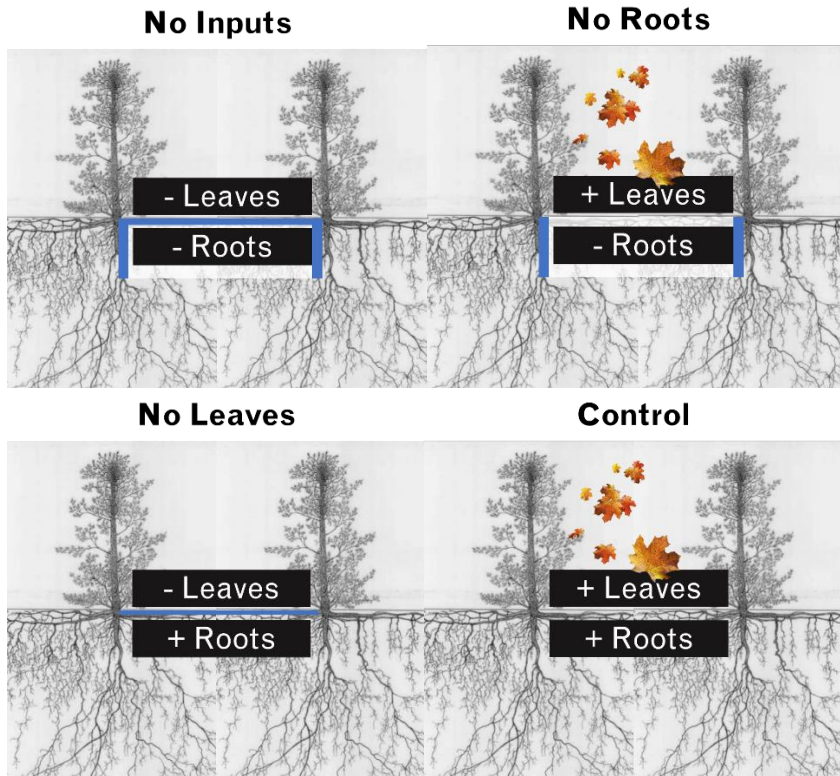


Figure 2. Project schematic. Soils from Detrital Inputs and Removal Treatments (DIRT) plots served as the basis for seedling mesocosm growth experiment. Hypotheses for the seedling mesocosm growth experiment.

Data analysis

All analyses and all model visualizations were created in R/RStudio with packages in R versions 4.1.3/2022.02.1. We performed ANOVAs with Tukey's HSD post-hoc tests on all normally distributed datasets; leaf % N data and number of leaves per seedling were not normally distributed and failed to conform with transformations - for those data, we ran a Kruskal Wallis non-parametric test (no post-hoc tests necessary for non-significant differences between treatments).

Results

Total biomass. Total seedling biomass (g) of *Q. rubra* plants grown in the No Roots, (+Leaves, -Roots) treatment was 27% greater than the biomass of plants grown in the No Inputs

(-Leaves, -Roots) treatment, (Figure 3, $F = 2.8201$, $P = 0.0412$). There was no statistical difference in seedling biomass between the No Inputs treatment, the No Roots treatment, or the the No Leaves and the Control treatments.

Aboveground biomass. Aboveground seedling biomass in the No Roots treatment was 32% greater compared to No Input treatment (Figure 1b, $F = 4.0584$, $P = 0.0086$). There were no statistically significant differences in aboveground seedling biomass between plants grown in these treatments and both the No Leaves and Control treatments. Belowground seedling biomass did not differ significantly among treatments (Figure 1c, $F = 0.4568$, $P = 0.7129$).

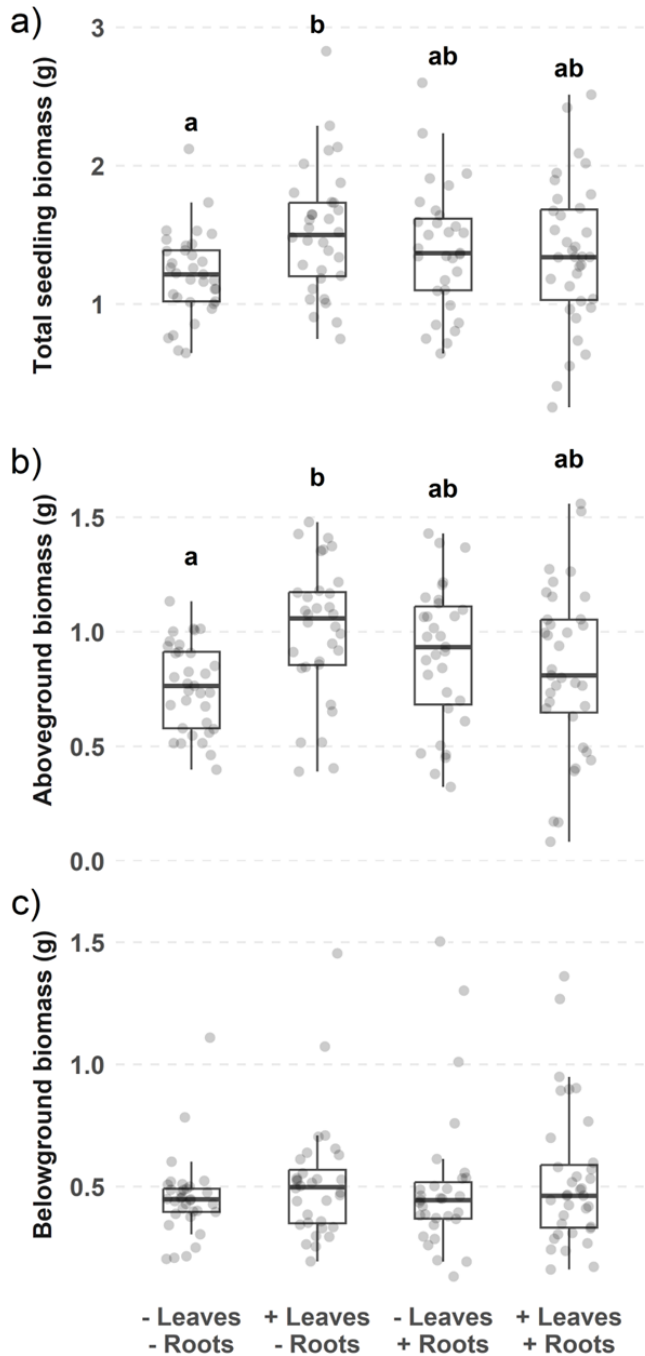


Figure 3. *Quercus rubra* seedling biomass. (a) Total seedling biomass (g) of *Q. rubra* plants grown in the No Roots, (+Leaves, -Roots) treatment increased by 27% compared to No Inputs (-Leaves, -Roots) treatments. ANOVA F -value = 2.8201, p -value = 0.0412. (b) +Leaf litter, -Root litter increased aboveground seedling biomass by 32% compared to no input plots, ANOVA F -value = 4.0584, p -value = 0.0086. (c) No treatment effect on belowground seedling biomass, ANOVA F -value = 0.4568, p -value = 0.7129.

Leaf area. Total leaf area of seedlings grown in the No Roots treatment (leaf litter present) was 21.5% greater than the leaf area seedlings grown in the No Input soils (Figure 2a, $F=2.4363$, $P=0.06791$). There were no other treatment differences. We removed one outlier that was 3 standard deviations from the mean (this plant was part of the No Roots treatment, sample code 3NRL).

Number of leaves. The number of leaves per seedling was not normally distributed ($W = 0.91886$, $p\text{-value} = 1.052e-06$) and several various transformations did not improve normality. Therefore, I used a Kruskal-Wallis test to examine how leaf number varied among treatments. It did not (Figure 3, $\chi^2 = 6.3661$, $p\text{-value} = 0.0951$).

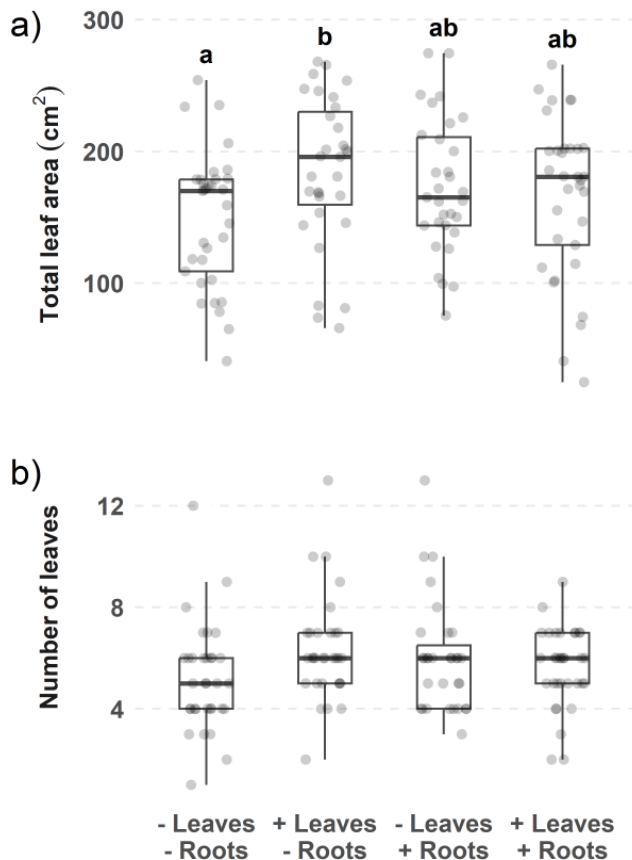


Figure 4. (a) Total leaf area increased by 21.5% for seedlings grown in soils with leaf litter present and root litter excluded compared to no input soils. One outlier has been removed for being > 3 standard deviations from the mean (3NRL). (b) No treatment effect on the number of leaves per seedling per treatment.

Nutrient composition. There were no differences in leaf total %N (Kruskal-Wallis $X^2 = 3.3133$, p -value = 0.3458) or leaf C:N (Figure 4 , ANOVA $F = 1.2320$, p -value = 0.3010) among the treatments.

