THE EFFECT OF NITROGEN DEPOSITION AND NITROGEN FIXATION BY *ROBINIA PSEUDOACACIA* ON EASTERN U.S. FORESTS

CARISSA MOORE
Rider University, 2083 Lawrenceville Rd, Lawrenceville, NJ 08648

MENTOR SCIENTISTS: DRS. MICHELLE WONG AND SARAH BATTERMAN
Cary Institute of Ecosystem Studies, Millbrook, NY 12545

Abstract. Biological nitrogen fixation provides the main source of new nitrogen into historically nitrogen-limited temperate forest ecosystems, increasing soil nitrogen levels and stimulating forest biomass productivity and carbon storage. Symbiotic nitrogen-fixing trees in particular can supply large amounts of new nitrogen relative to asymbiotic and non-biological sources due to their access to light energy. Across the United States, *Robinia pseudoacacia* is the dominant symbiotic nitrogen-fixing tree species and is mostly found in eastern forests. Over the past few decades, nitrogen deposition from anthropogenic sources has brought in large amounts of new nitrogen to eastern forests, potentially shifting the soil nutrient status away from nitrogen limitation. However, it is unclear how this increased nitrogen deposition has affected the role of *Robinia pseudoacacia* within eastern forest ecosystems, including its effect on soil nitrogen, forest biomass, and NPP. We hypothesize that increased nitrogen deposition would increase soil nitrogen availability, forest productivity and forest biomass carbon storage. We also hypothesize that nitrogen fixation and *Robinia* abundance will also increase these factor. Finally, we hypothesize that the positive effect of *Robinia* abundance will decline in interaction with high nitrogen deposition and abundance such that nitrogen deposition has the major effect on soil nitrogen, productivity and biomass with *Robinia* abundance only having a positive effect at lower nitrogen deposition levels. To test these hypotheses, we modified the Spe-CN model to include *Robinia pseudoacacia* and examine different scenarios of *Robinia* abundances and nitrogen deposition rates (historic, peak, and current). The model indicates that both nitrogen deposition and *Robinia* abundances increase ecosystem productivity, NPP, and soil nitrogen, supporting the hypotheses. In order to test this further, future focus will be on a more complex test using the Spe-CN model as well as comparisons of our results to current literature.

INTRODUCTION

The nitrogen cycle is a key piece of how life survives on this planet, affecting humans, plants, and many other organisms. Nitrogen is circulated throughout different parts of the Earth by both natural and anthropogenic processes, and can be limiting in some regions of the world, despite it being necessary for ecosystems to survive. To combat nitrogen limitation, some plants can form a relationship with bacteria to fix nitrogen, bringing in new nitrogen to the ecosystem. Specifically within eastern U.S. forests, *Robinia pseudoacacia* trees are the main symbiotic nitrogen fixers (Staccone et al. 2020). However, it is unclear how the role of *Robinia* in eastern forest ecosystems is changing due to anthropogenic nitrogen deposition.

Despite historic nitrogen limitation, humans have dramatically changed eastern U.S. forest nitrogen cycles by contributing to the input of large amounts of new nitrogen via nitrogen deposition. Nitrogen deposition has an effect on temperate ecosystems because they are often nitrogen limited (Vitousek and Howarth 1991). In the second half of the 20th century, anthropogenic nitrogen deposition from agriculture development and fossil fuel combustion increased in the eastern U.S. The large increase in new nitrogen caused forest ecosystems to switch from being historically nitrogen-limited, to being limited by another resource such as phosphorous or other compounds often necessary for plant growth (Verhoeven et al. 1996). Over the past several decades, the implementation of legislation such as the Clean Air Act has subsequently caused nitrogen deposition to decline (Du 2016), yet many ecosystems have a legacy of decades of peak deposition with substantial nitrogen build up in the soil.

