Changes in Nitrogen Cycling during Tropical Forest Secondary Succession on Abandoned Pastures

Senior Honors Thesis

Sanober R. Mirza

Department of Geography

University of Wisconsin-Madison

Advised by:

Erika Marín-Spiotta

May 3rd, 2018

Abstract

Nitrogen (N) plays two important roles in Earth's climate. As a plant nutrient, the availability of N affects plant growth and the uptake of carbon (C) from the atmosphere into plant biomass. The accumulation of C in long-lived biomass and in soils contributes to reducing the amount of CO_2, a greenhouse gas, in the atmosphere. Humans have altered the N cycle, affecting the potential for terrestrial ecosystems to sequester C and mitigate climate change. Land-use change, specifically deforestation and reforestation, can affect N availability for plant growth and N_2O production. Highly-weathered tropical soils are not expected to be N-poor, but long-term or intensive agricultural use can deplete soil nutrients, with implications for forest recovery. Despite the importance of N, there are still large gaps in our understanding of N dynamics during succession. Nitrogen limitation in pasture soils and early successional forests increases the demand for N-fixing tree species, but studies show a greater abundance of N-fixing species and in turn, fixation potential, in older wet tropical forests (Batterman et al. 2013). Nitrogen mineralization rates can be high in agricultural soils, yet lower in younger forests compared to mature ones (Templer et al. 2004). Here we used a chronosequence approach to study how N availability changes with reforestation on former pastures and identify potential factors explaining observed patterns. Our results showed that net N mineralization and nitrification rates peaked in later successional forests, indicating changes in soil N availability during reforestation of pastures. The reference forest rates were lower than those in the late successional forests, suggesting potential effects of differences in species composition with post-agricultural succession. Nitrogen mineralization and nitrification rates were related to the concentration of N in the free light fraction and to the presence of N-fixing trees. The successional trends in N cycling rates varied by sampling period, suggesting a complex relationship in the recovery of N availability during forest succession.

Introduction

Rapid deforestation is significantly altering biogeochemical cycles, specifically the carbon (C) and nitrogen (N) cycles. Alteration to N cycling can have direct effects on the global climate, as N can be lost from soils as nitrous oxide (N_2O), which is a potent greenhouse gas. Nitrogen also is important because not only is it the most abundant element in the atmosphere, but through its interactions with plant photosynthesis and microbial decomposition it can influence global C cycling (Cusack et al. 2010). All biological organisms need N to complete metabolic and growth processes as it is a component of fundamental biochemical building blocks of life, such as amino acids. This essential element has a strong influence on many aspects of the environment, including soil fertility.

Humans have continuously altered the N cycle, notably with the invention of the Haber-Bosch process which allowed for the production of synthetic fertilizers, with negative environmental effects (Paull 2009). Land-use change is another prevalent human activity that can significantly affect N storage in soils (Grace et al. 2014, Schipper and Sparling 2009, Roa-Fuentes et al. 2012). Specifically, deforestation and subsequent crop cultivation or intensive pasture use can accelerate losses of N into the atmosphere or adjacent water bodies (Davidson et al. 2007). Land-use driven reductions in N availability can influence C uptake in plant biomass, requiring additional inputs for maintenance of crop and forage yields or potentially affecting rates of forest growth during succession. For these reasons, understanding the effects of land-use change on soil N cycling is necessary, especially in tropical soils. Tropical soils play an important role in global biogeochemical cycles as they contain about 40% of terrestrial soil C (Jobággy and Jackson 2000). Carbon accumulation in soils can be strongly influenced by N availability, through its role in stimulating litter production and decomposition (Cusack et al. 2010, Frey et al. 2014). Differences in the type and amount of plant production, microbial communities, and soil properties between deforested and forest soils can affect N cycling (Vitousek & Reiners 2011).

Land-use change can dramatically cause shifts in ecosystem dynamics and nutrient cycling (Houghton et al. 2010, Pan et al. 2013). Although the specific influences of land-use change, such as deforestation, on biogeochemical cycles do not have a scientific consensus, pasture and forests store varying quantities of soil organic matter (Grace et al. 2014, Schipper and Sparling 2009). A large flux of N is released into the atmosphere during deforestation and other land-use changes due to soil nutrient depletion from the removal and combustion of biomass (Chazdon 2014) as well as release of N from physical disruption of the soil. When this happens, primary production can become N-limited and affect pasture dynamics (Roa-Fuentes et al. 2012) as well as rates of forest regrowth after pasture abandonment. Due to this limitation, pastures will slowly recover in their total N pool. It is still unclear what this slow recovery means for the N cycle and its processes in pastures.

There are several important N processes that occur in tropical soils. N mineralization is the conversion of organic N into ammonia or ammonium (NH_3/NH_4^+), making it available for other processes. Nitrification is the conversion of NH_3/NH_4^+ into NO_2^- or NO_3^-, a plant-accessible form of N for nutrient uptake. N mineralization has been shown to be higher in agricultural soils, yet lower in younger forests compared to mature ones (Templer et al. 2004). This provides an unclear picture of how N mineralization recovers in tropical forests immediately following disturbance as well as with the progression of time.

