

**Can an invasive species burn soil carbon? Black  
locust invasion and soil carbon in the Albany Pine  
Bush**

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## Abstract

SHARRY, JACQUELINE Can an invasive species burn soil carbon? Black locust invasion and soil carbon in the Albany Pine Bush. Department of Biological Sciences, June 2018.

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Invasive plant species can have dramatic and pervasive impacts on ecosystems, from species interactions, to resource availability, to biodiversity. One such invasive plant, the nitrogen-fixing black locust tree (*Robinia pseudoacacia*), has had significant effects on the soil chemistry in the globally rare inland pine barren ecosystem of the Albany Pine Bush. We have documented a loss of soil carbon following invasion by black locust, one that persists long after locusts are removed. We hypothesize that the nitrogen added through fixation by the black locust increases the carbon flux from soil to the atmosphere. Because decomposition rates are dependent on leaf litter quality and carbon:nitrogen ratio, addition of the nitrogen-rich locust leaves may accelerate the release of CO<sub>2</sub>. To test this, we added exotic locust or native scrub oak leaf litter to barren soil or locust soil in a factorial design and conducted a 16 week lab incubation. The carbon flux of the soil was monitored every 1-2 weeks using a LiCor 6400 portable photosynthesis analyzer. The invasion simulation treatment had a higher soil flux than the native simulation and treatments designed to control for the amount of carbon added and the original amount of carbon in the soil. These results indicate that adding nitrogen to the soil by nitrogen fixation accelerated the rate of decomposition and the release of CO<sub>2</sub> from the soil. Future measurements will determine whether there is less stored soil carbon in the soil. If more CO<sub>2</sub> is released to the atmosphere and stored soil carbon is lost because of species invasion, this may indicate another mechanism of carbon emissions and may provide more incentive for management of nitrogen fixing invasive species.

## INTRODUCTION

Invasive species are common and pervasive in many ecosystems, with the capability to change ecosystems in potentially harmful ways (Mack et al. 2000). They can decrease biodiversity (Hejda et al. 2009), drive out native species, change organismal interactions (Carpenter et al. 2005), impact nutrient dynamics (Ehrenfeld 2003; Laungani 2009; Knopps 2009), and change the amount of stored soil carbon (Suseela et al. 2016). N-fixing invasive species specifically cause changes in N-cycling in local and global ecosystems (Liao et al. 2008; Ehrenfeld 2010; Castro-Díez et al 2014) which has implications for biodiversity levels in these ecosystems and worldwide (Broadbent et al. 2017).

Invasive species may be particularly damaging as they impact soil biogeochemistry and alter the amount of resources in the soil available to plants. Some invasive species produce large amounts of litter with distinctive secondary compounds that can influence soil organic matter and microbial activity (Suseela et al 2016). Carbon, the building block of all organic matter and an element that microbes break down, is stored in soil. It is also dynamic in the soil, and though it can accumulate over decades or longer time scales, it can also be lost in some cases due to plant invasion, the addition of nitrogen, or the input of fresh carbon (Brumme and Beese 1992; Jackson, 2002; Luo et al. 2015).

Invasive species can have novel litter chemistry relative to native species, which can in turn change the rates of decomposition and nutrient cycling. Litter decomposition depends on climate, abundance of microarthropods and other decomposer organisms, and leaf litter quality (Seastedt et al. 1983; Perez-Harguindeguy et al. 2000). Leaf litter quality is defined as by carbon (C) content, nitrogen (N) content, and C-to-N ratio (Perez-Harguindeguy et al. 2000). Microbes need carbon and nitrogen to break down organic matter, but their activity depends on C:N ratio.

Generally, lower litter C:N ratios correlate with higher decomposition rates (Perez-Harguindeguy et al 2000). The accumulation or loss of soil carbon can be attributed to the function of microbial activity in soil and rate of decomposition. Decomposition releases carbon as CO<sub>2</sub> and nutrients in inorganic nitrogen forms such as N as ammonia (Schlesinger and Bernhardt 2013). Faster decomposition rates due to lower C:N ratio may lead to more release of CO<sub>2</sub> and loss of soil carbon (Neff et al. 2002).