Given this dramatic change in the nitrogen cycle, we seek to examine the effects on the function of *Robinia pseudoacacia* in eastern U.S. forests. With an increase in nitrogen deposition and soil nitrogen, nitrogen fixation will no longer be beneficial to plants because nitrogen fixation is costly (Phillips 1980), making the ability to fix nitrogen obsolete. Nitrogen deposition within temperate forests has been found to decrease asymbiotic nitrogen fixation (Zheng et al. 2019), and high soil nitrogen availability has been found to influence nitrogen fixation rates in tropical forests (e.g., Barron et al. 2011 Oecologia; Batterman et al. 2013 Journal of Ecology). Increased nitrogen deposition could negatively affect the population of *Robinia pseudoacacia* as they will no longer have the growth advantage of fixing nitrogen and would become out-competed by other tree species in the forest. Likely, the entire forest composition would change with less of a need for *Robinia pseudoacacia* and any other nitrogen fixers within the system. Legacy effects of nitrogen deposition could also maintain nitrogen richness. The composition change could be particularly harmful if the amount of nitrogen deposition over the next century drops due to efforts to curb the effects of climate change (Paris Agreement 2015), leaving only a percent of previous nitrogen fixers to provide for ecosystems.

Alternatively, the nitrogen fixation function of *Robinia pseudoacacia* in eastern forests could be positively affected by nitrogen deposition since *Robinia* is a nitrogen-demanding tree, possibly resulting in an increased abundance of *Robinia* and rates of fixation (Figure 2). Analysis of forest inventory data indicates that the growth of *Robinia pseudoacacia* is stimulated by nitrogen deposition more than any other tree species in eastern US forests (Horn et al. 2018).

In my project, I will address the following questions: With increased nitrogen deposition, has the role of *Robinia pseudoacacia* changed within the forest ecosystem? Specifically, how do shifting abundances of *Robinia pseudoacacia* due to nitrogen deposition and changing nitrogen deposition rates (historic, peak and current) affect total forest biomass and total soil nitrogen?

With these questions, there are three hypothesis that can be tested using the Spe-CN model. First, that higher nitrogen deposition could cause higher soil nitrogen, forest productivity and forest biomass. Nitrogen is an essential and historically limiting element within these forest systems, so the possibility that more of it could increase productivity is logical. Second, that nitrogen fixation and *Robinia* abundance will positively affect soil N, forest biomass, and NPP. Robina has the ability to input usable nitrogen into a forest system, with higher abundance there's more nitrogen being put into the system that can promote growth. Lastly, the interactive effect of nitrogen deposition and abundance will show a decrease in the effect of abundance and nitrogen fixation. Likely the abundance and nitrogen fixation will have more effect at lower nitrogen depositions. In order to test these hypotheses, I will use a combination of searching the literature for parameters, experiments, and a theoretical model that I will develop for *Robinia pseudoacacia*. By using data that I gather from the literature to parameterize the model, we will have a clear base of knowledge to work from for future predictions. I will use the model to test how nitrogen deposition interactions with *Robinia pseudoacacia* abundances affects soil properties, forest growth, and NPP. This information can be used for future projects as a place of comparison or a basis of new research.

METHODS AND MATERIALS

Study Area

For this study, we simulated eastern temperate United States forests using the Spe-CN model. While *Robinia pseudoacacia* does not comprise a majority of trees in these forests, it is a well-established nitrogen fixer that is currently thought to contribute the majority of symbiotically fixed nitrogen across the continental US (Staccone et al. 2020).

To test our hypotheses, the Spe-CN model was used in order to simulate *Robinia pseudoacacia* interaction and strategies in eastern United States forests (Crowley et al. 2016). The Spe-CN model uses individual trees and their plant traits to model the movement of carbon and nitrogen pools of the system. It was developed specifically for that portion of the United States and their specific tree species. The model was therefore appropriate for incorporating a *Robinia* species and evaluating our questions, and can give the current and past snapshots of *Robinia* within these forests.

Parameterizing the model

To parametrize Robina in the Spe-CN model, we aimed to find values from studies collected in eastern US forests, although if parameters were not available, some values were collected from studies conducted in temperate forests outside of the United States where *Robinia* is considered invasive (Rahmonov 2009). This should not have a profound effect on the data gathered from these experiments. Some of the parameters included the range of nitrogen concentrations in foliage and wood, turnover rates of foliage, forest biomass, and soils. For values found with no min and max, the given standard error was used to calculate three standard errors away to find the theoretical minimum and maximum. Also, for parameters that we were unable to find for specifically *Robinia*, as a best estimate, we used values from a tree with the most similar properties and processes.