Weathered tropical soils are not typically thought to be N-limited due to the large proportion of N-fixing tree species in the tropics. A main source of N in tropical soils is N fixation by symbiotic and free-living microbes (Menge & Hedin 2009). Studies have shown that there is variation in the number of N-fixing microbes, which are often affiliated with specific tree species, in reforested land (Mirza et al. 2014, Gehring 2005). Other studies find that the proportion of N-fixing tree species is greater in older forests (Batterman et al. 2013). However, more research needs to study the specific factors that affect how N availability varies between pastures and restored forests on tropical soils.

Deforestation causes a dramatic loss of N from pastures, creating the need for more N_2 from the atmosphere to be fixed and returned to the soil. This loss results in the increase in N-fixing microbes in the deforested area as the ecosystem becomes N-limited (Mirza et al. 2014). Tropical forests support more tree species that contain N-fixing microbes than pastures, leading to a higher fixation potential (Batterman et al. 2013). This introduces a contradiction, demonstrating N-limitation on pastures but the lack of N-fixing species compared to forests (Hedin et al. 2016). The effect of deforestation on N-fixing species and N mineralization is not clearly understood, due to conflicting hypotheses about fixation and N limitation.

On the other hand, during reforestation, mature forests have a wider variety of N-fixing trees in comparison to their younger counterparts due to greater time to develop higher biodiversity and accumulate more N-fixing tree species. Early successional forests are more N-limited after disturbances (Batterman et al. 2013). A relatively smaller number of tree species that support N-fixing microbes constrains younger forests and primary production (Weathers et al. 2013). This constraint supports the notion that there will be fewer outputs and tighter cycling in a new and still-developing ecosystem (Vitousek & Reiners 2011). However, forest regrowth in early successional forests brings the development of more plant species in the forest, increasing nutrient uptake and the need for plant-accessible N pools. Regeneration also has been shown to bring about more litterfall, which can restore pools of N (Vitousek & Reiners 2011). This restoration of N availability and the idea of early successional N-limitation pose a conflict that leads to the lack of certainty in how the N cycle and soil biomass recover with succession.

The objectives of this study were to identify variability in N fluxes and to investigate N stocks between land (e.g., forests) of different ages to gain a better understanding of the N cycle in tropical soils and its global importance. This study investigates two main research questions: (1) how do N cycling processes vary with forest succession? and (2) how are these processes related to N pools and the potential for N fixation? Specifically, our work contributes to addressing the uncertainties in current knowledge and understanding of N stocks and fluxes, as well as the factors that cause variation along pasture to forest chronosequences.

Methods

Site Description and Field Collection

To measure changes in N cycling during forest regrowth on pastures, we used a previously-established, well-replicated chronosequence in the tropical wet forest life zone in the Sierra de Cayey of southeastern Puerto Rico (18°010 N, 66°050 W) (Marín-Spiotta, Ostertag, & Silver 2007). We used a chronosequence approach, where sites of different ages are used to quantify changes in ecosystem processes over time. The forest chronosequence approach is a common ecological research approach that allows for the study of forests with similar environmental conditions at different ages as a proxy for how processes can vary with succession and growth (Matamala et al. 2008). The Cayey chronosequence used for this study had a total of 12 sites, which included three replicate sites each of active pastures (0 years since reforestation), 29-year-old and 69 year-old secondary forests, and primary reference forests with no history of human disturbance.

We collected three replicate soil samples at each field site using the following approach. At the center pole of each site, we randomly selected one angle from 0-119˚, 120-239˚, and 240-359˚ and a distance from 0-12 m. At these three coordinates, we collected a soil sample from 0-10 cm depth using a 2.25-cm diameter soil corer by compositing five soil plugs. Field-moist soils were shipped from Puerto Rico in a cooler to the University of Wisconsin-Madison within 1 day of collection. In the lab, they were sieved through 4-mm sieve, with large roots and rocks removed, and then kept refrigerated to maintain field moisture until the incubation began, within 7 days of collection. Soils were collected in August of 2017 and in January of 2018, six months after category-5 Hurricane Maria hit the island.

<u>Soil Incubations</u>

To understand how N changes during succession, we conducted lab measurements of N cycling rates. To measure rates of N mineralization from organic N to NH_4^+, we measured inorganic N accumulation during lab incubations on fresh soils (Hart et al. 1994). Soils were incubated for 13 days, with extractions of nitrate and ammonium at T_0 and T_{13}. Soils were incubated in specimen cups, covered with polyethylene film and placed in a darkened, climate-controlled room at 25 ˚C for 13 days. The samples were regularly checked to adjust moisture content to keep samples at field capacity. Briefly, 50 mL of 2.0 *M* KCl was added to 10 g of each sample, and these samples were shaken for 70 minutes at 160 rpm. Samples were then filtered into labeled specimen cups using funnels and pre-wet Whatman No. 1 filters. Extracts were frozen until analysis on a Lachat instrument for NH_4^+ and NO_3^-.