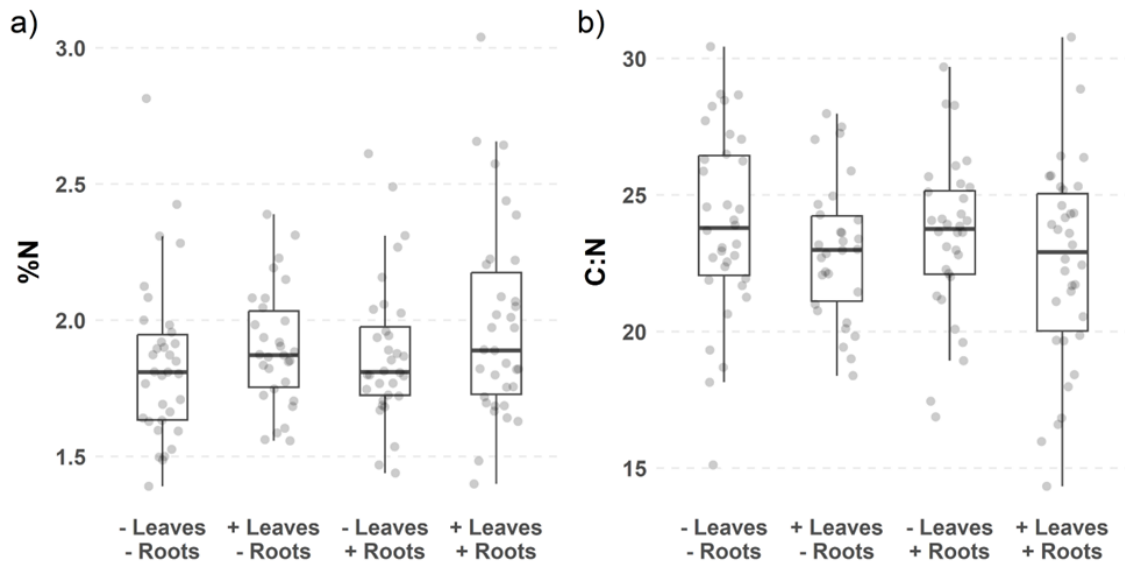


Figure 5. Carbon and nitrogen analysis of plant leaves. (a) Treatment has no effect on % nitrogen of dry leaves, *Kruskal-Wallis chi-squared* = 3.3133, p -value = 0.3458. (b) Treatment has no effect on the ratio of carbon to nitrogen in seedlings, ANOVA F -value = 1.3429, p -value = 0.2635

Discussion

Forests are responding to a diversity of global changes that alter the quality and quantity of leaf and root inputs into soils (Norby & Zak, 2011). These new soil environments could differentially facilitate the growth of seedlings and lead to changes in leaf and root traits as seedlings emerge (Veen et al. 2019; Gillespie et al. 2021). While we know that global changes such as elevated $[CO_2]$ will change the relative contribution of roots and leaves to soil quality (Norby et al., 2004; Norby & Zak, 2011), it is difficult and expensive to experimentally manipulate global changes such as elevated $[CO_2]$ in ecosystems for long enough to see large nutrient changes in soil pools. We took advantage of a long-term soil input manipulation, the DIRT experiment, to explore how long-term differential inputs of leaves and roots to forest soils might impact seedling (*Q. rubra*) growth, leaf and root traits, and root/leaf biomass production.

We found that seedlings had 27% more biomass when grown in soils where leaf litter was added and roots inputs were removed relative to treatments where root and leaf litter inputs were removed (no inputs). We also found that seedlings had 21.5% more leaf area when grown in soils where leaf litter was added and roots inputs were removed relative to treatments where root and leaf litter inputs were removed. We found no significant effect of any of our soil treatments on seedling leaf C:N or % total N. Overall, we draw three main conclusions: (1) Red oak seedlings grown in soils where only leaf, not root, litter was added had 32% more aboveground biomass than seedlings grown in soils where root and leaf litter was excluded. (2) Interestingly, there were no differences in belowground biomass among treatments (3) There were no statistically significant differences among treatments in seedling leaf area, leaf % nitrogen, leaf C:N or leaf number.

In support of our prediction that seedling biomass and leaf area would be lowest in soils with no inputs, we found that seedling biomass and leaf area were lowest in soils where there were no leaf or root inputs. Interestingly, there was a significant difference between the no input treatment and the plus leaf litter input treatment where there was more biomass in the plus leaf litter treatment (leaf litter treatment excludes root litter). Biomass and leaf area was similar in the plus leaf, plus root, and plus leaf and root treatments and the plus root and plus leaf and root treatments were no different from the no input treatment. Further, the number of leaves per seedling and plant nutrient contents did not significantly differ among treatments. These results unexpectedly contrast our hypotheses because belowground litter can contribute more to soil nutrients than aboveground litter (Zhu et al., 2021). Instead, we observed the opposite – in the absence of root litter, leaf litter increased seedling biomass. We found this result surprising as we predicted that, when roots and litter were both added to the soil, soil fertility would increase and seedling biomass, leaf number and leaf nutrient content would be enhanced (Gillespie et al., 2021). However, previous studies have also found that the addition of root litter can negatively impact seedling success. Aldorfova, and colleagues (2022) found that root litter

additions negatively influenced biomass in a constructed, two-phase garden experiment with seven common European annual species. They suggested that pathogens that were transferred with the root addition or nutrient immobilization led to a reduction in community biomass. They found that leaf litter additions increased soil nutrients but did not impact seedling production. It is possible that root pathogens varied in our treatments and could lead to a decline in seedling biomass relative to the leaf litter only treatment; this would be an area to follow up on in future work. It is also possible the addition of lignified root litter slowed the release of N from this material, thereby having a negative effect on seedling growth. Data from the UMBS DIRT soils mineralization incubations in 2018 suggest that the presence of root litter in the absence of leaf litter increases soil nitrogen compared to no root treatment soils (where leaves are present). In these soils, it may be unlikely that root litter immobilized nutrients, decreasing seedling biomass. To date, differences in and among DIRT plots have not been analyzed; rhizosphere pathogens remain a possibility and provide direction for future research. Senesced root and shoot litter can influence plant soil feedbacks physically, chemically, and biotically. There are many potential mechanisms behind these litter-mediated plant soil feedbacks which could alter nutrient cycling in the DIRT soils and affect seedling growth in these soils including close associations between decomposer communities and specific plant species (Austin et al., 2014), species-specific nutrient release patterns over time (Perez et al., 2013), and toxic compounds released during decomposition (Mazzoleni et al., 2015). These litter-mediated plant soil feedbacks, both above and belowground, are another mechanism to explore in future studies (Veen et al., 2019).

There are numerous DIRT experiments occurring in a variety of ecosystem types – grassland, coniferous forest, deciduous forests -- around the world and thus among site comparisons can be context dependent. Yet, an overall pattern across sites emerges - excluding aboveground litter alters soil conditions, though the response and direction is site specific. At the Harvard Forest site after 20 years of litter manipulations (1990 - 2010), doubling litter primed the soil community and increased litter decomposition – labile C did not increase in

SOM, microbial activity biomass (PLFA profiles), and the fungal: bacterial of the microbial community increased (Pisani et al., 2016). These results were observed only in the double litter plots suggesting there is a litter quantity threshold necessary. Unfortunately, we did not select this treatment for our seedling experiment. While the changes observed at the Harvard Forest DIRT site were observed at other dirt sites, the results are not universal (Crow, Lajtha, Filley, et al., 2009; Lajtha, Bowden, et al., 2014; Sulzman et al., 2005). For example, while Lajtha et al., (2014) observed similar microbial shifts including lack of C accretion in SOM and increased soil respiration in double litter treatments, they observed no change in soil mineral C (Lajtha, Townsend, et al., 2014); these results are not the same at UMBS. However, excluding leaf litter in their study negatively impacted soil C (decreased it by 18%). Further, they found that decreasing root litter (no roots treatment) reduced soil C by 9%; adding leaf litter did not change soil C but excluding both roots and leaf input altered soil C (Lajtha, Townsend et al., 2014). At the Bousson temperate forest site, Bowden et al., 2014 found leaf litter exclusions reduced soil C (for both O and mineral horizons combined) by 24% in No Litter plots and 33% in No Input treatments. The No Root treatments did not reduce soil C as may have been expected. Taken together, soil C content generally decreases with litter exclusion treatments across a variety of DIRT sites. This pattern suggests that soil C content and stability may be more affected by litter exclusions than by litter addition (Bowden et al., 2014; Fekete et al., 2014; Leff et al., 2012; Sayer, 2006). In our results, excluding leaf litter, calculated as the difference between No Input treatments and No Root treatments, led to lower total seedling biomass. This change potentially reflects the pattern observed in other DIRT sites, where excluding aboveground litter affects soil properties which may have cascading effects to future seedling growth.

There are confounding factors that might have influenced our results including DIRT methods, non-native earthworm presence at UMBS, rodent predation of acorns during the mesocosm experiment, and other unmeasured variables. Since we sourced soils from a long-term field experiment, our existing knowledge is subject to established sampling techniques –

for example, live plant material is removed from the DIRT plots annually and biomass data are not recorded. Tracking the differences in aboveground plant biomass each year could further validate our conclusion that aboveground plant biomass increases when seedlings are grown in plots where belowground litter is excluded from entering the plots. Additionally, belowground biomass of those plants that grow throughout the year and are then removed is not removed, because that would introduce additional disturbance to the long-term field study. Currently, we do not know the impact of those fine roots on all the DIRT treatment soil profiles analyzed here. Non-native earthworms significantly affect soil structure and nutrient cycling at UMBS (Crumsey et al., 2015). When earthworms invaded the site, soil C storage declined and offset increased C inputs from leaf litter addition (Crumsey et al., 2015). The DIRT plots at UMBS do not exclude earthworms or measure their effect on soil profiles, including litter loss quantities to them in litter addition plots. These background ecosystem characteristics may influence seedling responses - if earthworms at UMBS significantly reduce nutrient inputs to plots, then our significant responses cannot be attributed to treatment effect alone. During the mesocosm growth portion of the experiment, acorn predation by rodents led to the loss of approximately 50% of the seedlings that we originally planted. Finally, we did not measure variables such as post seedling soil nutrient analyses, microbial community analysis of DIRT treatment soils or soils post seedling growth, or root decomposition rates which could have impacted our results (Aldorfová et al., 2022; Gillespie et al., 2021; Veen et al., 2019).