Since litter quality (C:N ratio) can influence decomposition rates, changes in ecosystem nitrogen dynamics through nitrogen fixation, fertilization, and invasion can influence microbial activity (Broadbent et al. 2017). Accelerated decomposition because of additional nitrogen through fertilization may cause the loss of stored soil carbon to the atmosphere (Neff et al. 2002). In the Colorado Rocky Mountains, soil carbon stored for many years was released as a result of indirect or direct fertilization (Neff et al. 2002). Nitrogen fixation, for example, can accelerate decomposition rates in a warm-temperate forest floor (Gallardo and Schlesinger 1994). Invasive species that fix nitrogen may provide another mechanism of nitrogen addition in ecosystems.

In the Albany Pine Bush Preserve, the black locust tree (*Robinia pseudoacacia*) is an invasive species that inputs atmospheric nitrogen into the nutrient poor, sandy soils of the Preserve through the process of nitrogen fixation. As an early successional species, it outcompetes most native species (Rice et al. 2004). It establishes well in nutrient poor soils and can also add up to 75 kg N ha<sup>-1</sup> yr<sup>-1</sup> (Boring and Swank 1984). The black locust not only adds nitrogen to the soil but may also impact carbon dynamics. The black locust is the target of intense management and restoration efforts by the Albany Pine Bush Preserve in its efforts to restore native biodiversity and ecosystem function.

Previous research at the Albany Pine Bush has shown that there is significantly less carbon in invaded locust soils than in barren soils that have never been invaded by the black locust (Jeffrey Corbin, *unpublished data*; Figure 1). This difference in soil percent C between barren and locust soil may be due to increased C respiration by microbes decomposing N-rich black locust litter. This study seeks to examine the differences in microbial respiration that occur as a result of differences in C:N ratio of the inputs from leaves of the locust compared to native oak leaves. We hypothesized that areas that experience black locust invasion have lower soil percent C than never-invaded soils because the increase in nitrogen accelerates the rate of microbial respiration through decomposition which leads to a loss of stored carbon from the soil. We tested this hypothesis by exposing Pine Bush soils to several different types of litter in a laboratory incubation, and monitored carbon loss.

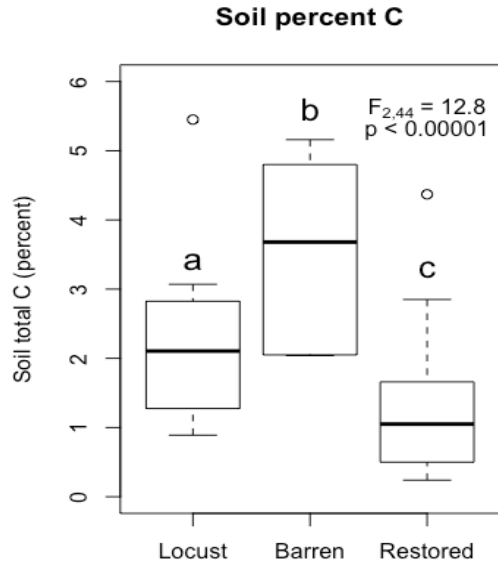


Figure 1. Soil Percent C in locust, barren, and restored sites of the Albany Pine Bush. This is unpublished data from Jeff Corbin collected in 2015.

## MATERIALS AND METHODS

### Study Area

The Albany Pine Bush Preserve is a globally rare Inland Pitch Pine-Scrub Oak ecosystem, located in Albany and Schenectady counties (592,800 Easting; 4,730,100 Northing) (Rice et al. 2004). It receives 0.92 m average annual rainfall and temperatures range from -6 °C to 22 °C. The sandy, nutrient poor soils are a former glacial lake bottom substrate, and its dominant species include pitch pine, scrub oak, and dwarf chestnut oak along with a variety of perennial herbaceous plants and grasses.