Calculation of Rates

We used the calculations outlined in Robertson et al. 1999 to find the rates of mineralization and nitrification from the measured NH_4^+ and NO_3^- at T_i and T_f of the incubation. The mineralization and nitrification equations are the following, respectively:

$$N_{mineralized} = [(Nitrate_f + Ammonium_f) – (Nitrate_0 + Ammonium_0)]/T_{days}$$

$$N_{nitrified} = (Nitrate_f – Nitrate_0) /T_{days}$$

Statistics

Before running statistics on our data, we checked for normality. We ran one-way ANOVA tests to determine if there was a significant age effect on net N mineralization and net nitrification rates with secondary succession for both samples from August 2017 and January 2018. One-way ANOVA tests were also used to test for differences between age groups (e.g., pasture vs. 69-year-old forest, pasture vs. reference forest). In order to test for relationships between net mineralization and nitrification rates with other measures of N in soil, we performed correlation analyses.

Relationship of Soil N Cycling Rates with Other Environmental Factors

To compare the N process rates with other components of the N cycle in the soils, we analyzed data on total soil N, free light fraction N (loose organic N from litterfall that is not sorbed to soil aggregates or minerals), and the relative abundance and average basal area of N-fixing tree species at each sampling site. We tested for correlations between N mineralization rates and total soil N from depths of 0-10 cm (Marín-Spiotta et al. 2009) as well as the proportion of bulk soil N recovered in the particulate organic matter pool or free light fraction (fLF) (0-10 cm) (Marín-Spiotta et al. 2008). Our significance level was p-value < 0.05 unless indicated otherwise. In addition, we used data on abundance of N-fixing tree species

(Leguminosae) at the sites to determine whether there were any patterns between potential for N fixation and measures of N availability to plants.

Results

Net N Mineralization and Net Nitrification Rates

In August 2017, soil net N mineralization rates peaked in the later stages of secondary succession (p-value < 0.006), with average rates of 0.55 ± 0.78, 1.87 ± 1.09, and 5.06 ± 2.42 mg N/kg d for active pastures, 29-year-old secondary forests, and 69-year-old secondary forests, respectively (Figure 1a). The 29-year old secondary forests had similar mineralization rates to the pastures, which had lower rates than both the mature 69-year-old secondary forests and reference forests (2.42 ± 0.91 mg N/kg d). Pastures had significantly lower net mineralization than both mature 69-year-old secondary forests and reference forests. Mature 69-year-old forests' net mineralization rates were higher than 29-year-old forest and reference forests.

Net N mineralization rates from the January 2018 samples did not differ with age. Rates averaged -0.23 ± 0.55, 0.39 ± 0.53, 1.02 ± 0.63 and 0.68 ± 1.28 mg N/kg d for active pastures, 29-year-old secondary forest, 69-year-old secondary forests, and reference forests, respectively (Figure 1). Generally, rates in 2018 were more variable than in 2017.

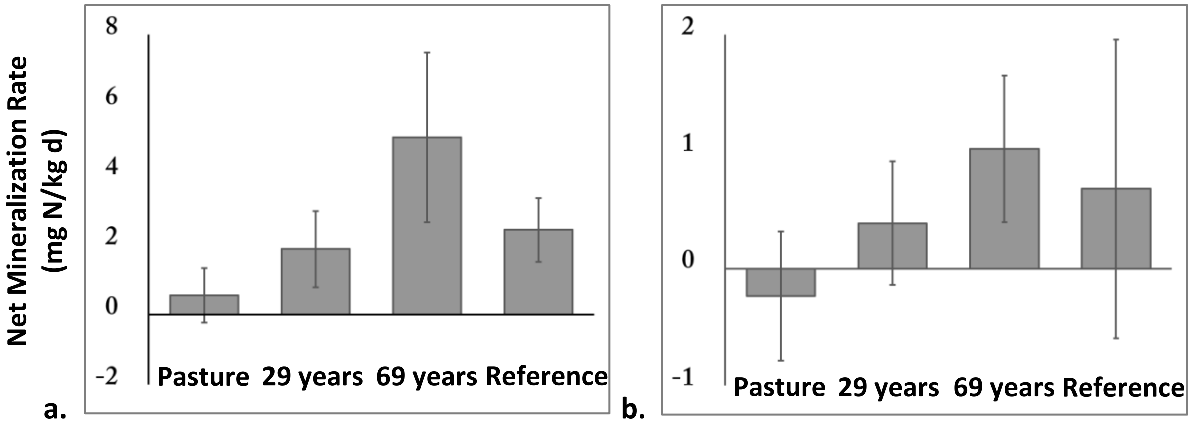

Figure 1. a. The average net N mineralization rates of active pasture, 29-year-old forest, 69-year-old forest, and reference forests from August 2017 increased with succession and peaked in mature secondary forests. **b.** The average net mineralization rates of the four different forest age categories from January 2018, showing a similar but insignificant pattern.

Soil net nitrification rates from August 2017 showed similar trends to net mineralization rates, but with fewer significant differences among forest age categories. Nitrification rates peaked in the 69-year old secondary forests (6.54 ± 2.21 mg N-NO_3/kg d). Reference forests (1.95 ± 1.21 mg N-NO_3/kg d) had greater rates than pastures (0.93 ± 0.49 mg N-NO_3/kg d) but lower rates than the late successional forests. The 29-year-old secondary forests had intermediate values (3.55 ± 1.39 mg N-NO_3/kg d). Overall there was a significant age effect ($p < 0.04$).

