CONSEQUENCES OF LONG-TERM FERTILIZATION ON WETLAND MICROBIAL FUNCTION

by

Megan Koceja

A Senior Honors Project Presented to the
Honors College
East Carolina University
In Partial Fulfillment of the
Requirements for
Graduation with Honors
by
Megan Koceja
Greenville, NC
May, 2019

Approved by:
Ariane L Peralta, PhD
Department of Biology | Thomas Harriot College of Arts and Sciences

Consequences of long-term fertilization on wetland microbial function

Megan E Koceja

Department of Biology, East Carolina University, Greenville N.C. 27858-4353, U.S.A.

ABSTRACT - Anthropogenic disturbances have led to increased deposition of nitrogen (N) and phosphorus (P) into soils. Nutrient enrichment of soils is known to increase plant biomass and also increase rates of microbial litter decomposition. Through decomposition, microorganisms release carbon (C) previously stored as organic C in soils into the atmosphere as carbon dioxide, a greenhouse gas. As these gaseous C emissions increase, global warming potential increases along with them. Understanding soil-microbe-plant interactions and their influence on decomposition rates is essential for understanding the causes of climate change and its mitigation. This study explores how shifts in organic C, N, and P caused by long-term nutrient enrichment and litter-type composition influence soil microbial function (e.g. decomposition). It is hypothesized that long-term nutrient enrichment causes shifts in soil microbial community structure that lead to higher rates of litter decomposition. Further, plant litter with a lower carbon C to N ratio (compared to high C:N ratio litter) is expected to decompose faster due to an available N source provided to nutrient-starved microbes. This study began at the long-term experimental fertilization and disturbance by mowing experiment at East Carolina University's West Research Campus. In each of eight replicate mowed/fertilized and mowed/unfertilized plots, replicate bags of two different litter types (high C:N ratio rooibos tea and low C:N ratio green tea) were buried for 111 days. By using rooibos and green tea as a model plant litter with known C:N ratios, we are able to draw a link between litter type and decomposition rates by litter mass loss. Results revealed that soil microbes are capable of decomposing rooibos tea litter

(higher C:N ratio) more quickly in fertilized compared to unfertilized. However, green tea litter (lower C:N ratio) decomposition rates were similar between fertilized and unfertilized plots. Overall, as predicted, the green tea litter decomposed faster than the rooibos tea litter. The outcomes of this study will provide insight into long-term effects of nutrient additions on soil microbial diversity and composition, related rates of decomposition, and the potential for climate change mitigation as nutrient enrichment continues to increase.

Acknowledgments

I would like to express my sincerest gratitude to my research mentor Dr. Ariane L Peralta for her extensive support and guidance throughout this process. I also extend thanks to Gina Bledsoe for her knowledge and encouragement, Dr. Suelen Tullio for her support in ECU's Environmental Research Lab, Dr. Carol Goodwillie for support in the field and feedback on this thesis, Peralta Lab members Allison Fisk and Emma Richards, the Department of Biology, and East Carolina University. Funding was received by an East Carolina University Undergraduate Research and Creativity Award. Without the people and organizations above, this study would not have been possible.

Table of Contents

List of Figures

Introduction

Humans constantly modify their environments as industrialization and urbanization continue. Some ways in which humans modify their landscapes are through the burning of fossil fuels, deforestation, and intense agricultural activity (Huisingh et al. 2015). These anthropogenic disturbances have led to increased atmospheric deposition of chemicals such as nitrogen (N) and phosphorus (P) into soils (Guignard et al. 2017). Since N and P are important limiting nutrients in many ecosystems, this increased nutrient deposition can cause a fertilization effect on plant-microbial interactions due to increased biomass and shifts in plant and microbial community structure (Cherif and Loreau 2009; Harpole et al. 2016; Leff et al. 2015). Changes in community composition of plants and microbes directly influence ecosystem functions associated with C cycling. This can alter the balance of C stored in soils and plant biomass with C released as carbon dioxide (CO_2) through respiration. Specifically, this fertilization effect can increase C biomass, which fuels heterotrophic microbial growth and reproduction and leads to increased respiration of CO_2 (Hoosbeek et al. 2004). Following this line of thought, it is essential to explore underlying factors that alter C cycling as a consequence of changes to plant-microbe interactions. For example, how do changes in plant litter composition and biomass affect the functioning of microbes that are dependent on C resources and nutrients (such as N and P) accessed from their environment? If increased nutrient deposition ultimately leads to more C loss through respiration than C fixed through primary production, then climate change is exacerbated as global warming potential ultimately increases.