One of the strengths of the DIRT network is the factorial comparison between aboveground and belowground litter input in isolation. Because we did not observe greater seedling growth from root litter isolated soils (no leaf litter plots compared to no root plots), we conclude that additional belowground soil processes may complicate how litter inputs from roots and leaves impact seedling growth. For example, root priming may occur; King et al. (2022, unpublished data from Zak lab) found that the activity of some microbial enzymes in the organic layer, were higher in in the leaf litter and no root treatments relative to the no litter input

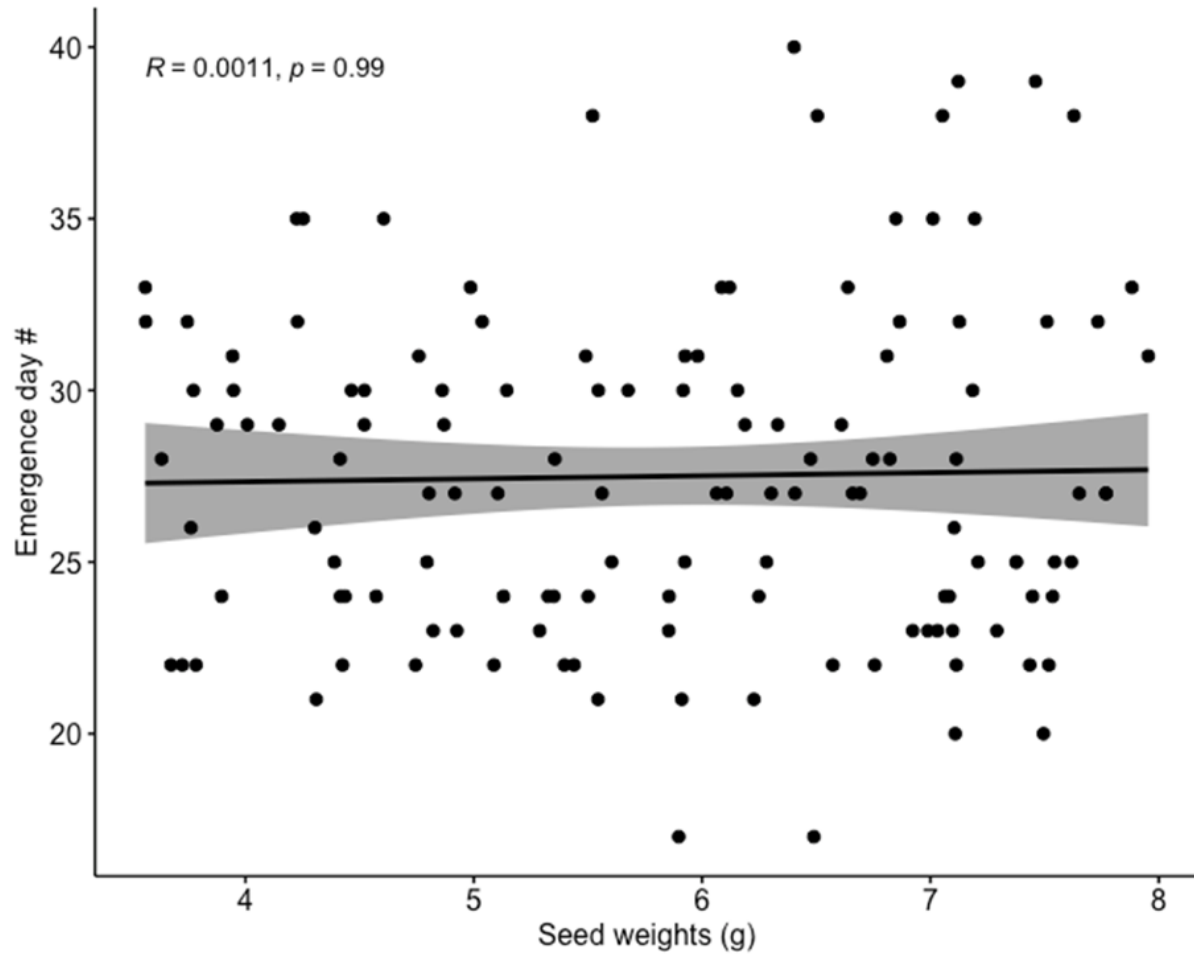
treatment (see appendix). In the soils where roots were eliminated, priming may not impact soil nutrient availability. Thus, microbial activity and root exudates may impact soil nutrient turnover, occlusion, and availability in ways we did not measure leading to our observed results.

Alternatively, there may be another mechanism at play leading to our observed patterns – root pathogen loads could be higher in the root addition plots leading to negative plant soil feedbacks and slower seedling growth. Long term soil input manipulations did not affect seedlings to the degree or in the ways we originally hypothesized. If our results had supported our hypotheses, we would have observed seedling growth, leaf nutrient contents, and leaf area scale with relative inputs into the treatment soils (with the fewest inputs in No Input plot soils, and the greatest inputs into control plot soils). Instead, our mesocosm experiment suggests the need for additional studies; these could include expanding tree seedling species and broadening the DIRT treatments selected to include wood and fertilized plots. Looking more closely at plant soil feedbacks could be important for understanding legacy effects of plants and soils in the DIRT network. Future analyses of root chemistry and other nutrient availability in UMBS DIRT plots might increase our understanding of above and belowground decomposition and further inform future seedling and forest research (Zhang & Wang, 2015). By continuing to leverage long-term field studies and support research stations, we can continue to enable students to delve into these unanswered and potentially unanswerable questions. With that being said, I would like to echo Dr. Francis Hole’s remarks when asked how long he hoped the DIRT experiment would last, and how long I hope ecosystem science research baffles scientists, from undergraduates to experienced researchers: “oh, I am hoping for 1000 years at least”.

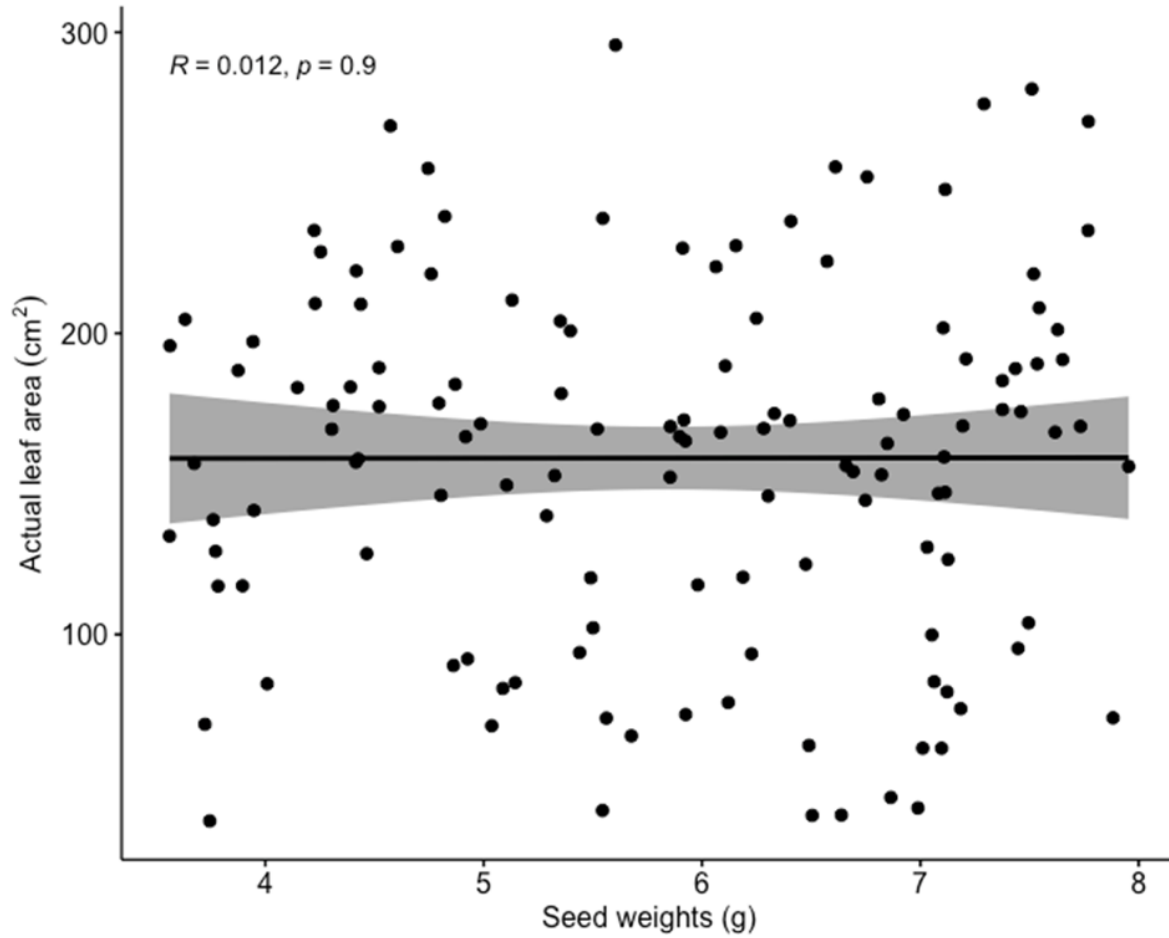
Appendices

Table 1. Subset DIRT network treatments: this experiment subset 4 unique DIRT treatments from the UMBS plots, they are described below. Throughout this paper, I will maintain this treatment order as a sort of continuum depicting fewest to most inputs into the plots - the no inputs treatments have the fewest, and the control plots have the greatest. 'No Roots,' and 'No Leaf,' serve as short-hand for no belowground litter and no aboveground litter.

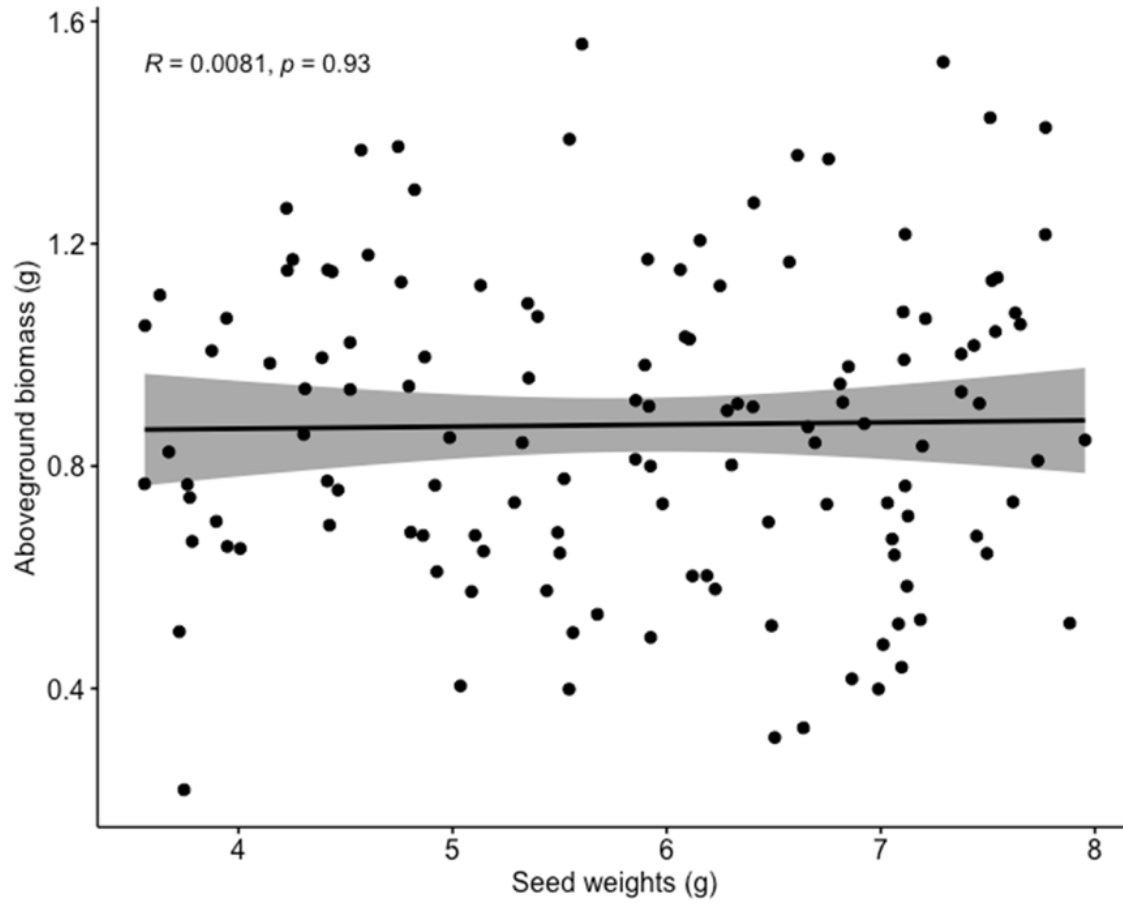
Treatment	Description
No Inputs, (-/-)	Aboveground and belowground litter inputs were excluded from the plots (aboveground litter excluded with 1-mm mesh cloth covering and polyethylene tarp, belowground litter inputs excluded up 140 cm depth).
No Roots, (+,-)	Roots are excluded with a plastic, water-permeable tarp-like_barrier (akin to an in-ground garden barrier) that extends from the soil surface to 140 cm depth.
No Leaf (-,+)	An 80% sunblock shade cover overlaid with a regular outdoor poly tarp prevents aboveground litter inputs from entering the plots. The cover always remains on the plots outside of seasonal sampling (2x annually).
Control, (+,+)	Natural above and belowground litter inputs can enter the plots. Seedlings and herbaceous material are removed from the plots 2x/year, in the autumn and spring.