The soil net nitrification rates from January 2018 also did not show any differences among age groups. The average rates were 0.42 ± 0.42, 1.50 ± 0.62, and 1.84 ± 0.68 mg N/kg d for active pastures, 29-year-old secondary forest, and 69-year-old secondary forest, respectively (Figure 2). Reference forests had an average net nitrification rate of 0.65 ± 0.49 mg N/kg d. Comparing the two collections, 69-year-old secondary forests and reference forests had lower net mineralization and nitrification rates in January 2018 compared to August 2017.

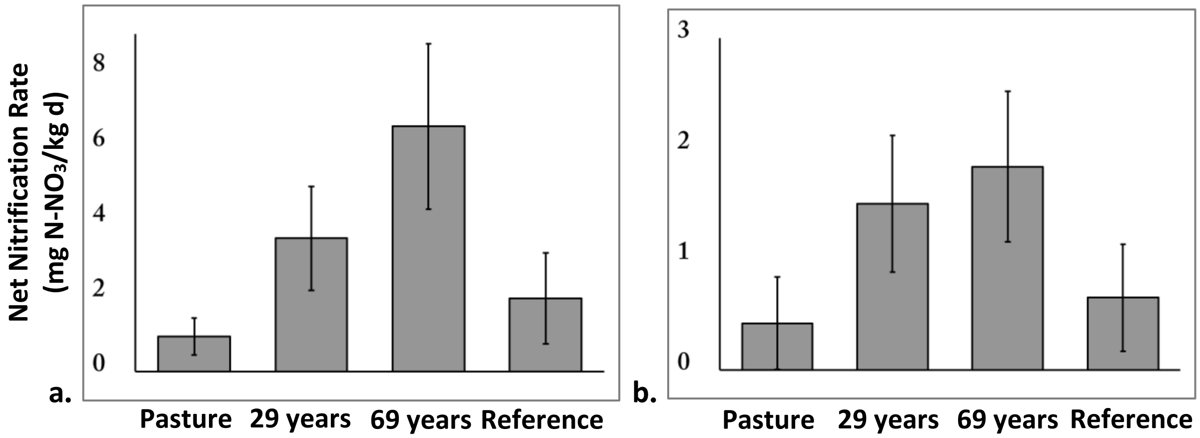

Figure 2. a. The average net N nitrification rates of forest age categories of active pasture, 29-year-old secondary forest, 69-year-old secondary forest, and reference forests from August 2017 increased with secondary succession, while peaking in mature secondary forests. **b.** The average net nitrification rates of the four different forest age categories from January 2018, showing a similar yet insignificant pattern.

Correlations between net N mineralization and nitrification and other environmental variables

We tested for correlations of soil mineralization and nitrification rates with other environmental factors, mainly total bulk soil N stocks (0-10 cm), free light fraction organic matter N concentrations (g/m^2) for depth 0-10 cm, average basal area of N-fixing tree species, and the relative abundance of N-fixing trees.

For the August 2017 sampling, neither net N mineralization or net nitrification rates were correlated with bulk soil N content in surface soils (tN/ha). Net N mineralization rates were not correlated with free light fraction N, while soil nitrification rates showed a positive correlation with free light fraction N ($R^2 = 0.63$, $p < 0.01$) (Figure 3a). Net mineralization rates had a positive correlation with the relative abundance of N-fixing tree species ($R^2 = 0.54$, $p < 0.007$). The relationship for net N nitrification rates was marginally significant ($R^2 = 0.24$, $p < 0.10$). Mineralization rates also had a positive linear relationship with the basal area of N-fixing tree species ($R^2 = 0.50$, $p < 0.01$) and nitrification rates had an insignificant trend. There was a strong positive correlation between net N mineralization rates and net N nitrification rates ($R^2 = 0.71$, $p < 0.0006$) (Figure 4a).

For the January 2018 net N mineralization and nitrification rates, there was no significant relationship between calculated N processes rates and the relative abundance of N-fixing tree species. Similar to the sampling from August 2017, there were no relationships between either net mineralization or net nitrification rates and bulk soil N from 0-10 cm depth. Free light fraction N showed a positive correlation with net N nitrification rates ($R^2 = 0.70$, $p < 0.005$) (Figure 3b), but net N mineralization rates showed no relationship with free light fraction N. There was no relationship between net N mineralization or nitrification rates and the average basal area of N-fixing tree species. However, the positive correlation between net mineralization and nitrification rates was maintained, although the relationship was weaker compared to August 2017 sampling ($R^2= 0.35$, $p < 0.054$) (Figure 4b).