Though there has recently been a large focus placed on the human modifications to physical landscapes, drastic alterations to underlying elemental cycles are also pervasive.

Elemental cycles can change as a consequence of variations in C to N to P (C:N:P) concentrations due to changes in community composition, physical movement of elements that subsidize nutrient-poor areas, changes in predation, and a multitude of other means (Rappaport et al. 2018; Leroux et al. 2012). Perhaps one of the most easily accessible concepts of this is that of deforestation. Large-scale removal of trees inherently releases the C previously stored by trees and producers within soils. Deforestation also removes trees that previously fixed C through primary production. This major ecosystem C loss is also accompanied by volatilization of other nutrients such as N and P, which then can get deposited to subsidize other ecosystems. This example of physical disturbance is one of many frequently used examples of how human intervention directly changes elemental cycling. However, the effects of anthropogenic disturbance on community composition and ecosystem function in the context of climate change depends on a multitude of factors.

While it is relatively well documented that humans alter their environment and inherently affect C storage and release, there are few studies focused on the interacting effects of environmental change (i.e., nutrient enrichment) on underlying structure-function relationships. Specifically, how do changes in internal C:N:P community stoichiometry and external C:N:P additions inherently affect elemental cycling? As microbial and plant community composition shift, their functions within their ecosystems change. Microorganisms, specifically, have an immeasurable impact on ecosystem processes (Van Der Heijden et al. 2008). This includes the process of decomposition, the reduction of a formerly living organism into simpler forms of matter. Microbial colonies within terrestrial soil are a major player in regulating the amount of C release and storage in a certain ecosystem through decomposition and respiration (Su, R. et al. 2015). However, the rate of these microbial processes is limited by N and P availability. This

leads one to question how plant and soil C:N:P composition affects rates of biogeochemical processes such as decomposition. As microbial respiration involves the decomposition of plant litter and relies on nutrients within the environment to carry out this function, it is only logical to assume that changes in the nutrient stoichiometry of both plants and surrounding soils would influence microbial community composition and function, causing changes in elemental cycling.

One way in which plant composition is influenced by nutrient addition is through its biomass. Prior studies have shown that long-term nutrient addition enhances plant biomass and increases C inputs into soils (e.g., Harpole et al. 2016). These increased plant inputs also increase CO_2 outputs via microbial respiration (Hoosbeek et al. 2004; Lange et al. 2015). If microbes are not limited by N and P, the increase in plant derived organic C inputs are more quickly metabolized and CO_2 emissions increase. In addition to amount of plant biomass, however, plant inputs could also vary in C:N or C:P ratios, which could also cause changes in microbial nutrient use and respiration rates (Figure 1). Because microbes are often dependent on nutrients present for their individual functioning, it is pertinent to understand how changes in available nutrients within plant litter alter microbial communities and the CO_2 outputs that they facilitate.

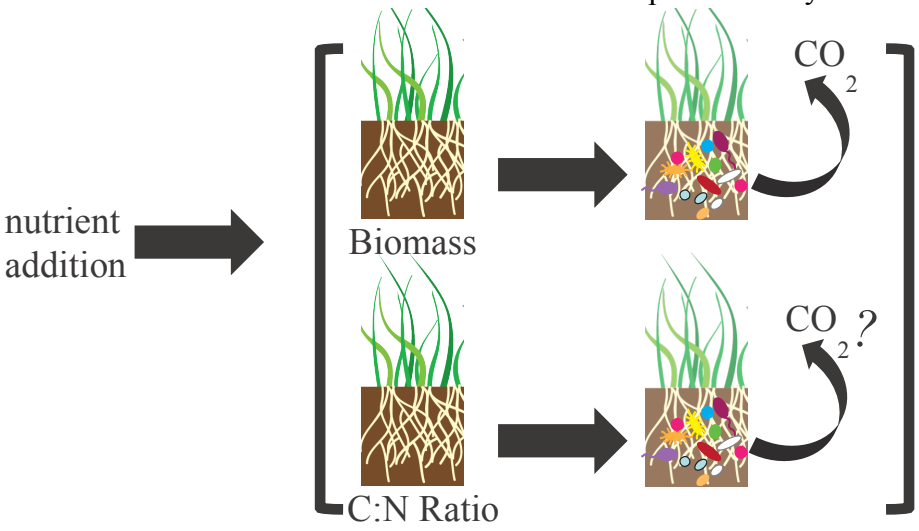

Figure 1. Depiction of known and unknown relationships between nutrient additions, change in plant biomass, change in C:N ratio, and CO_2 emissions.