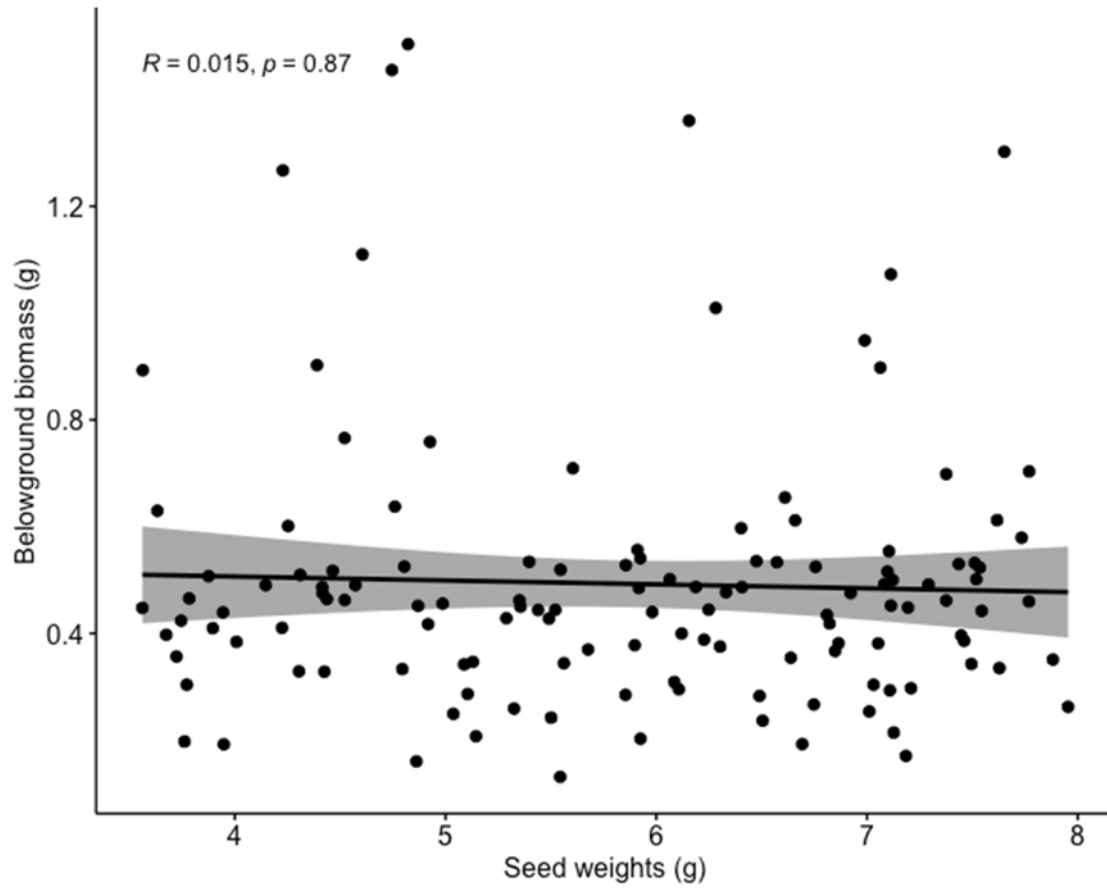
Appendix 2. Seed weights (g) are not correlated with emergence (number of days plants visible above ground since planting), both datasets are non-normally distributed, Spearman's Rho statistic, $r = 0.0065$, $p = 0.94$, line shows best fit \pm standard error.



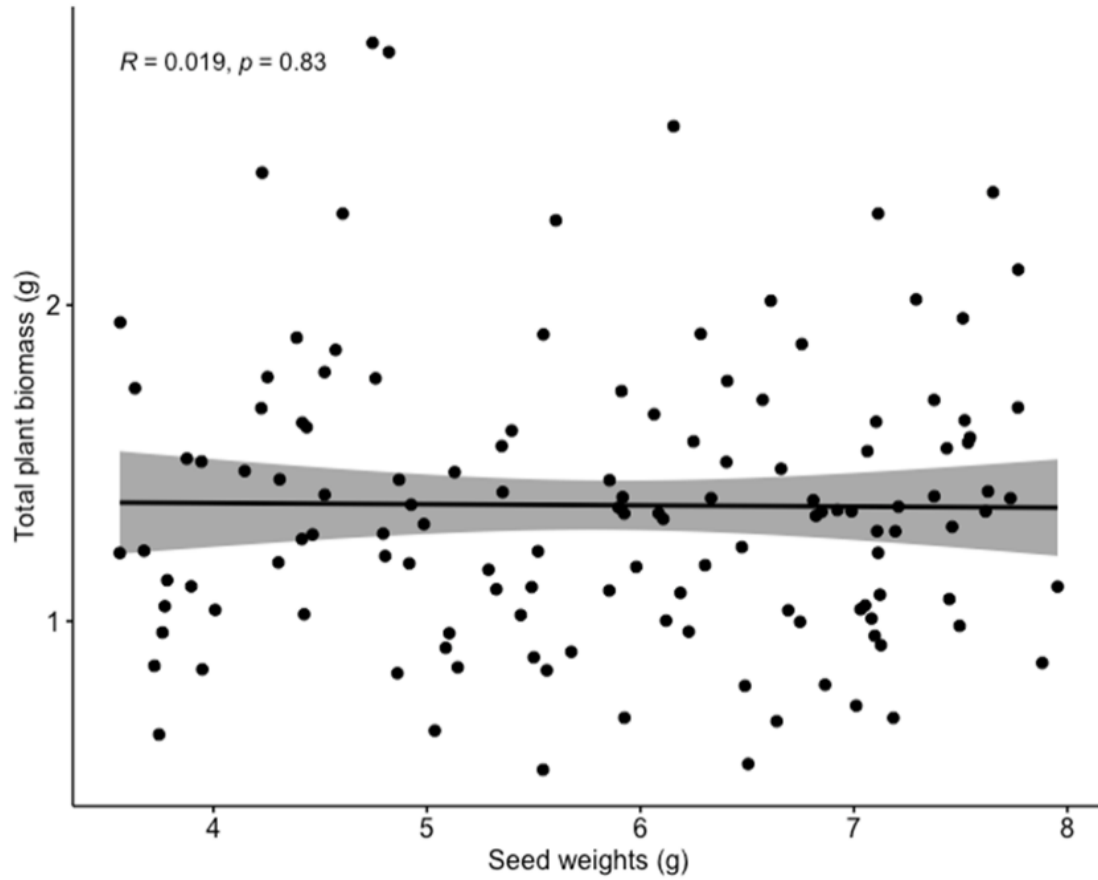
Appendix 3. Seed weights (g) are not correlated with leaf area (cm²), seed weight data are non-normally distributed, Spearman's correlation results: $R = 0.012, p = 0.90$, line shows best fit \pm standard error.



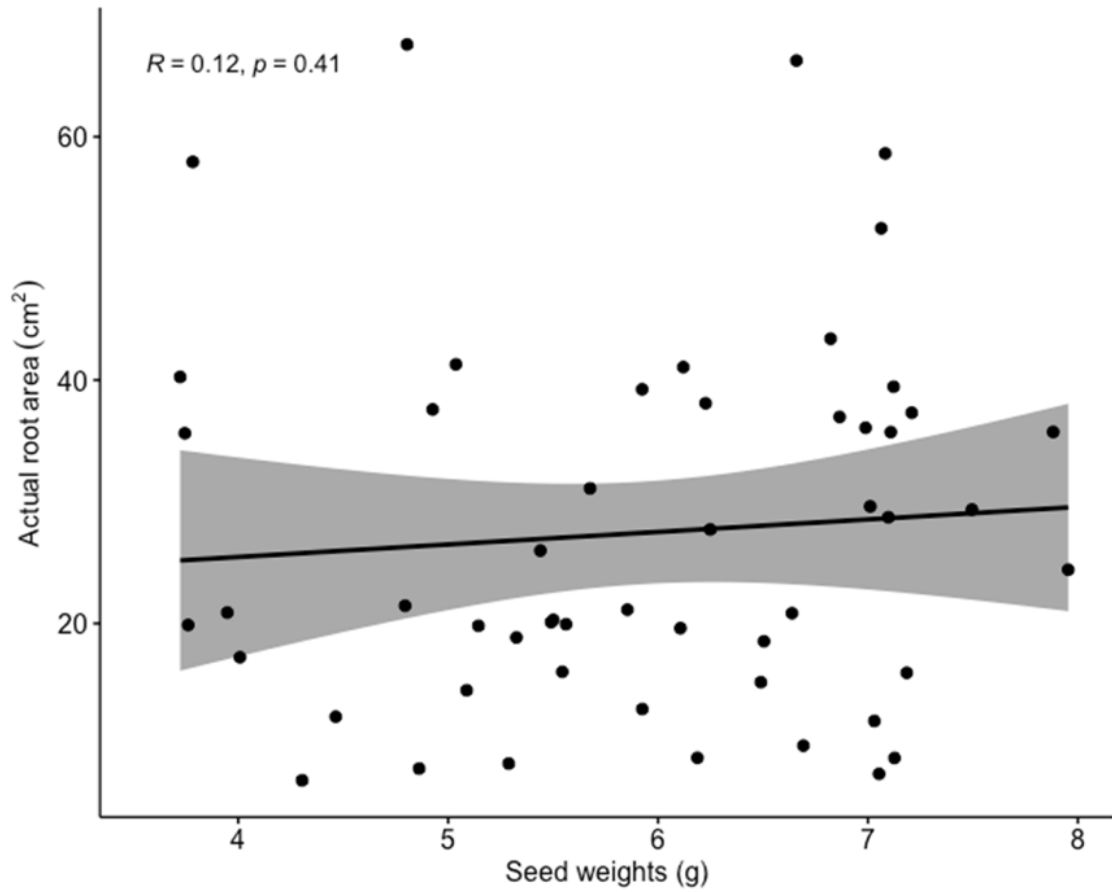
Appendix 4. Seed weights (g) are not correlated with aboveground biomass (g), seed weights are non-normally distributed, Spearman's correlation result, $r = 0.0081$, $p = 0.93$, line shows best fit \pm standard error.