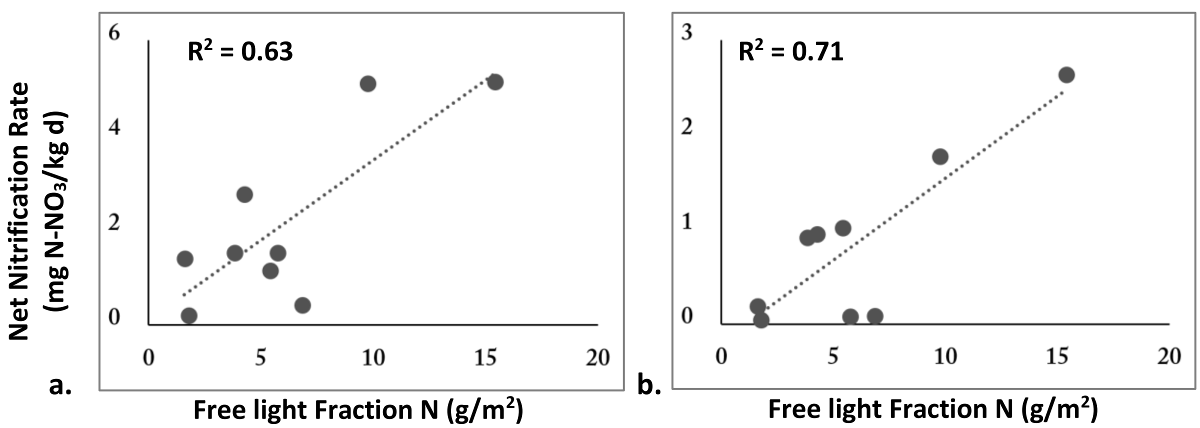

Figure 3. a. The positive relationship between net nitrification rates and the amount of N in the free light fraction from the August 2017 soils. **b.** From the January 2018 sampling, the positive relationship between net nitrification rates and the free light fraction N was maintained.

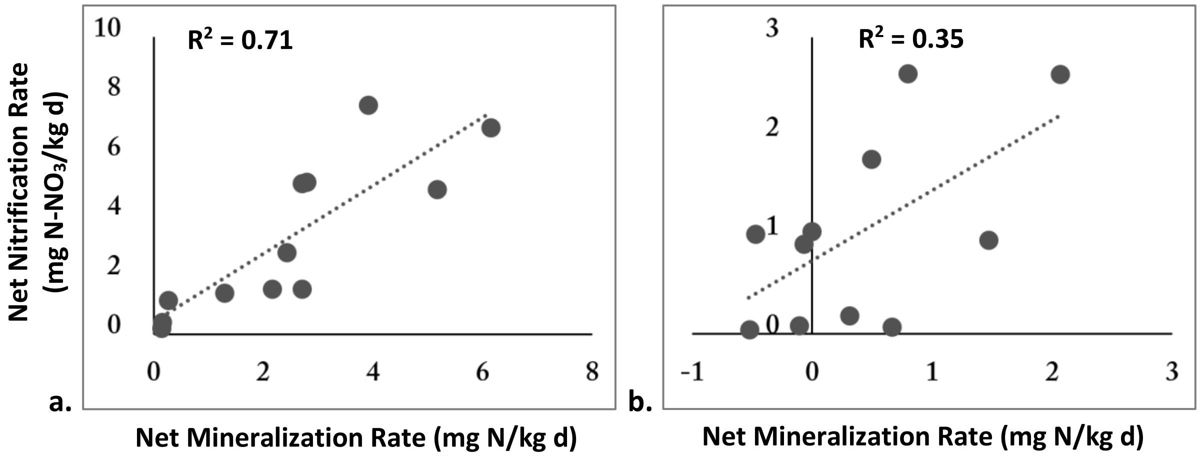

Figure 4. a. The positive correlation between net mineralization and nitrification rates from August 2017. **b.** The same correlation was maintained but weakened for the January 2018 sampling soils.

Discussion

Measures of nitrogen availability increased with secondary succession on former pastures

The results from our study have several implications about N cycling and its relationship to land-use change in tropical settings. Although there was variation, we saw net N mineralization and nitrification rates increase with secondary succession and peak in mature secondary forests. This demonstrated changes in the conversion of N into plant-available forms at each successional stage, corresponding with increased biomass during secondary succession. We found plant-accessible N to be more available in mature secondary forests compared to early successional forests, consistent with other studies (Batterman et al. 2013).

Pastures and primary forests were significantly different for our August 2017 sampling, demonstrating the higher N cycling in primary reference forests compared to deforested areas. Pastures had lower rates than early successional forests, implying they are more N-limited after deforestation. This increase showed how more N activity occurs with forest regeneration.

However, these patterns varied between our two samplings, emphasizing the importance of repeated sampling over time as suggested in other studies (Smith, Marín-Spiotta, & Balser 2015).

The observed differences in both mineralization and nitrification rates between 69-year-old secondary forests and primary reference forests suggest that there are still differences between mature secondary forests in N cycling processes from mature primary forests. Older secondary forests may have greater rates compared to primary forests because they are still recovering from disturbance and the loss of nutrients from deforestation on a shorter temporal scale. These differences question the ideas of succession and forest regeneration, implying the possibility that forests need more than 70 years to recover after deforestation and are influenced by pasture duration and management. Although it is known that human-driven land-use change can deplete soil N reserves, it could be altering forests beyond what is recoverable in the given timescale. However, there were still differences in species composition across our successional forest sites (Rivera, R.J. unpublished data), which in turn could affect nutrient cycling.