This study seeks to understand how long-term nutrient enrichment and litter-type composition influence decomposition rates. We hypothesize that litter with a lower C to N ratio (compared to high C:N ratio litter) would lead to higher decomposition rates due an available N source provided to nutrient-starved microbes. We further hypothesize that long-term nutrient additions will cause shifts in soil microbial community structure that lead to higher rates of litter decomposition. Before controlled experiments can be conducted on how the nutrient addition effects on nutrient stoichiometry of plant communities influence microbial function, however, a small-scale model is necessary. A small-scale model will allow us to understand if indeed differences in single species plant litter stoichiometry affect microbial function. This study's small-scale model can later be expanded upon to measure ecosystem-scale changes in CO_2 fluxes from soils. By studying the rate of decomposition occurring in an area that has both anthropologically fertilized and unfertilized land, information can be gathered and understood about the connection between humans, plant and microbial community composition, microbial decomposition, and C cycling.

Methods

The methodology for this experiment combined the efforts of a long-term fertilization experiment at East Carolina University's West Research Campus and a tea bag index developed by Keuskamp et al. 2013 to collect decomposition data.

EXPERIMENTAL DESIGN OF WEST RESEARCH CAMPUS

In the West Research Campus (WRC), there is a long-term ecological experiment being conducted that studies the factors that shape patterns of diversity and community composition. This study began in 2002 and specifically targets the effects of mowing and fertilization on plant and microbial diversity. At the WRC, there are eight replicate blocks set up in a fully factorial experiment to test the impact of fertilizer addition (fertilized, unfertilized) and disturbance by mowing (mowed, unmowed) (Figure 2). The four plots within each block are representative of land that is mowed/fertilized, mowed/unfertilized, unmowed/fertilized, and unmowed/unfertilized. Within each plot, there are three designated quadrats in which annual soil and plant sampling takes place (Figure 2). For this study, decomposition experiments were focused in the mowed plots only.

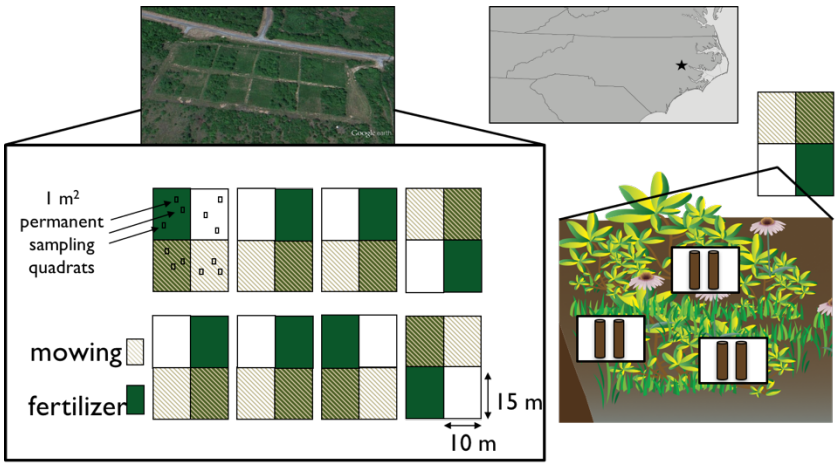

Figure 2. Experimental design of long-term ecological experiment to test the impacts of fertilizer and disturbance by mowing on plant and microbial communities at East Carolina University's West Research Campus.

DESCRIPTION OF TEA BAG INDEX

In attempt to further understand the effects of microbial communities on C storage and release, scientists in the Netherlands developed a universal protocol to measure decomposition rate in any type of environment. The Tea Bag Index (TBI) compares the decomposition rates of two different plant litters, green and rooibos Lipton™ tea bags (Keuskamp et al. 2013). The index outlines a key difference between green and rooibos tea through chemical analyses: different C:N ratios. Green tea has a measured mean C:N ratio of 12.229 while rooibos tea is measured to have a much higher mean C:N ratio of 42.870. Simply, green tea has a larger source of available N within its litter than rooibos tea. Considering both the established method for measuring decomposition and the model plant litter with known C:N ratios, the TBI was used in this study to examine how long-term fertilization affects decomposition rates. By placing differing plant litters into differing fertilized and unfertilized environments of the WRC, we can begin to disentangle the effects of nutrient availability on microbial respiration and therefore, C release and storage potential.