The Preserve, intersected by highways and neighborhoods, is broken up into management units with different site histories. Soil was collected from 8 different such units during October of 2017. Four of these sites, KBE, KBW, King's Road Barrens, and Blueberry Hill Central, had never been invaded by the Black Locust tree ("Barren"). KBW Barrens was last burned in 2014, KR Barrens was burned in 2013, KBE Barrens was burned in 2006, and BH Central was last burned in 1996. The other four sites, Siver, BH3, BH5, KR, were mature black locust forests ("Locust") with individual trees that were at least 40 years old. None of the locust stands had been burned. The barren soils generally had higher nitrogen and carbon content than the locust soils (Table 1), consistent with earlier findings (Figure 1).

Table 1. Nitrogen and carbon content of sampled plots. List too which ones are barrens and which are locust. (In the table legend here.)

Sites	Percent Nitrogen	Percent Carbon
BH Central	0.22	4.8
KBE Barrens	0.20	4.9
KBW Barrens	0.23	5.16

KR Barrens	0.11	3.93
BH5	0.12	1.35
KR	0.16	2.66
BH5 (proxy for BH3)	0.16	2.15

### **Soil and Litter Collection**

Soil was collected from each site using a 56.71 cm<sup>3</sup> soil core to a depth of 10 cm. The soils were sieved (2mm) to remove rocks, twigs, insects, roots, and other naturally occurring objects. A subsample of soil was weighed before and after drying (104 degrees Celsius) to measure gravimetric water content. Two types of litter, scrub oak leaves, and locust leaves, were collected from various areas of the Albany Pine Bush in December 2016, dried (60 degrees C), and ground into a homogenous mixture. Litter C and N content was determined by mass spectrometer analysis at Union College's Stable Isotope Chemistry Laboratory.

### **Incubation Set Up**

To test the difference in carbon flux due to locust invasion, four treatments were set up. Barren-locust, the experimental treatment, simulated invasion of the black locust tree into native barren soils. Barren-oak simulated natural native barren soils, and so controlled for soil type. Locust-locust controlled for litter type. To control for the greater amount of carbon in locust litter compared to the native oak litter, the ratio of litter to soil was increased to match the ratio of carbon in locust litter. Each site-litter treatment combination had four replicates made from each of the four Barren and Locust sites from the soil was collected. Each replicate was also repeated twice, for a total of eight jars per treatment and 32 jars in total.

Each jar received 110 grams (dry-weight) of soil. The amount of litter added to each treatment was one-third the mass of annual litter biomass ( $\text{g/m}^2$ ) in pine-oak stands and black locust stands as determined by Rice et al. (2004). It is important to note that roughly 2 times more litter mass falls onto locust stands per year than oak stands (Rice et al. 2004). We converted g litter biomass per  $\text{m}^2$  to g litter biomass per g soil using soil bulk density values (Table 2, Table 3). Per 110 grams of barren soil, 1.375 grams of locust litter were added; per 110 grams of locust soil, 1.188 grams of locust litter were added; Per 110 grams of barren soil, 0.616 grams of oak litter were added. We controlled for the difference in carbon added to treatments that received locust litter (Table 3) using our barren-match treatment. For the match control treatment, 1.3552 grams of oak litter were added, 2.2 times the ratio for the barren-oak treatment. A factor of 2.2 was based on the initial carbon and nitrogen measurements from previous mass spectrometer analysis (Table 3).

The jars were incubated in mason jars ranging in volume from 237-241 mL. The jars were kept room temperature, away from natural light. The lids were vented to allow air circulation and maintain natural  $\text{CO}_2$  and  $\text{O}_2$  concentrations. Water was added twice weekly, maintaining the soil's original weight, to prevent the soil from drying out.

Table 2. Bulk density measurements used to calculate mass of annual litter fall.