To project possible outcomes of different nitrogen deposition scenarios for historic, peak, and current, we used estimates collected from the literature (2, 6.7, and 20 kg N ha-1 yr-1) (Crowley et al 2016 and S. Batterman, personal communication, July 2020). We also examined the effects of differences in abundances of Robina, and used abundances that ranged from very low (1%) to very high (100%) of trees in a forest stand, and two intermediate levels of abundance (25 and 50%). We conducted model runs to test how soil properties, growth patterns, and carbon storage could change with different nitrogen deposition amounts and Robinia abundances. Since the model needs 100% abundance in order to run properly, Robinia was paired with Red maple for our runs. Gary Lovett, our point person for the model, explained that Red maple was found near Robinia in the wild quite often and would give semi-realistic values. There were twelve total scenarios run based in 3 different nitrogen deposition levels (background of 2, current of 6.7 and peak of 20 kg N ha-1 yr-1) and four abundances of Robinia pseudoacacia (representing stands with 1%, 25%, 50% and 100% Robinia), with Red Maple (*Acer rubrum*) as the co-occurring species making up the rest of the forest stand. The rates 2 and 6.7 kg N ha-1 yr-1 were already being used in the model as numbers found through research in the Catskills (Crowley et al. 2016). The original high deposition number was 11.1 kg N ha-1 yr-1, but that was for the Catskills which is known to be a pristine ecosystem. The rate of 20 kg N ha-1 yr-1 is a rate more likely to be found in a less pristine system, suggested by Sarah Batterman who mainly works with nitrogen and has a clear understanding of deposition trends within the US. I plotted the data into graphs using R for visualization of the results.

We also added N fixation into Spe-CN because of *Robinia's* high foliar N which led to a high N demand that was not met by the rates of N deposition that we specified. To do this, we examined Boring and Swank 1984, who found that at forest ages of 4, 17, and 38 had fixation rates of 48, 75, and 33 kg/ha, respectively.

N fixation was added by adding in nitrogen directly to the plant nitrogen pool that could be used for growth. When there was extra nitrogen, it was added into the soil nitrogen pool. The nitrogen fixation is also scaled based on the abundance of *Robinia pseudoacacia*, putting 30 kg N ha^{-1} yr^{-1} into the system at 100% abundance and putting a fraction in based on lower abundance (i.e. 50% * 30 kg N ha^{-1} yr^{-1} = 15 kg N ha^{-1} yr^{-1}).

RESULTS

Overall, *Robinia* abundance had a stronger effect on total soil N, annual NPP, and biomass C compared to nitrogen deposition alone. We found the highest forest productivity and highest soil N with the highest *Robinia* and nitrogen deposition scenario, with no negative effects of N deposition on *Robinia's* function in northeastern forests. The effect of *Robinia* was additive based on the level of nitrogen deposition.

An increase of nitrogen deposition from 2 to 20 kg N ha^{-1} yr^{-1} led to an increase of total soil N 1.5 times with a *Robinia* abundance of 1%. For the highest *Robinia* abundance of 100%, the increase from low to high nitrogen deposition increased soil N by 1.1 times. The change from 1% to 100% abundance in the low deposition led to an increase of soil N by 2.4 times while the same change under the same abundance under high deposition led to an increase of soil N by 1.7 times.

However, the effects of N deposition were less strong for NPP. An increase of nitrogen deposition from 2 to 20 kg N ha^{-1} yr^{-1} only led to an increase of NPP 1.3 times. We found a strong increase in NPP as *Robinia* abundance increased.

Nitrogen deposition had the least effect on biomass carbon. The change from 1% low to high deposition was about 1.04. That means there was only a very slight increase from low to high nitrogen deposition.

DISCUSSION

In this study, we tested the interactions of nitrogen deposition and Robinia abundances on total soil N, forest NPP, and forest biomass. Our goal was to ask how increased nitrogen deposition and Robinia abundance would affect forest productivity, measured in total soil N, forest NPP, and biomass C. We had several hypotheses: first, that higher nitrogen deposition would increase the forest productivity factors. Second, we hypothesized that nitrogen fixation and Robinia abundance will affect the outcomes positively as they increase. Lastly, that the interactive effects between the abundance and nitrogen deposition would show a decrease in the influence of abundance and nitrogen fixation when both are increasing.