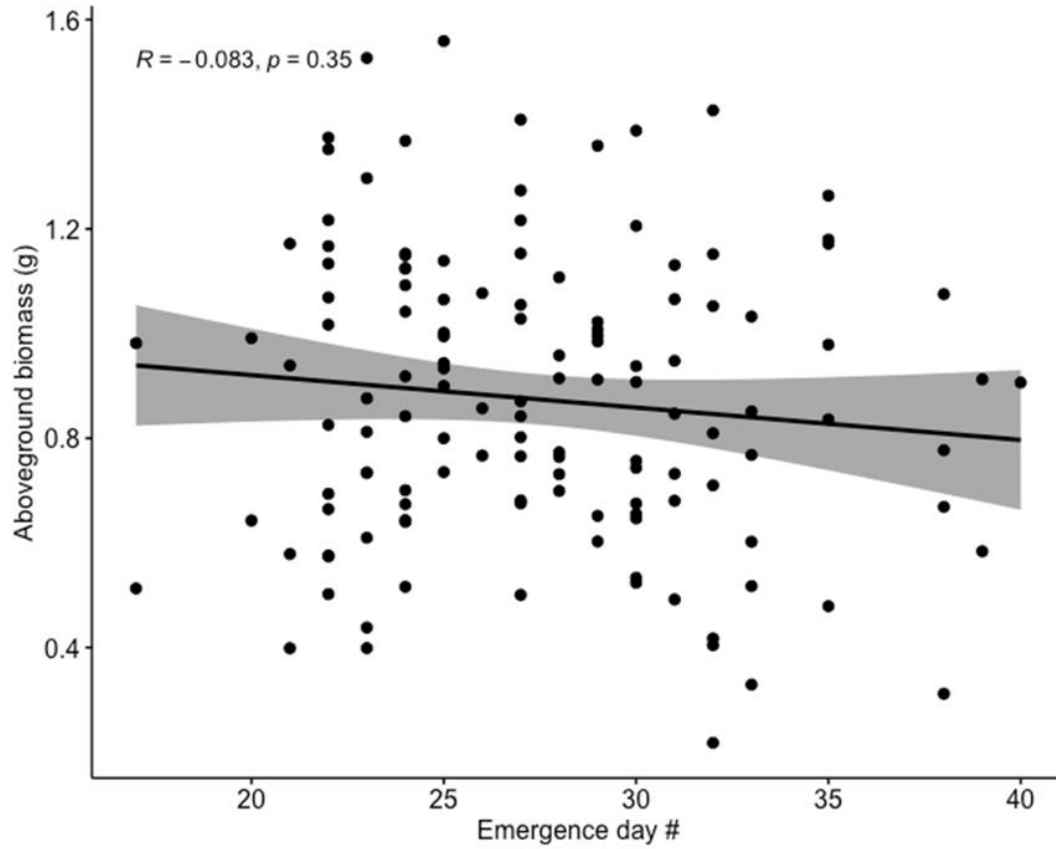
Appendix 5. Seed weights (g) are not correlated with belowground biomass (g), both datasets are non-normally distributed, Spearman's correlation result, $r = 0.015, p = 0.87$, line shows best fit \pm standard error.



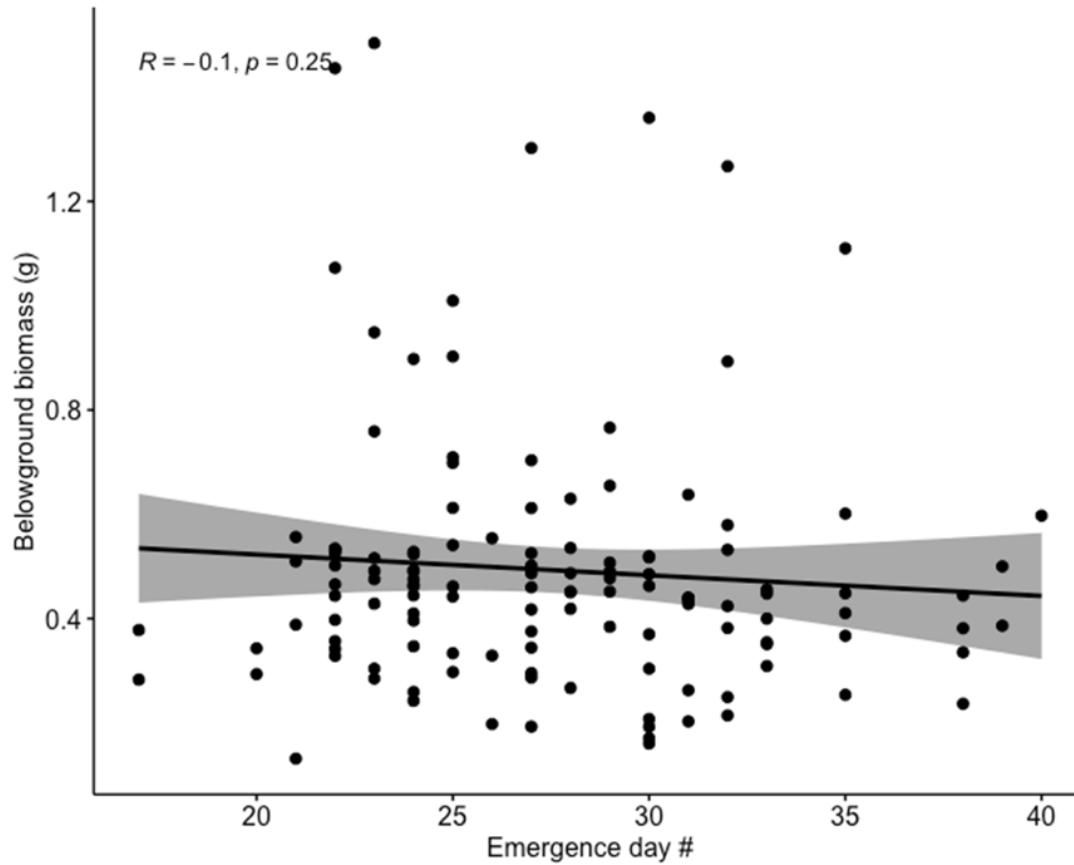
Appendix 6. Seed weights (g) are not correlated with total plant biomass (g), both datasets are non-normally distributed, Spearman's correlation result, $r = 0.015, p = 0.87$, line shows best fit \pm standard error.



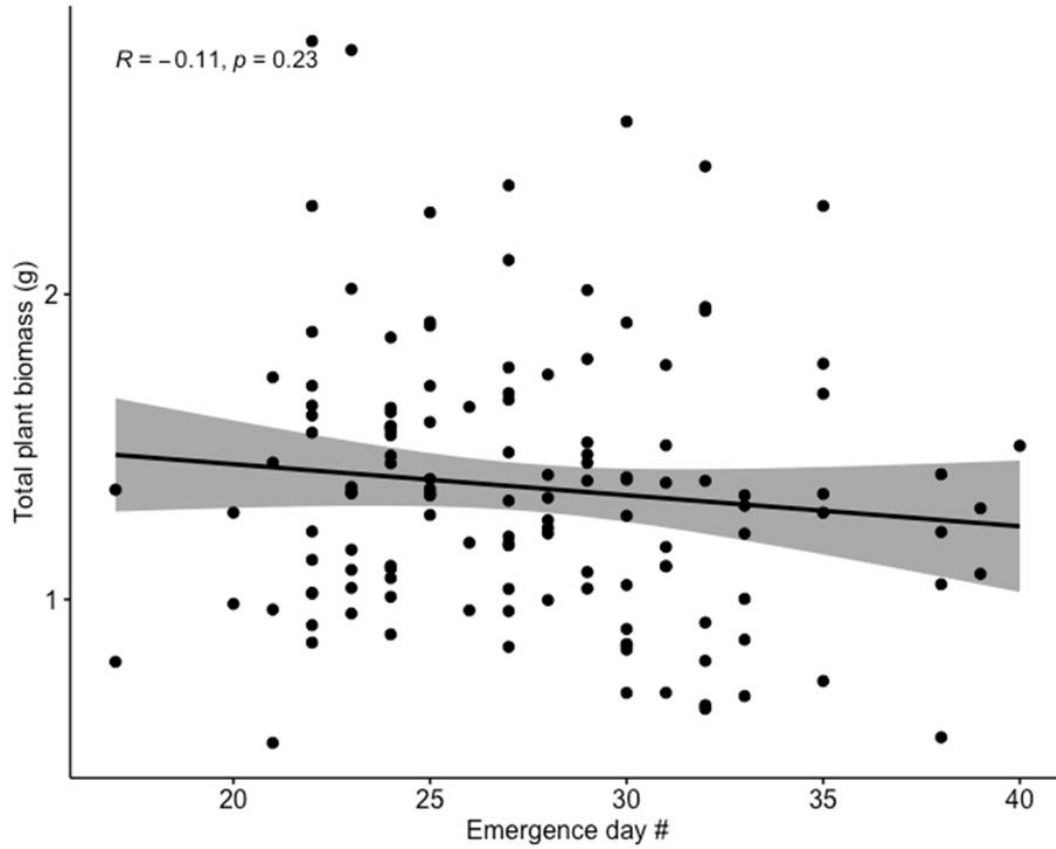
Appendix 7. Seed weights (g) are not correlated with actual root area (cm²), both datasets are non-normally distributed, Spearman's correlation result, $r = 0.12, p = 0.40$, line shows best fit \pm standard error.