Diameter of cylinder	3.8 cm
Depth of measurement	5 cm
Volume cylinder	18.05 cm
Locust dry weight of soil	51.88
Barren dry weight of soil	51.77
Locust bulk density	0.915



Barren bulk density	0.913
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Table 3. Average amounts of litter, carbon, and nitrogen added to each incubation jar for each treatment.

Treatment	Grams of litter/ 110 grams of soil	Grams of carbon per 110 grams of soil	Grams of nitrogen per 110 grams of soil
Barren-locust	1.375	0.670	0.033
Barren-oak	0.616	0.310	0.005
Locust-locust	1.188	0.599	0.029
Barren match	1.3552	0.681	0.011

### Carbon flux measurements

. The carbon dioxide released from the soil was measured using a converted Li-Cor 6400 portable photosynthesis system (Figure 2). The Li-Cor 6400 has an infrared gas analyzer that detects real-time carbon dioxide concentrations. To convert the system from a photosynthesis system to a soil CO<sub>2</sub> flux system, the leaf chamber was detached and reconfigured so that each mason jar could screw on directly below the sensor head. The software was set on a soil chamber configuration, and CO<sub>2</sub> concentration (ppm), pressure, and temperature were recorded every 5 seconds. The soil CO<sub>2</sub> flux was measured once per week for the first 8 weeks of the experiment and once every two weeks for the remaining 8 weeks. Measurements were taken over 3 minutes for the first 8 weeks of the experiment. After 8 weeks, the measurement time was increased to 4 minutes to account for the decreased rate of respiration.

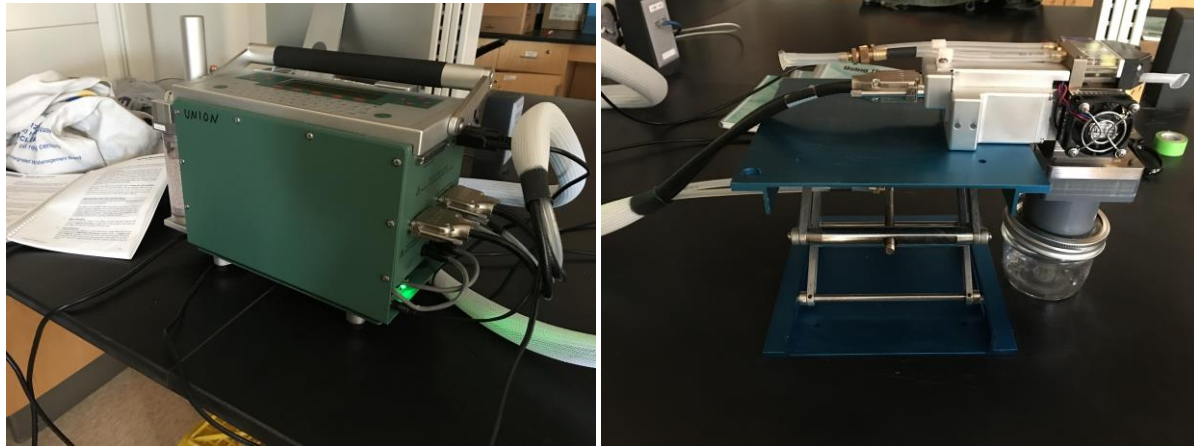


Figure 2. The LiCor-6400 adapted for a mason jar to screw on directly below the sensor head.

## Analysis

The slope of the CO<sub>2</sub> collecting in each jar (ppm/second) was converted to mass loss of carbon ( $\mu\text{g C g soil}^{-1}$ ) using the ideal gas law and soil mass. The molecular weight of carbon was 12.01 ug/umol and the atmospheric pressure and temperature were recorded by the LiCor. Nearly all  $R^2$  were  $>0.98$ , but where the  $R^2$  value was low ( $<0.90$ ), the timing used to calculate the slope was adjusted until  $R^2$  was above 0.90.