Figure 3. TBI protocol

1. Use one bag of Lipton green tea (EAN: 87 22700 05552 5) and one Lipton rooibos tea (EAN: 87 22700 18843 8) per replicate.
    a. To obtain better estimates of TBI, bury more replicates per site.
2. Measure the initial weight of the tea bag and subtract the weight of an empty bag (see also Table 1) to determine the initial weight of the tea.
3. Mark the tea bags on the white side of the label with a permanent black marker.
4. Bury the tea bags in 8-cm deep, separate holes while keeping the labels visible above the soil and mark the burial site with a stick.
5. Note the date of burial, geographical position, ecotype and experimental conditions of the site.
6. Recover the tea bags after c. 90 days
7. Remove adhered soil particles and dry in a stove for 48 h at 70°C (not warmer!).
8. Remove what is left of the label but leave the string, weigh the bags and subtract the weight of an empty bag without the label to determine the weight after incubation.
    a. To get a more precise estimation, open the bag and weigh its content; combust the content at 550°C and subtract what is left from the content weight.
9. Calculate stabilization factor S and decomposition rate k using eqn 1b-1b.
10. More (facultative) instructions and tips on how to incorporate the TBI in scientific experiments can be found on our website: http://www.decolab.org/tbi

Figure 3. Tea Bag Index protocol of *Tea Bag Index: a novel approach to collect uniform decomposition data across ecosystems.* This protocol was adapted for this study to be applied to the long-term fertilization study at ECU's West Research Campus.

FIELD EXPERIMENTAL METHODS

The field protocol for this experiment was adapted from the Tea Bag Index Protocol (Figure 3) and applied within the eight replicate mowed/fertilized and mowed/unfertilized plots of the WRC (Keuskamp et al. 2013). The experimental setup involved three bags of Lipton™ green tea and three bags of Lipton™ rooibos tea per replicate plot. Green and rooibos replicate bags were buried within the same quadrat (6 teabags per quadrat per replicate plot = 96 total bags). The 96 tea bags were measured for their initial weight of string, label, bag, and contents using a 0.0001g analytical balance. In the field, the tea bags were buried around 8cm-deep using a hand trowel in separate holes. Soil was lightly packed around the tea bags, while keeping the labels visible above the soil. Green and rooibos tea bags were buried in rows 20 cm apart and marked with flags labeled with the corresponding sample numbers.

Tea bags were recovered after 111 days. Hand trowels were used to loosen soil adjacent to tea bag locations, and care was taken not to pierce the bags. Two of three green tea bags at each replicate site were placed into a prelabeled Ziploc bag to return to lab to weigh for mass loss. The third green tea bag at each replicate site was placed into a pre-labeled, sterile Whirl-pak bag to later characterize tea-associated bacterial communities (Figure 4). The same procedure was followed with rooibos tea bags at each replicate site. If all three tea bags were not retrieved at each replicate site, a separate tea bag was not placed in a Whirl-pak bag.

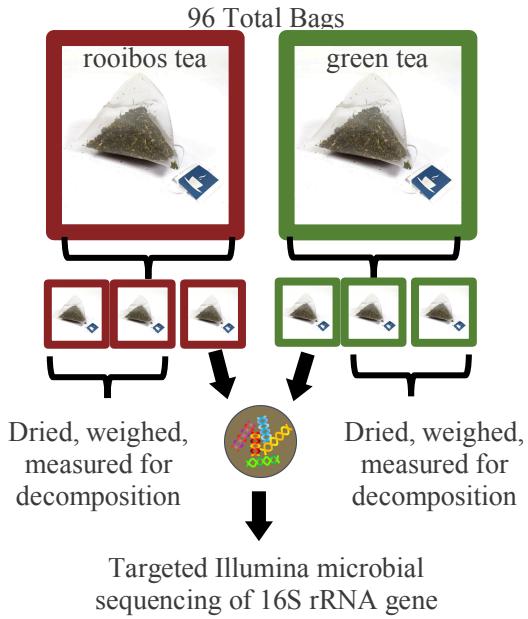

Figure 4. Schematic depiction of tea litter processing methodology.

TEA BAG PROCESSING

Upon returning to the lab, the tea bags placed in Ziploc bags for decomposition analysis were separated from adhered soil particles, placed into individual aluminum weighing dishes, and dried in a stove for 48 hours at 70 °C. To quantify mass loss, the tea bags were reweighed following drying. Tea bags were placed on the same 0.0001g analytical balance used to initially weigh the tea bags and weighed for their final weight of dried tea bag, string, label, and contents. Following drying, tea bags were held in a small desiccator while waiting to be weighed as to prevent any unintended moisture from entering the tea samples.

DATA ANALYSIS

Decomposition was measured through mass loss of buried tea bags. Initial and final tea bag weights were used to determine percent of loss, and those values were used in an analysis of variance followed by a Tukey's HSD (honestly significant difference) to identify specific pair-wise differences. Outcomes of tests were reflected in a series of box plots.