Soil nitrate increased with secondary succession, indicating an increase in nitrification rates. A similar study carried out Davidson et al. 2007 in Brazil suggests that N availability increases with succession in former pastures in tropical sites. Our results differ from a study in the Dominican Republic which found that net mineralization and nitrification rates were higher in agricultural sites (Templer et al. 2004). These differences between our study and others can suggest that local factors, such as topography, nutrient content, different soil properties, and parent material, can greatly influence N cycling processes across the tropics region.

Free light fraction N is a better predictor of soil N availability than bulk soil N

Recent plant organic N inputs serve as a source for N mineralization and nitrification processes, seen through the relationships between our measured rates and free light fraction N. In contrast, we did not observe any relationships between bulk soil N and both net mineralization and nitrification rates. This result suggests that total soil N has less of a relation to these N cycling processes, while the relationship we found with the free light fraction N demonstrates that this organic matter N potentially serves as a source for nitrification and mineralization processes. Bulk soil N is often used a measure of N cycling in studies (Springob & Kirchmann 2003), but our results suggest that organic matter free light fractions, which represent recent inputs that are not very decomposed, could be better measures of N cycling in tropical settings.

Net N mineralization rates increased with abundance of N-fixing trees

The relative abundance of N-fixing tree species was a better predictor of soil N mineralization rates compared to nitrification rates. This relationship suggests that there is a potential relationship between two different aspects of the N cycle, with fixation capturing atmospheric N_2 and mineralization converting organic N to ammonia or ammonium for plant uptake. Our results corroborate past studies that have shown relationships between N pools and fluxes in tropical soils (Erickson et al. 2001). Observed relationships between these two components of the N cycle can demonstrate the connections between different N processes in tropical forests. N fixation can influence the pools for both mineralization and nitrification processes. Greater rates of N fixation potential with greater abundance of N-fixing trees could lead to more nitrification and mineralization through higher N capture in the soil. One limitation is that we cannot determine if the N-fixing trees at our sites are currently fixing N due to their facultative nature, so the abundance of tree species is not a direct measure of N fixation.

However, it is still important to understand these relationships in N cycle processes to better quantify how change in species composition from human practices affect each stage and form of N, and in turn its role in global climate change.

Temporal changes in N cycling rates

The majority of the significant relationships as well as the significant age effect for net mineralization and nitrification rates were not maintained across our two sampling times. Specifically, the January 2018 sample had lower overall net mineralization and nitrification rates, with less relationships between these rates and other N pools. Since our site is tropical, solar radiation and temperature do have negligible variation throughout different times in the year, although there are seasonal differences in precipitation and litterfall that would affect microbial activity and N availability.

However, our sampling in January 2018 took place six months after category 5 Hurricane Maria made landfall on the island. Although six months after the hurricane did not give us a sample from immediately after the disturbance, our sites still suffered severe canopy loss and treefall. Hurricanes can affect biogeochemical cycling through canopy openness, which can lead to drier soil and slower microbial processes as well as large amounts of litter inputs, which can be integrated into N cycling (Ostertag et al. 2003). Studies have also shown that hurricanes can alter species composition, while also having different effects on different age forests (Flynn et al. 2010). This is supported by our findings that the older forests, 69-year-old secondary forests and primary reference forests, had significantly different net mineralization and nitrification rates compared to before the hurricane. In the case of our study, this type of major disturbance in these secondary forests may have altered the composition of the forests, in turn causing a significant decrease in both N net mineralization and nitrification rates.

There was a substantial loss of N from the system as well as vegetation to support N cycling, resulting in lower net mineralization and nitrification rates. Other pools of N were affected as well, demonstrated in the lack of relationship with our calculated rates. Our study seemed to have conflicting results with previous hurricane studies. An experimental hurricane-simulation study that manipulated canopy openness and debris addition in Luquillo Experimental Forest in Puerto Rico found that these combined effects of a simulated hurricane led to greater N availability and nitrate concentrations 9-12 months later (Shiels et al. 2015). Six months since Hurricane Maria, measures of N availability and nitrification had decreased compared to our pre-hurricane rates. This could imply that our sampling was too early to see the recovery effects after Hurricane Maria or that there are still major differences between experimental and observational hurricane effect studies as well as our understanding of how hurricane disturbance affects forest nutrient cycling. However, some studies suggest that after more time, the N process values can return to pre-hurricane levels (Silver et al. 1996). Overall, our results demonstrate the potential of hurricanes and other disturbance events, such as land use, to affect tropical soil N dynamics, with implications for climate change mitigation via C uptake in regenerating forests.

Conclusion

Alterations to the N cycle affect N availability as well as the potential of terrestrial ecosystems to sequester C. Most of our understanding about N cycling comes from temperate ecosystems, despite the importance of tropical forests in the global N cycle and the prevalence of human alterations to tropical ecosystems. This study quantified differences in N cycling between pastures and successional forests to evaluate the influence of land-use change on N availability in tropical landscapes. We found that net N mineralization and nitrification rates increased with secondary succession on abandoned pastures, while hurricane disturbance caused variation in these results. We also quantified relationships with measured N process rates and saw that different measures and stocks of soil N provide information about tropical N cycling and its complexity. Our results implied that tropical forest succession affects N cycling post-disturbance, whether human or natural disturbance, as well throughout various stages of succession. With information on N availability and plant biomass, the findings from this study have implications for current land-management practices in the tropics and for improving predictions of human land use on the global N and C cycles, as well as understanding how extreme weather can alter ecosystem processes.