We analyzed C lost from each jar in two ways. First, a repeated measures analysis of variance test was performed comparing the effect treatments, measurement date and the interaction between treatment and date on carbon flux. Second, the total carbon flux during the 5 month experiment was summed, scaled by time between measurements, for each jar. T-tests with randomized block design, in which "sites" were blocks and replicates of each site were nested factors were used for planned contrasts of total C lost from the barren-locust treatment versus barren-oak treatment and from barren-locust versus barren-match. A one-way t-test in which replicates of each site were nested factors was used for barren-locust versus locust-locust. All analyses were performed using R software (v. 3.4.3).

## RESULTS

The carbon flux in each treatment generally decreased over time (Figure 3). The carbon flux was significantly different between all treatments ( $p\text{-value} = 0.0026$ ) and all dates measured ( $p\text{-value} = 0.0001$ ) (Table 4). The interaction between treatment and date was not significant ( $p\text{-value} = 0.19$ ) (Table 4, Figure 3).

The experiment-wide averages of each treatment ranged from 1.624 to 2.705. Total carbon flux in the barren-locust treatment was significantly higher than in the barren-oak treatment ( $p\text{-value} < 0.0001$ ) (Table 5). The carbon flux was significantly higher in the barren-locust invasion simulation than the locust-locust control treatment ( $p\text{-value} = 0.007$ ) (Table 6, Figure 3, Figure 4). The barren-locust treatment also had a significantly higher total carbon flux than the barren match control treatment ( $p\text{-value} = 0.0022$ ) (Table 5, Figure 4).