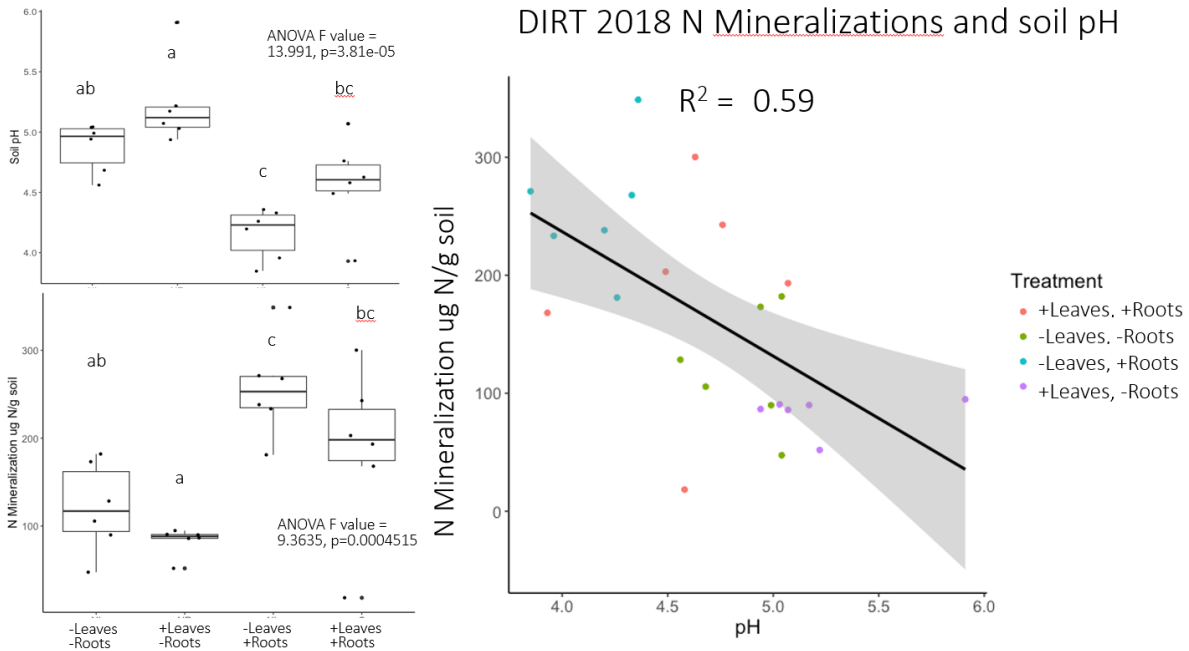
Appendix 8. Emergence day number are not correlated with aboveground biomass (g), emergence data are non-normally distributed, Spearman's correlation result, $r = -0.083$, $p = 0.35$, line shows best fit \pm standard error.



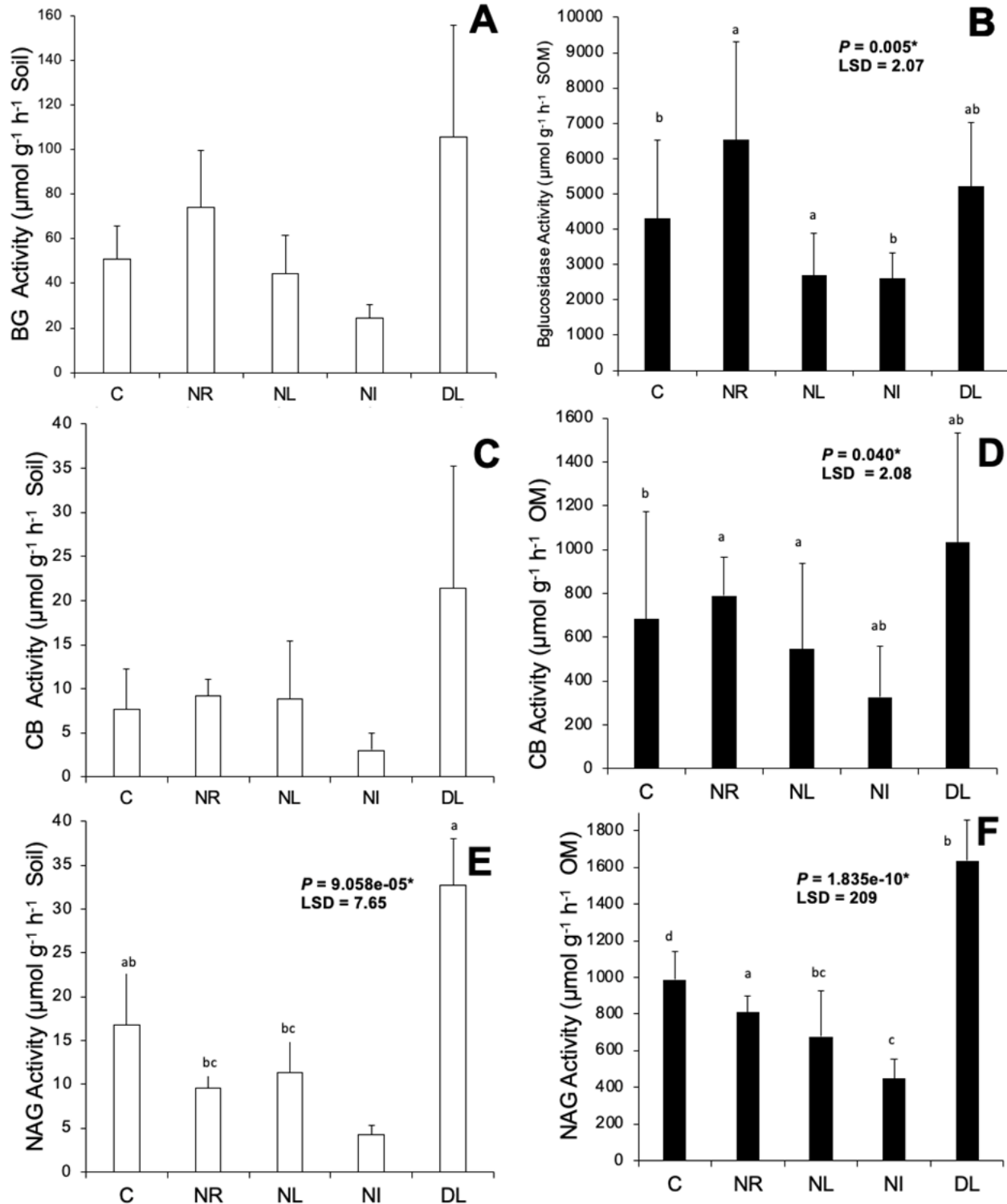
Appendix 9. Emergence day number is not correlated with belowground biomass (g) neither dataset is non-normally distributed, Spearman's correlation result, $r = -0.010$, $p = 0.25$, line shows best fit \pm standard error.



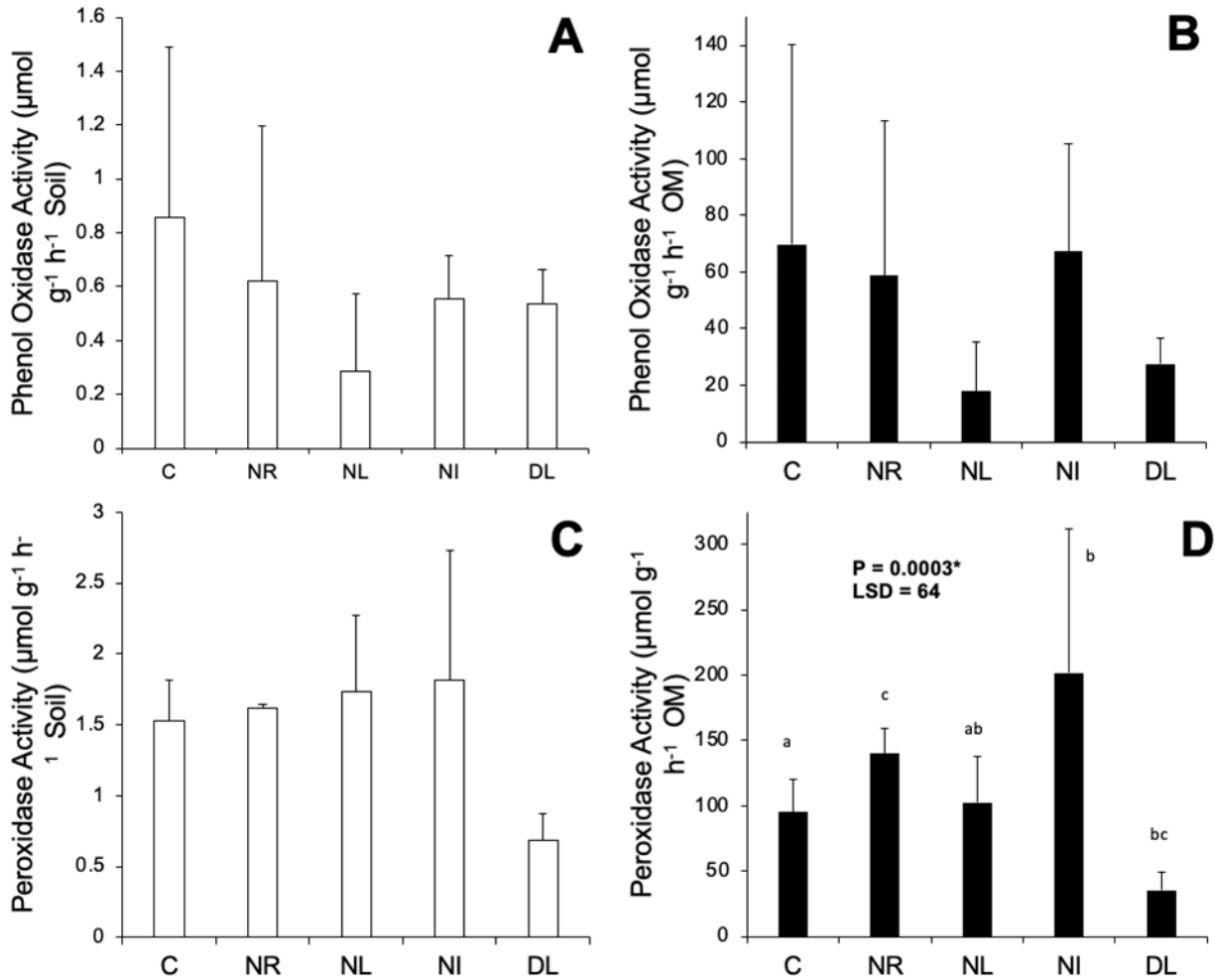
Appendix 10. Emergence day number is not correlated with total plant biomass (g) neither dataset is non-normally distributed, Spearman's correlation result, $r = -0.011$, $p = 0.23$, line shows best fit \pm standard error.



Appendix 11. DIRT soil mineralization data and soil pH data, 2018 (Nadelhoffer Lab). Results depict subset of treatments. Soil pH declines between isolation of above vs. belowground soil inputs, the No Roots and No Leaves treatment. ANOVA results, F-value = 13.991, p-value = 3.81e-05. N Mineralization values (ug N g soil) increase between the No Roots and No Leaves treatments, ANOVA results F-value = 9.3635, p-value = 0.0004515.