Acknowledgments

I first would like to thank my mentor, Erika Marín-Spiotta, for encouraging me to pursue this project. I wish to thank Elliot Vaughan, Ricardo Rivera, Ann Olsson, Emily Stanley and Elizabeth Runde for field and lab assistance and funding from the UW-Madison Holstrom Scholarship and NSF CAREER BCS-1349952.

References

Batterman, S. Batterman, S. A., Hedin, L. O., Breugel, M. Van, Ransijn, J., Craven, D. J., & Hall, J. S. (2013). forest secondary succession. *Nature*, *502*(7470), 224–227. doi:10.1038/nature12525

Chazdon, R. (2014). *Second Growth: The Promise of Tropical Forest Regeneration in an Age of Deforestation*. Chicago: University of Chicago Press.

Cusack, D. F., Silver, W. L., Torn, M. S., & McDowell, W. H. (2010). Effects of nitrogen additions on above- and belowground carbon dynamics in two tropical forests. *Biogeochemistry*, *104*(1-3), 203–225. doi:10.1007/s10533-010-9496-4

Davidson E. A., De Carvalho C. J., Figueira A. M., Ishida F. Y., Ometto J., et al. 2007. Recuperation of nitrogen cycling in Amazonian forests following agricultural abandonment. *Nature* 447:995–98

Erickson H., Keller, M., Davidson, E. (2001). Nitrogen Oxide Fluxes and Nitrogen Cycling during Postagricultural Succession and Forest Fertilization in the Humid Tropics. *Ecosystems,* 4(1): 67-84.

Flynn, D., Uriarte, M., Crk, T., Pascarella, J., Zimmerman, J., Aide, T., Ortiz, M. (2010). Hurricane Disturbance Alters Secondary Forest Recovery in Puerto Rico. *Biotropica*. 42(2): 149-157.

Frey, S. D., Ollinger, S., Nadelhoffer, K., Bowden, R., Brzostek, E., Burton, a., … Wickings, K. (2014). Chronic nitrogen additions suppress decomposition and sequester soil carbon in temperate forests. *Biogeochemistry*, 305–316. doi:10.1007/s10533-014-0004-0

Gehring, C., Vlek, P. L. G., Souza, L. A. G. De, & Denich, M. (2005). Biological nitrogen fixation in secondary regrowth and mature rainforest of central Amazonia, *Agriculture Ecosystems & Environment* 111, 237–252. doi:10.1016/j.agee.2005.06.009

Grace, J., Mitchard, E., & Gloor, E. (2014). Perturbations in the carbon budget of the tropics. *Global Change Biology*, *20*(10), 3238–55. doi:10.1111/gcb.12600

Hart, S. C., Nason, G.E., Myrold, D. D., & Perry, D. A. (1994). Dynamics of Gross Nitrogen Transformations in an Old-Growth Forest : The Carbon Connection Author (s): Stephen C . Hart , G . E . Nason , David D . Myrold and David A . Perry Reviewed work (s): Published by : Ecological Society of America Stable URL : *Ecology*, *75*(4), 880–891.

Hedin, L. O., Brookshire, E. N. J., Menge, D. N. L., Barron, A. R., Hedin, L. O., & Jackbrookshire, E. N. (2016). The Nitrogen Paradox in Tropical Forest Ecosystems, *Annual Review of Ecology, Evolution, and Systematics* 40(2009), 613–635. doi:10.1146/annurev.ecolsys.37.091305.110246

Houghton, R. A., House, J. I., Pongratz, J., Werf, G. R. Van Der, Defries, R. S., Hansen, M. C., & Qu, C. Le. (2012). Carbon emissions from land use and land-cover change, *Biogeosciences* 4, 5125–5142. doi:10.5194/bg-9-5125-2012

Jobbágy, E. G., & Jackson, R. B. (2000). The vertical distribution of soil organic carbon and its relation to climate and vegetation. *Ecological Applications*, *10*(April), 423–436. doi:10.1890/1051-0761(2000)010[0423:TVDOSO]2.0.CO;2

Marín-Spiotta, E., Ostertag, R., & Silver, W. (2007). Long-term patterns in tropical reforestation: Plant community composition and aboveground biomass accumulation. *Ecological Applications* 17(3), 828–839.

Marín-Spiotta, E., W.L. Silver, C.W Swanston, and R. Ostertag. 2009. Soil organic matter dynamics during 80 years of reforestation of tropical pastures. *Global Change Biology* 15:1584-1597. doi: 10.1111/j.1365-2486.2008.01805.x

Marín-Spiotta, E., C.W. Swanston, M.S. Torn, W.L. Silver and S.D. Burton. 2008. Chemical and mineral control of soil carbon turnover in abandoned tropical pastures. *Geoderma* 143: 49-62

Matamala, A. R., Jastrow, J. D., Miller, R. M., & Garten, C. T. (2016). Temporal Changes in C and N Stocks of Restored Prairie : Implications for C Sequestration Strategies Stable URL : http://www.jstor.org/stable/40062268, *Ecological Applications* 18(6), 1470–1488.