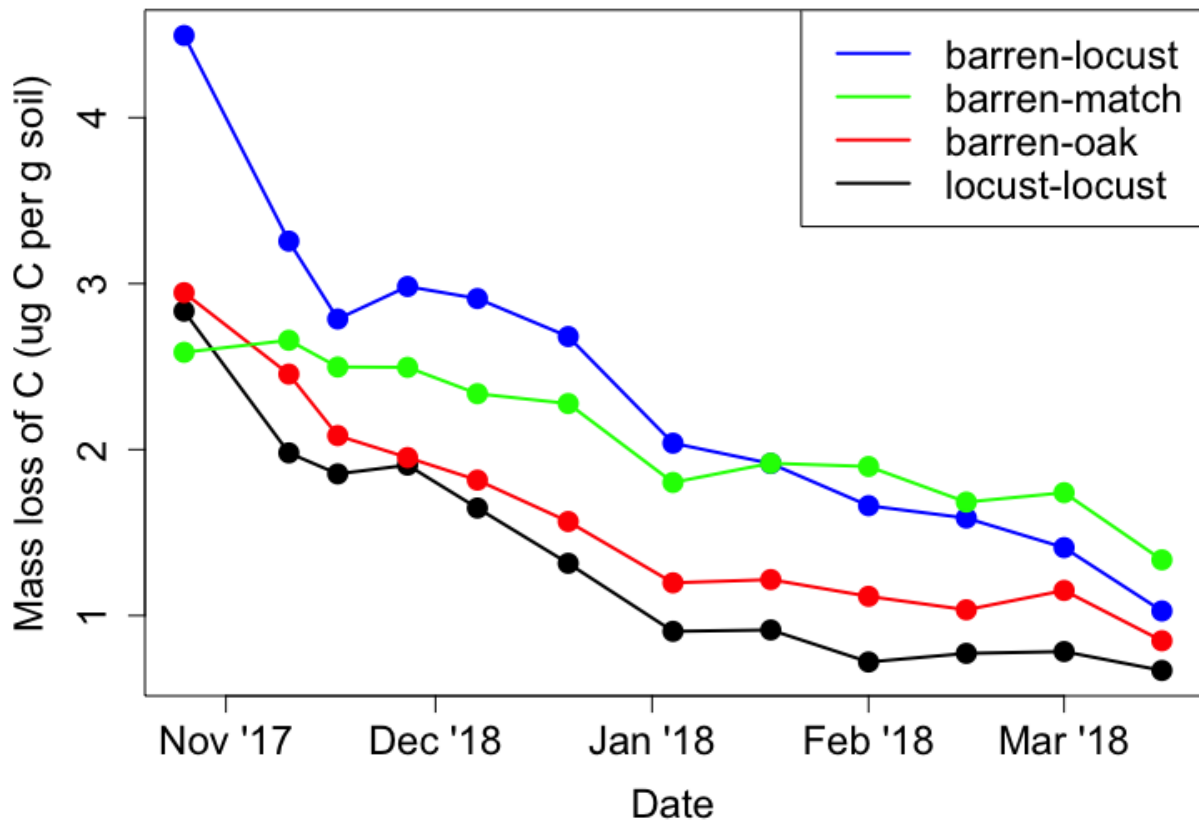


Figure 3. Carbon flux values for each treatment from October 26, 2017 to March 15, 2018.

Table 4. Repeated measures ANOVA analysis of treatment, date, and the interaction of treatment and date. The analysis took account of repeated samples and that these samples are not independent of each other.

Source	d.f.	F	p-value
Treatment	3	9.716	0.0026
(Residuals)	12		
Date	9	26.4	0.0001
Treatment*Date	27	1.3	0.19
(Residuals)	108		