We found that Robinia had a much stronger effect on total soil N, forest NPP, and forest biomass compared to nitrogen deposition alone in contrast to our hypothesis. This implies that Robinia has an important role in forest recovery and productivity, likely due to the high nitrogen fixation rates that we added into the model which were an order of magnitude higher than the nitrogen deposition rates we simulated. One caveat is that we lack empirical data on the regulation of nitrogen fixation in response to nitrogen richness. However, we note that because we parameterized nitrogen fixation rates based on field measurements, they are likely representative of field conditions. We found that two of our three hypothesis were supported by our results. Nitrogen fixation and abundance had a very positive effect on the outcomes we were looking into. Nitrogen deposition had a positive effect on the outcomes, but it was much less than Robinia's effect on those same outcomes. The unsupported hypothesis is the last hypothesis. We found that together abundance and nitrogen fixation had a higher effect on total soil N, NPP, and forest biomass.

Although we were unable to directly test if nitrogen deposition would impact the abundance of *Robinia*, data from Horn et al. (2018) suggest that *Robinia* is still characterized by high growth rates and recruitment even with higher rates of nitrogen deposition. These results hint that *Robinia* may not be negatively affected by the influx of nitrogen from non-natural sources. This could mean that *Robinia* would continue to be a useful part of the ecosystem and would not die out, but that the role of *Robinia* may change, as some nitrogen-fixing trees have been found to play other roles in nutrient cycling (Perakis and Pett-Ridge et al. 2019). Although *Robinia* is considered invasive in some ecosystems, it may play an important role in future forests. Another very important takeaway is that *Robinia* has a major capability to sequester carbon, as suggested by the increase in forest NPP and biomass. From an anthropogenic standpoint, this is important in terms of trying to slow down climate change. In figure 4, the change in nitrogen deposition has little effect on the amount of carbon that can possibly be stored by *Robinia*. This implies that *Robinia* is important in the big picture of trying to solve climate change, especially since the usage of trees is on the forefront of that conversation.

Future research should focus on simulating the function of *Robinia* using a more complex forest system. That would mean looking into literature and finding which trees *Robinia* would most likely grow near in the wild. With this, I would test a few natural abundances of these trees to see how *Robinia* would interact with trees it would normally grow near. Another possible future study to compliment this one would look into the possibility of age being a factor in carbon sequestration and overall contribution to forest productivity. Some species do more work in certain parts of their life cycles, thus it would be important to know when *Robinia* can be of the most use to an ecosystem.

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APPENDIX

TABLE 1. Classification of studies found and used as parameters within the Spe-CN model for *Robinia pseudoacacia.*

Parameter	Study/Studies	Values	Parameter value used
Leaf N (g/kg)	NERC	3.25%	2.83%
	Hirschfeld et al. 1984	2.06%	
	Buzhdyygan et al. 2015	3.22%	
	Mantovani et al. 2015	2.80%	
		3.00%	
	Rahmonov et al. 2009	2.65%	
Litter N (g/kg)	Buzhdyygan et al. 2015	1.15%	2.82%
	Rahmonov et al. 2009	2.13%	
	Rice et al. 2004	2.60%	
		1.30%	
		5.20%	
		4.50%	
		2.20%	
	White et al. 1987	2.20%	
		1.25%	
	Tateno et al. 2007	5.65%	
Resorption	Deng et al. 2019	0.399	0.399
Lignin (g/kg)	Tateno et al. 2007	27%	20.10%
	White et al. 1987	18.1%	
		15.20%	
Fine root	Boring and Swank 1984	1.4%	1.4%
Fine wood	Boring and Swank 1984	1.13%	1.13%

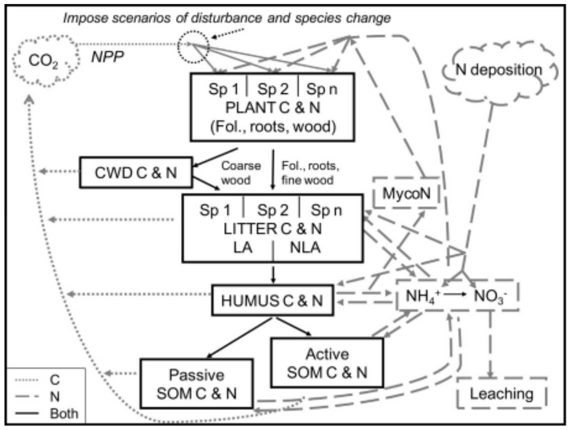

FIGURE 1. A picture of the carbon and nitrogen interactions as examined by the Spe-CN model (Crowley et al. 2016).