Appendix 12. Samantha King enzyme activity results 1 (2022). Mean β -glucosidase (BG), cellobiohydrolase (CB), and N-acetylglucosaminidase (NAG) activity across treatments ($n = 3$). White bars indicate enzyme activity per gram soil; black bars represent enzyme activity per gram soil organic matter (SOM). One standard deviation is shown by the length of each error bar. Treatments with the same letter are not significantly different. * indicates statistically significant differences in means based on ANOVA testing ($\alpha = 0.05$).



Appendix 13. Samantha King enzyme activity results 2 (2022). Mean phenol oxidase and peroxidase activity across treatments (n=3). White bars indicate enzyme activity per gram soil; black bars represent enzyme activity per gram soil organic matter (SOM). One standard deviation is shown by the length of each error bar. Treatments with the same letter are not significantly different. * indicates statistically significant differences in means based on ANOVA testing ($\alpha = 0.05$).

References

- Aldorfová, A., Dostálek, T., & Münzbergová, Z. (2022). Effects of soil conditioning, root and shoot litter addition interact to determine the intensity of plant–soil feedback. *Oikos*, 2022(6), 1–12.
<https://doi.org/10.1111/oik.09025>
- Austin, A. T., Vivanco, L., González-Arzac, A., & Pérez, L. I. (2014). There's no place like home? An exploration of the mechanisms behind plant litter-decomposer affinity in terrestrial ecosystems. *New Phytologist*, 204(2), 307–314. <https://doi.org/10.1111/nph.12959>
- Berhongaray, G., Cotrufo, F. M., Janssens, I. A., & Ceulemans, R. (2019). Below-ground carbon inputs contribute more than above-ground inputs to soil carbon accrual in a bioenergy poplar plantation. *Plant and Soil*, 434(1–2), 363–378. <https://doi.org/10.1007/s11104-018-3850-z>
- Bever, J. D. (1994). Feedback between plants and their soil communities in an old field community. *Ecology*, 75(7), 1965–1977. <https://doi.org/10.2307/1941601>
- Bonan, G. B. (2008). Forests and Climate Change: Forcings, Feedbacks, and the Climate Benefits of Forests. *New Series*, 320(5882), 1444–1449.
- Bowden, R. D., Deem, L., Plante, A. F., Peltre, C., Nadelhoffer, K., & Lajtha, K. (2014). Litter Input Controls on Soil Carbon in a Temperate Deciduous Forest. *Soil Science Society of America Journal*, 78(S1), S66–S75.
<https://doi.org/10.2136/sssaj2013.09.0413nafsc>
- Bünemann, E. K., Bongiorno, G., Bai, Z., Creamer, R. E., De Deyn, G., De Goede, R., ... & Brussaard, L. (2018). Soil quality—A critical review. *Soil Biology and Biochemistry*, 120, 105–125.
- Crow, S. E., Lajtha, K., Bowden, R. D., Yano, Y., Brant, J. B., Caldwell, B. A., & Sulzman, E. W. (2009). *Increased coniferous needle inputs accelerate decomposition of soil carbon in an old-growth forest.*
<https://doi.org/10.1016/j.foreco.2009.01.014>
- Crow, S. E., Lajtha, K., Filley, T. R., Swanston, C. W., Bowden, R. D., & Caldwell, B. A. (2009). Sources of plant-derived carbon and stability of organic matter in soil: Implications for global change. *Global Change Biology*, 15(8), 2003–2019. <https://doi.org/10.1111/j.1365-2486.2009.01850.x>
- Crumsey, J. M., Capowiez, Y., Goodsitt, M. M., Larson, S., Le Moine, J. M., Bird, J. A., Kling, G. W., & Nadelhoffer, K. J. (2015). *Exotic earthworm community composition interacts with soil texture to affect redistribution and retention of litter-derived C and N in northern temperate forest soils.* 126(3), 379–395. <https://doi.org/10.1007/s>
- Fekete, I., Kotroczó, Z., Varga, C., Nagy, P. T., Várbíró, G., Bowden, R. D., Tóth, J. A., & Lajtha, K. (2014). Alterations in forest detritus inputs influence soil carbon concentration and soil respiration in a central-european deciduous forest. *Soil Biology and Biochemistry*, 74, 106–114. <https://doi.org/10.1016/j.soilbio.2014.03.006>

- Feng, J., He, K., Zhang, Q., Han, M., & Zhu, B. (2022). Changes in plant inputs alter soil carbon and microbial communities in forest ecosystems. *Global Change Biology*, *28*(10), 3426–3440.
<https://doi.org/10.1111/gcb.16107>
- Finzi, A. C., Norby, R. J., Calfapietra, C., Gallet-Budynek, A., Gielen, B., Holmes, W. E., Hoosbeek, M. R., Iversen, C. M., Jackson, R. B., Kubiske, M. E., Ledford, J., Liberloo, M., Oren, R., Polle, A., Pritchard, S., Zak, D. R., Schlesinger, W. H., & Ceulemans, R. (2007). Increases in nitrogen uptake rather than nitrogen-use efficiency support higher rates of temperate forest productivity under elevated CO₂. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(35), 14014–14019.
<https://doi.org/10.1073/pnas.0706518104>
- Gillespie, L. M., Hättenschwiler, S., Milcu, A., Wambsganss, J., Shihan, A., & Fromin, N. (2021). Tree species mixing affects soil microbial functioning indirectly via root and litter traits and soil parameters in European forests. *Functional Ecology*, *35*(10), 2190–2204. <https://doi.org/10.1111/1365-2435.13877>
- Gross, C. D., & Harrison, R. B. (2019). The case for digging deeper: Soil organic carbon storage, dynamics, and controls in our changing world. *Soil Systems*, *3*(2), 1–24. <https://doi.org/10.3390/soilsystems3020028>
- Hardiman, B. S., Bohrer, G., Gough, C. M., Vogel, C. S., Curtis, P. S., Vogel, S., Curtis, S., & Hardiman, S. (2011). The role of canopy structural complexity in wood net primary production of a maturing northern deciduous forest. Published by : Ecological Society of America Stable URL : <http://www.jstor.org/stable/23034858> . Your use of the JSTOR archive indicates your a. *Ecological Society of America*, *92*(9), 1818–1827.
<http://onlinelibrary.wiley.com/doi/10.1890/10-2192.1/abstract>
- Hassan, K., Golam Dastogeer, K. M., Carrillo, Y., & Nielsen, U. N. (2022). Climate change-driven shifts in plant–soil feedbacks: a meta-analysis. *Ecological Processes*, *11*(1), 1–13. <https://doi.org/10.1186/S13717-022-00410-Z/FIGURES/5>
- Huang, W., & Spohn, M. (2015). Effects of long-term litter manipulation on soil carbon, nitrogen, and phosphorus in a temperate deciduous forest. *Soil Biology and Biochemistry*, *83*, 12–18.
<https://doi.org/10.1016/j.soilbio.2015.01.011>
- Jing, Y., Tian, P., Wang, Q., Li, W., Sun, Z., & Yang, H. (2021). Effects of root dominate over aboveground litter on soil microbial biomass in global forest ecosystems. *Forest Ecosystems*, *8*(1). <https://doi.org/10.1186/s40663-021-00318-8>
- Keller, A. B., Brzostek, E. R., Craig, M. E., Fisher, J. B., & Phillips, R. P. (2021). Root-derived inputs are major contributors to soil carbon in temperate forests, but vary by mycorrhizal type. *Ecology Letters*, *24*(4), 626–635.
<https://doi.org/10.1111/ele.13651>

- Lajtha, K., Bowden, R. D., Crow, S., Fekete, I., Kotroczó, Z., Plante, A., Simpson, M. J., & Nadelhoffer, K. J. (2018). The detrital input and removal treatment (DIRT) network: Insights into soil carbon stabilization. *Science of the Total Environment*, 640–641, 1112–1120. <https://doi.org/10.1016/j.scitotenv.2018.05.388>
- Lajtha, K., Bowden, R. D., & Nadelhoffer, K. (2014). Litter and Root Manipulations Provide Insights into Soil Organic Matter Dynamics and Stability. *Soil Science Society of America Journal*, 78(S1), S261–S269. <https://doi.org/10.2136/sssaj2013.08.0370nafsc>
- Lajtha, K., Townsend, K. L., Kramer, M. G., Swanston, C., Bowden, R. D., & Nadelhoffer, K. (2014). Changes to particulate versus mineral-associated soil carbon after 50 years of litter manipulation in forest and prairie experimental ecosystems. *Biogeochemistry*, 119(1–3), 341–360. <https://doi.org/10.1007/s10533-014-9970-5>
- Leff, J. W., Wieder, W. R., Taylor, P. G., Townsend, A. R., Nemergut, D. R., Grandy, A. S., & Cleveland, C. C. (2012). Experimental litterfall manipulation drives large and rapid changes in soil carbon cycling in a wet tropical forest. *Global Change Biology*, 18(9), 2969–2979. <https://doi.org/10.1111/j.1365-2486.2012.02749.x>
- Liu, R., Zhang, Y., Hu, X. F., Wan, S., Wang, H., Liang, C., & Chen, F. S. (2021). Litter manipulation effects on microbial communities and enzymatic activities vary with soil depth in a subtropical Chinese fir plantation. *Forest Ecology and Management*, 480(1101), 118641. <https://doi.org/10.1016/j.foreco.2020.118641>
- Lu, X., Gilliam, F. S., Yue, X., Wang, B., & Kuang, Y. (2023). Shifts in Above- Versus Below-Ground Carbon Gains to Terrestrial Ecosystems Carbon Sinks Under Excess Nitrogen Inputs. *Global Biogeochemical Cycles*, 37(3), e2022GB007638. <https://doi.org/10.1029/2022GB007638>
- Ma, Y., Filley, T. R., Szlavecz, K., & McCormick, M. K. (2013). Controls on wood and leaf litter incorporation into soil fractions in forests at different successional stages. <https://doi.org/10.1016/j.soilbio.2013.10.043>
- Ma, Y., McCormick, M. K., Szlavecz, K., & Filley, T. R. (2019). Controls on soil organic carbon stability and temperature sensitivity with increased aboveground litter input in deciduous forests of different forest ages. 47907.
- Maloof, J. N., Nozue, K., Mumbach, M. R., & Palmer, C. M. (2013). LeafJ: An ImageJ Plugin for Semi-automated Leaf Shape Measurement. *J. Vis. Exp*, 71, 50028. <https://doi.org/10.3791/50028>
- Man, M., Pierson, D., Chiu, R., Tabatabaei Anaraki, M., vandenEnden, L., Ye, R. X., Lajtha, K., & Simpson, M. J. (2022). Twenty years of litter manipulation reveals that above-ground litter quantity and quality controls soil organic matter molecular composition. *Biogeochemistry*, 159(3), 393–411. <https://doi.org/10.1007/s10533-022-00934-8>
- Mangan, S. A., Schnitzer, S. A., Herre, E. A., MacK, K. M. L., Valencia, M. C., Sanchez, E. I., & Bever, J. D. (2010). Negative plant-soil feedback predicts tree-species relative abundance in a tropical forest. *Nature*, 466(7307),