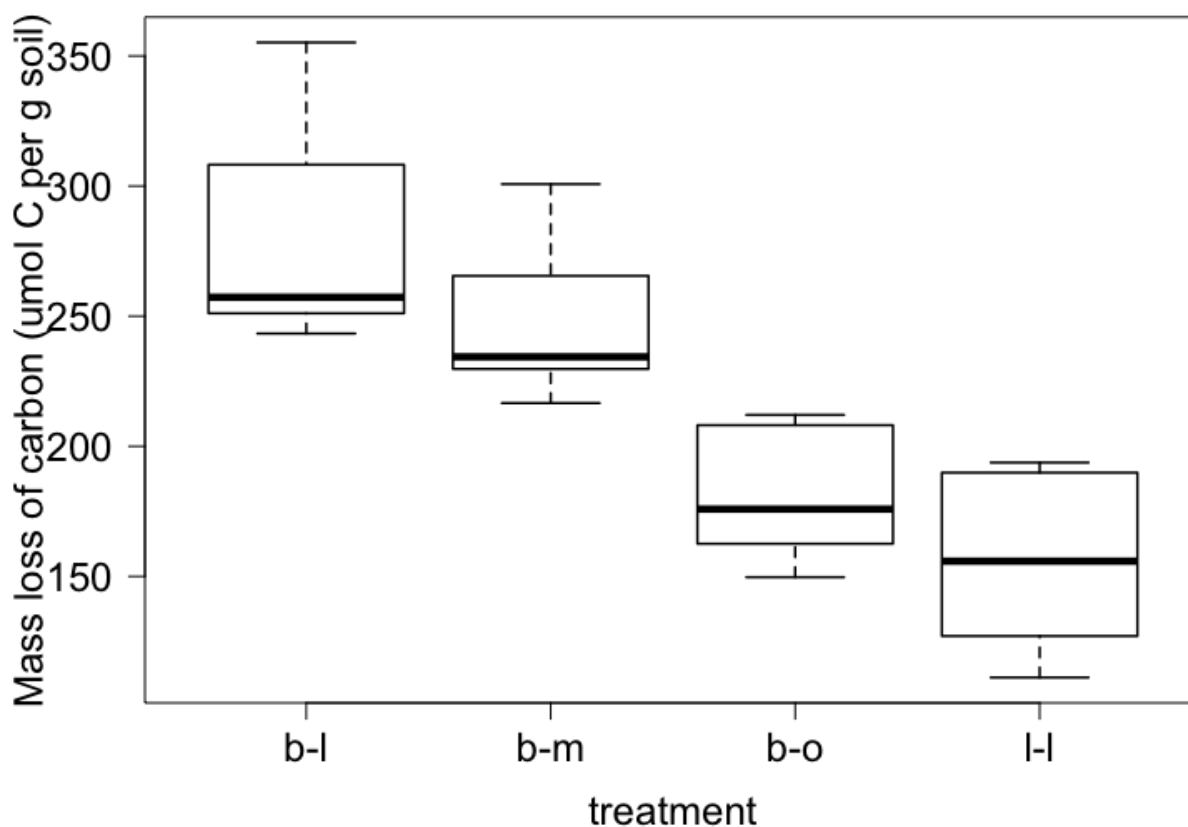


Figure 4. Total carbon loss of all treatments summed over five months of measurements. B-l indicates barren-locust, b-m indicates barren-match, b-o indicates barren-oak, and l-l indicates locust-locust.

Table 5. Planned contrast t-test using a randomized block design in which “sites” are blocks and replicates of each site are nested factors.

Barren-Locust versus Barren-Match			
	d.F.	F	p-value
Litter	1	22.12	0.0022
Residuals	7		
Barren- Locust versus Barren-Oak			
Litter	1	16.11	0.007

Residuals	6		
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Table 6. One-way t-test comparing Barren-Locust versus Locust-Locust in which replicates of each site are nested factors.

	d.F.	F	p-value
Soil	1	16.11	0.007
Residuals	6		

## DISCUSSION

Previous studies suggested that N addition and litter with lower C:N ratios increase decomposition and soil C respiration rate (Neff et al. 2002; Brumme and Beese 1992) so we hypothesized that locust invasion, simulated by the barren-locust treatment, would have a higher carbon flux than an uninvaded stand, simulated by the barren-oak treatment. Our results supported this hypothesis as the barren-locust treatment had a higher carbon flux than the barren-oak treatment (Table 6, Figure 3). The expected mechanism was that, in the barren-locust treatment, more nitrogen rich litter was added to the soil than in the barren-oak treatment. This added nitrogen likely increased soil respiration and therefore, resulted in higher total carbon flux.

If this increase in soil respiration was responsible for the loss of stored soil carbon observed at the Albany Pine Bush (Figure 1), then the larger C flux in the barren-locust treatment must be driven by loss of soil C, rather than loss of locust litter C. Our two controls served to test this mechanism. If the observed increase in C flux in the barren-locust treatment were due simply to the respiration of locust litter, not the respiration of stored soil C, then the C flux in the locust-locust treatment should be equal to the barren-locust treatment. This was not the case: the barren-

locust treatment had a higher carbon flux rate than the locust-locust treatment (Table 6, Figure 3). This suggests that the barren-locust treatment did not have higher flux rates just because of the decomposition of the locust leaves but because of the higher N content of the locust leaves. We do acknowledge that different amounts of locust litter were added to these two treatments because of the differences in soil bulk density between the locust soil and barren soil, but these differences were not large enough to account for the dramatic difference in carbon flux.

Another possible alternative mechanism to explain the loss of soil C from the barren-locust treatment is that the higher loss was a function of the quantity of litter, rather than its chemistry. We also controlled for the amount of carbon added in litter with the barren match treatment. This treatment added the same amount of carbon in litter as the barren-locust treatment so that only C:N ratio varied between the two treatments. Because the total carbon flux was higher with locust litter than when the same amount of carbon was added as oak litter, we can conclude that it was the C:N ratio that drove the flux value. Therefore our results suggest that the higher N content and lower C:N ratio of the invasive black locust litter increased soil respiration and burned stored soil carbon. Future mass-spectrometer analysis will confirm whether the barren-locust soils have a lower carbon content than at the beginning of the experiment.