Menge, D., S. and Hedin, L. (2009). Facultative versus Obligate Nitrogen Fixation Strategies and Their Ecosystem Consequences. *The American Naturalist*. 174(4): 465-477.

Mirza, B. S., Potisap, C., Nüsslein, K., Bohannan, B. J. M., & Rodrigues, L. M. (2014). Response of Free-Living Nitrogen-Fixing Microorganisms to Land Use, *Applied and Environmental Microbiology* 80(1), 281–288. doi:10.1128/AEM.02362-13

Ostertag, R., Scatena, F., Silver, W. (2003). Forest Floor Decomposition Following Hurricane Litter Inputs in Several Puerto Rican Forests. *Ecosystems*. 6:261-273. DOI: 10.1007/s10021-002-0203-8.

Pan, Y., Birdsey, R. a., Phillips, O. L., & Jackson, R. B. (2013). The Structure, Distribution, and Biomass of the World's Forests. *Annual Review of Ecology, Evolution, and Systematics*, *44*, 593–622. doi:10.1146/annurev-ecolsys-110512-135914

Paull, John. (2009). A Century of Synthetic Fertilizer. *Journal of Bio-Dynamics Tasmania*. 16-21.

Rivera, R. J. (2018). [Cayey Species Composition]. Unpublished raw data.

Roa-Fuentes, L L., Martinez-Garza, C., Etchevers, J., & Campo, J. (2003). Recovery of Soil C and N in Tropical Pasture: Passive and Active Restoration. *Land Degradation & Development* 26: 201–210

Robertson G.P, Wedin D, Groffman P. M, Blair J. M, Holland E. A, Nadelhoffer K. J, Harris D. 1999. Soil Carbon and Nitrogen Availability: Nitrogen Mineralization, Nitrification, and Soil Respiration Potentials, sec. Potential Nitrogen Mineralizatoin – Laboratory Incubations. Pp. 264-265. In Robertson, G. P et al., eds., *Standard Soil Methods for Long-Term Ecological Research.* Oxford University Press, New York, New York USA.

Schipper, L. A., & Sparling, G. P. (2009). Accumulation of soil organic C and change in C:N ratio after establishment of pastures on reverted scrubland in New Zealand. *Biogeochemistry*, 104(1-3), 49–58. doi:10.1007/s10533-009-9367-z

Shiels, A., Gonzalez, G., Lodge, D., Willig, M., Zimmerman, J. (2015). Cascading Effects of Canopy Opening and Debris Deposition from a Large-Scale Hurricane Experiment in a Tropical Rain Forest. *Bioscience*, 65:871-881.

Silver, W., Scatena, F., Johnson, A., Siccama, T., Watt, F. (1996). At What Temporal Scales Does Disturbance Affect Belowground Nutrient Pools? *Biotropica*, 28(4a): 441-457.

Smith, A. P., Marín-Spiotta, E., Balser, T. (2015). Successional and seasonal variations in soil and litter microbial community structure and function during tropical post-agricultural forest regeneration: a multiyear study. *Global Change Biology,* 21: 3532–3547. doi: 10.1111/gcb.12947.

Springob, G. & Kirchmann, H. (2003). Bulk soil C to N ratio as a simple measure of net N mineralization from stabilized soil organic matter in sandy arable soils. *Soil Biology and Biochemistry*, 35(4): 629-632. https://doi.org/10.1016/S0038-0717(03)00052-X

Templer, P. H., Groffman, P. M., Flecker, A. S., & Power, A. G. (2004). Land use change and soil nutrient transformations in the Los Haitises region of the Dominican Republic, *Soil Biology and Biochemistry* 37, 215–225. doi:10.1016/j.soilbio.2004.07.031

Vitousek, P. M., & Reiners, W. A. (2011). Ecosystem Succession Nutrient Retention: Hypothesis, *BioScience* 25(6), 376–381.

Weathers K., Strayer D., and Likens, G. (2013). *Fundamentals of Ecosystem Science.* San Diego: Elsevier.

Appendix

	August 2017	**January 2018**
1. Net nitrification rates vs. Net N mineralization rates	*Significant positive correlation*	*Significant positive correlation*
2. Net N mineralization rates vs. Relative Abundance of N-fixing tree species	*Significant positive correlation*	*No relationship*
3. Net N mineralization rates vs. Average Basal Area of N-fixing tree species	*Significant positive correlation*	*No relationship*
4. Net N mineralization rates vs. bulk soil N	*No relationship*	*No relationship*
5. Net N mineralization rates vs. free light fraction organic N	*No relationship*	*No relationship*
6. Net N nitrification rates vs. Relative Abundance of N-fixing tree species	*Marginal positive trend*	*No relationship*
7. Net N nitrification rates vs. Average Basal Area of N-fixing tree species	*Marginal positive trend*	*No relationship*
8. Net N nitrification rates vs. bulk soil N	*No relationship*	*No relationship*
9. Net N nitrification rates vs. free light fraction organic N	*Significant positive correlation*	*Significant positive correlation*

Table 1. A summary table of all the relationships that we tested for and their results relative to the two sampling times.