- 752–755. <https://doi.org/10.1038/nature09273>
- Mazzoleni, S., Bonanomi, G., Incerti, G., Chiusano, M. L., Termolino, P., Mingo, A., Senatore, M., Giannino, F., Carteni, F., Rietkerk, M., & Lanzotti, V. (2015). Inhibitory and toxic effects of extracellular self-DNA in litter: A mechanism for negative plant-soil feedbacks? *New Phytologist*, *205*(3), 1195–1210. <https://doi.org/10.1111/nph.13121>
- Mccarthy-Neumann, S., & Nez, I. (2013). Plant-soil feedback links negative distance dependence and light gradient partitioning during seedling establishment. *Ecology*, *94*(4), 780–786. <https://doi.org/10.1890/12-1338.1>
- Milbau, A., Vandeplas, N., Kockelbergh, F., & Nijs, I. (2017). Both seed germination and seedling mortality increase with experimental warming and fertilization in a subarctic tundra. *AoB PLANTS*, *9*(5), 1–13. <https://doi.org/10.1093/aobpla/plx040>
- Morin, X., Fahse, L., Jactel, H., Scherer-Lorenzen, M., García-Valdés, R., & Bugmann, H. (2018). Long-term response of forest productivity to climate change is mostly driven by change in tree species composition. *Scientific Reports*, *8*(1), 1–12. <https://doi.org/10.1038/s41598-018-23763-y>
- Nadelhoffer, K. J., Boone, R. D., Bowden, R. D., Canary, J. D., Kaye, J., Micks, P., Ricca, A., Aitkenhead, J., Lajtha, K., & McDowell, W. H. (2004). The DIRT experiment: litter and root influences on forest soil organic matter stocks and function. *Forests in Time: The Environmental Consequences of 1000 Years of Change in New England, January*, 300–3015.
- Nave, L E, Vance, E. D., Swanston, C. W., & Curtis, P. S. (2009). Impacts of elevated N inputs on north temperate forest soil C storage, C/N, and net N-mineralization. *Geoderma*, *153*, 231–240. <https://doi.org/10.1016/j.geoderma.2009.08.012>
- Nave, Lucas E., Le Moine, J. M., Gough, C. M., & Nadelhoffer, K. J. (2019). Multidecadal trajectories of soil chemistry and nutrient availability following cutting vs. burning disturbances in Upper Great Lakes forests. *Canadian Journal of Forest Research*, *49*(7), 731–743. <https://doi.org/10.1139/CJFR-2018-0211>
- Nave, Lucas E., Nadelhoffer, K. J., Le Moine, J. M., van Diepen, L. T. A., Cooch, J. K., & Van Dyke, N. J. (2013). Nitrogen Uptake by Trees and Mycorrhizal Fungi in a Successional Northern Temperate Forest: Insights from Multiple Isotopic Methods. *Ecosystems*, *16*(4), 590–603. <https://doi.org/10.1007/s10021-012-9632-1>
- Norby, R. J. (2011). Ecological and evolutionary lessons from free air carbon enhancement (FACE) experiments. *Annual Review of Ecology, Evolution, and Systematics*, *42*. <https://doi.org/10.1146/annurev-ecolsys-102209-144647>
- Norby, R. J., & Iversen, C. M. (2006). *Nitrogen Uptake, Distribution, Turnover, and Efficiency of Use in a CO₂-Enriched Sweetgum Forest*. *87*(1), 5–14.

- Norby, R. J., Ledford, J., Reilly, C. D., Miller, N. E., & O'Neill, E. G. (2004). Fine-root production dominates response of a deciduous forest to atmospheric CO₂ enrichment. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(26), 9689–9693. <https://doi.org/10.1073/pnas.0403491101>
- Norby, R. J., & Zak, D. R. (2011). Ecological and evolutionary lessons from free air carbon enhancement (FACE) experiments. *Annual Review of Ecology, Evolution, and Systematics*, *42*. <https://doi.org/10.1146/annurev-ecolsys-102209-144647>
- Perez, G., Aubert, M., Decaëns, T., Trap, J., & Chauvat, M. (2013). Home-Field Advantage: A matter of interaction between litter biochemistry and decomposer biota. *Soil Biology and Biochemistry*, *67*, 245–254. <https://doi.org/10.1016/j.soilbio.2013.09.004>
- Pisani, O., Lin, L. H., Lun, O. O. Y., Lajtha, K., Nadelhoffer, K. J., Simpson, A. J., & Simpson, M. J. (2016). Long-term doubling of litter inputs accelerates soil organic matter degradation and reduces soil carbon stocks. *Biogeochemistry*, *127*(1), 1–14. <https://doi.org/10.1007/s10533-015-0171-7>
- Rasse, D. P., Rumpel, C., & Dignac, M. F. (2005). Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation. *Plant and Soil*, *269*(1–2), 341–356. <https://doi.org/10.1007/S11104-004-0907-Y/METRICS>
- Reich, P. B., Hobbie, S. E., Lee, T., Ellsworth, D. S., West, J. B., Tilman, D., Knops, J. M. H., Naeem, S., & Trost, J. (2006). *Nitrogen limitation constrains sustainability of ecosystem response to CO₂*. <https://doi.org/10.1038/nature04486>
- Ricart, R. D., Pearsall, D. R., & Curtis, P. S. (2020). Multidecadal shifts in forest plant diversity and community composition across glacial landforms in northern lower Michigan, USA. *Canadian Journal of Forest Research*, *50*(2), 126–135. https://doi.org/10.1139/CJFR-2019-0138/SUPPL_FILE/CJFR-2019-0138SUPPL.ZIP
- Ring, ; D R, Barnes, B. V, Zogg, G. R., Lapin, M., Ring, R. R., Pearsall, D. R., & Ring ; D, R. R. R. (1995). *Landscape ecosystems of the University of Michigan Biological Station. School of Natural Resources & Environment*. 66, + Appendix.
- Rinnan, R., Michelsen, A., & Jonasson, S. (2008). Effects of litter addition and warming on soil carbon, nutrient pools and microbial communities in a subarctic heath ecosystem. *Applied Soil Ecology*, *39*(3), 271–281. <https://doi.org/10.1016/j.apsoil.2007.12.014>
- Santos, F., Nadelhoffer, K., & Bird, J. A. (2016). Rapid fine root C and N mineralization in a northern temperate forest soil. *Biogeochemistry*, *128*(1–2), 187–200. <https://doi.org/10.1007/s10533-016-0202-z>
- Sayer, E. J. (2006). Using experimental manipulation to assess the roles of leaf litter in the functioning of forest ecosystems. *Cambridge Philosophical Society*, *81*, 1–31. <https://doi.org/10.1017/S1464793105006846>
- Staaf, H., & Berg, B. (1981). *PLANT LITTER INPUT TO SOIL*. <https://about.jstor.org/terms>

- Sulzman, E. W., Brant, J. B., Bowden, R. D., & Lajtha, K. (2005). Contribution of aboveground litter, belowground litter, and rhizosphere respiration to total soil CO₂ efflux in an old growth coniferous forest. *Biogeochemistry*, 73(1), 231–256. <https://doi.org/10.1007/s10533-004-7314-6>
- Tanner, E. V. J., Sheldrake, M. W. A., & Turner, B. L. (2016). Changes in soil carbon and nutrients following 6 years of litter removal and addition in a tropical semi-evergreen rain forest. *Biogeosciences*, 13(22), 6183–6190. <https://doi.org/10.5194/bg-13-6183-2016>
- Van Der Putten, W. H., Bardgett, R. D., Bever, J. D., Martijn Bezemer, T., Casper, B. B., Fukami, T., Kardol, P., Klironomos, J. N., Kulmatiski, A., Schweitzer, J. A., Suding, K. N., Van De Voorde, T. F. J., & Wardle, D. A. (2013). SPECIAL FEATURE-ESSAY REVIEW PLANT-SOIL FEEDBACKS IN A CHANGING WORLD Plant-soil feedbacks: the past, the present and future challenges. *Journal of Ecology*, 101, 265–276. <https://doi.org/10.1111/1365-2745.12054>
- vandenEnden, L., Frey, S. D., Nadelhoffer, K. J., LeMoine, J. M., Lajtha, K., & Simpson, M. J. (2018). Molecular-level changes in soil organic matter composition after 10 years of litter, root and nitrogen manipulation in a temperate forest. *Biogeochemistry*, 141(2), 183–197. <https://doi.org/10.1007/s10533-018-0512-4>
- Veen, G. F., Fry, E. L., ten Hooven, F. C., Kardol, P., Morriën, E., & De Long, J. R. (2019). The Role of Plant Litter in Driving Plant-Soil Feedbacks. *Frontiers in Environmental Science*, 7(October). <https://doi.org/10.3389/fenvs.2019.00168>
- Wang, J. J., Pisani, O., Lin, L. H., Lun, O. O. Y., Bowden, R. D., Lajtha, K., Simpson, A. J., & Simpson, M. J. (2017). Long-term litter manipulation alters soil organic matter turnover in a temperate deciduous forest. *Science of the Total Environment*, 607–608, 865–875. <https://doi.org/10.1016/j.scitotenv.2017.07.063>
- Whitney, G. G. (1987). *An Ecological History of the Great Lakes Forest of Michigan* Author (s): Gordon G . Whitney
Published by : British Ecological Society Stable URL : <https://www.jstor.org/stable/2260198> REFERENCES
Linked references are available on JSTOR for this article : 75(3), 667–684.
- Yuan, Z., Ali, A., Jucker, T., Ruiz-Benito, P., Wang, S., Jiang, L., Wang, X., Lin, F., Ye, J., Hao, Z., & Loreau, M. (2019). Multiple abiotic and biotic pathways shape biomass demographic processes in temperate forests. *Ecology*, 100(5), 1–10. <https://doi.org/10.1002/ecy.2650>
- Zhang, T., Niinemets, Ü., Sheffield, J., & Lichstein, J. W. (2018). Shifts in tree functional composition amplify the response of forest biomass to climate. *Nature*, 556(7699), 99–102. <https://doi.org/10.1038/nature26152>
- Zhang, X., & Wang, W. (2015). The decomposition of fine and coarse roots: their global patterns and controlling factors OPEN. *Nature Publishing Group*. <https://doi.org/10.1038/srep09940>
- Zhu, H., Gong, L., Ding, Z., & Li, Y. (2021). Effects of litter and root manipulations on soil carbon and nitrogen in a

Schrenk's spruce (*Picea schrenkiana*) forest. *PLoS ONE*, 16(2 February), 1–18.

<https://doi.org/10.1371/journal.pone.0247725>