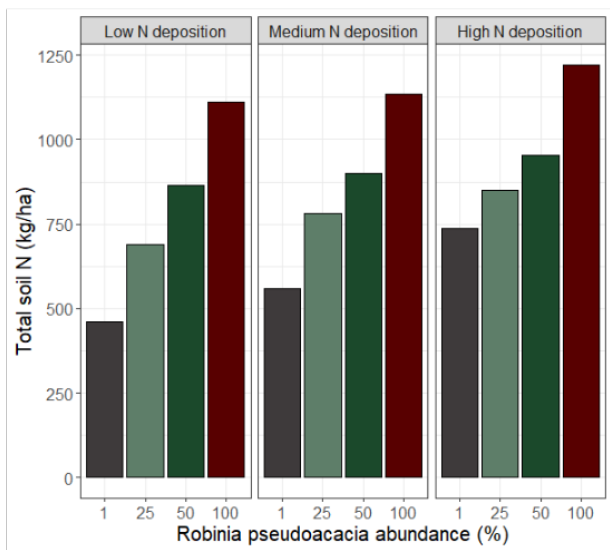

FIGURE 2. Effects of varying nitrogen deposition and Robinia abundances on total soil nitrogen.

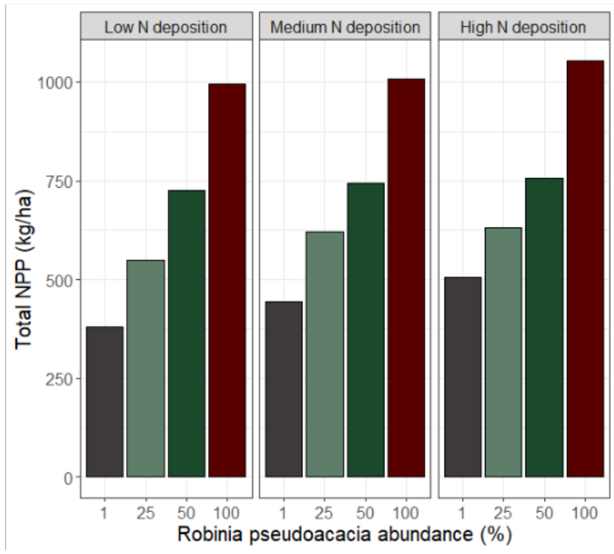

FIGURE 3. Effects of varying nitrogen deposition and Robinia abundances on annual forest NPP.

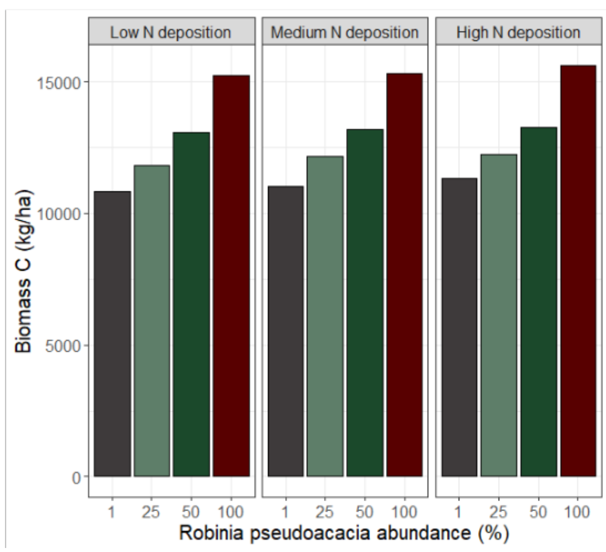

FIGURE 4. Effects of varying nitrogen deposition and Robinia abundances on forest biomass C.