Soil carbon is one of the largest pools of carbon in our global system so understanding soil carbon storage and turnover is necessary to study the global carbon cycle (Jackson et al. 2002). Carbon cycling is complex as evidenced by results of previous studies which find that nitrogen addition may increase (Lovett et al. 2013, Rodriguez et al. 2014, Wang et al. 2014, Craig 2015), decrease (Neff et al. 2002, Jackson 2002, Brumme and Beese 1992), or have no effect on soil C storage. Other studies are consistent with the pattern we observed as evidence shows that invasive plant species do have the capacity to destabilize native soil C pools which is

of concern for the global carbon cycle (Tamura and Tharayil 2014). However complex carbon cycling and soil C storage is, as atmospheric carbon increases to 409 ppm, it is increasingly important to understand mechanisms of storage and release of soil carbon (ProOxygen, 2018). Any reasons for fluctuation in soil carbon may contribute to climate change.

The Albany Pine Bush is a barren environment with nutrient poor soils (Rice et al. 2004). The average flux of the different soil treatments in our study ranged from 1.623 to 2.704 which is low compared to ecosystems such as temperate forests. Temperate forests have slightly higher ecosystem respiration rates ranging from approximately 2.77 to 3.53 while arid ecosystems such as the tundra or desert had flux values typically less than 1 (Oertel et al. 2016). The Albany Pine Bush soils also have low carbon content soils. But this mechanism of carbon emissions by nitrogen fixing invasive systems observed in our study could be especially consequential in carbon rich soils that either grow nitrogen fixing species or are subject to invasion by nitrogen fixers. Ecosystems such as the agricultural fields of the Midwestern United States and boreal biomes have carbon rich soils and large amounts of stored carbon. The arctic permafrost regions of the Northern Hemisphere store 1672 Pg, 50% of the Earth's carbon that is stored below ground (Tarnocai et al. 2009).

As the climate warms, shrubs and trees, many of which are nitrogen fixers, are predicted to expand their ranges northward and establish in subarctic tundras (Rousk and Michelsen, 2016). Nitrogen fixing species will provide a source of N input greater than current litterfall inputs (Rousk and Michelsen, 2016). Hiltbrunner et al (2014) hypothesized that expansion of alder, a nitrogen fixing genus of plant, will reduce C storage because of the inhibition of the establishment of late-successional stages of plants. If nitrogen fixers in such habitats alter soil



carbon dynamics in the same way as the black locust in the Albany Pine Bush, this could be impactful for global carbon emissions.

In the carbon rich soils of the Midwest, soy is an abundantly produced nitrogen-fixing plant. Studies in Brazil and other tropical places, where soybean production has expanded, have found that conversion of forest to agricultural land has contributed to carbon emissions (Nagy et al. 2017, Noojipady et al. 2017). Data from the Brazilian government estimates that the carbon dioxide emissions from conversion to soybean agricultural land of the Cerrado ecosystem in Brazil is more than half of the total emissions from the UK in 2009 (Word Wildlife Federation 2011). Part of these carbon emissions can be attributed to deforestation but the nitrogen fixing nature of soybeans may play a role as well. Soybeans are the second most planted field-crop in the U.S. and are mainly grown in carbon rich Midwestern U.S. soils (USDA 2017) so if these nitrogen-fixing legumes increase the rate of soil respiration, released stored soil carbon dioxide into the atmosphere this could be harmful not only because of greenhouse gas emissions but also because of the decrease in soil nutrients in these important agricultural areas.

In sum, our study offers a novel mechanism for carbon emissions that contribute to climate change. It also provides more incentive for the strategic and successful management of invasive species as a large portion of invasive species are nitrogen fixers (Daehler 1997). These types of invasive plant species may not just impact the ecosystems they invade by decreasing biodiversity, changing soil chemistry, and leaving soil legacies that even restoration cannot remedy (Mack et al. 2000), they also may have an effect on the global ecosystem and climate change. Finally, it provides more incentive for agricultural management of nitrogen-fixing legumes such as soybeans to ensure that sustainable practices are being employed, including consideration of carbon budgets.

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