Calculations of decomposition rate (k) and stabilization factor (S) for each tea bag were accomplished according to formulas found within the TBI protocol. Parameter k represents short-term dynamics of new input and parameter S is indicative for long-term carbon storage (Keuskamp et al. 2013). These values were used to draw comparisons between k and S values found within the WRC wetland ecosystem to those of global ecosystems submitted to the Tea Bag Index protocol (Keuskamp et al. 2013). Outcomes of these calculations were plotted on a graph, and locations of groupings were compared to those of the larger multi-ecosystem index.

All statistical calculations were completed in the R environment (R v3.5.0, R Core Development Team 2018) and graphs constructed using the ggplot2 package.

Results

To examine how litter type and nutrient addition influenced decomposition rates, we measured mass loss of green and rooibos tea bags following an 111-day incubation in fertilized and unfertilized soils. The interactive effects of treatment and litter type were not significant. Overall mass loss of green tea litter was ~24% higher than that of overall rooibos tea litter (ANOVA, litter type: F=187.8, P<0.0001; Figure 5). In addition to litter type, fertilization effect significantly influenced mass loss (ANOVA, treatment: F=18.2, P<0.0001; Figure 5). Following a post-hoc Tukey's HSD test, it was determined that green tea had similar mass loss between fertilized and unfertilized plots (P_{adj}=0.2), while fertilization increased mass loss of rooibos tea in the mowed plots compared to litter decomposition at the unfertilized/mowed plots (P_{adj}<0.05).

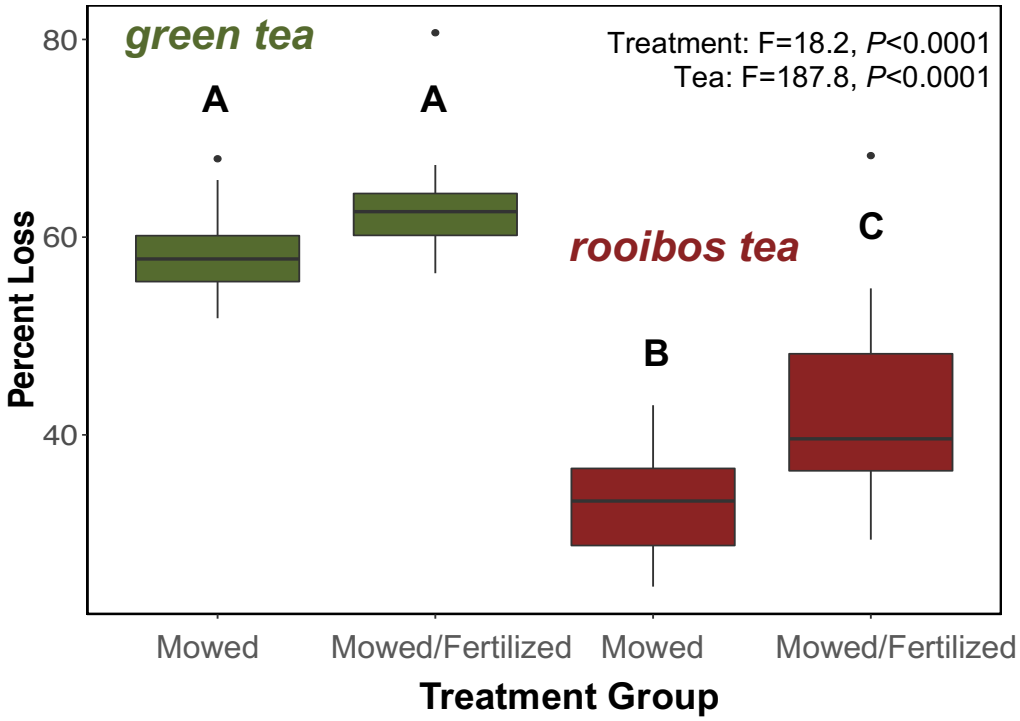

Figure 5. Cumulative mass loss of green and rooibos tea in fertilized and unfertilized plots. Different letters above bars are considered significantly different at P < 0.05 (based on Tukey's HSD test).

Following mass loss calculations, both decomposition rate (*k*) and stabilization factor (*S*) were calculated for each retrieved tea bag (Figure 6). The mowed/unfertilized compared to fertilized samples were generally higher along the stabilization factor axis. We compared the decomposition rates and stabilization factors from the WRC experiment to a broader context of ecosystems represented in the Keuskamp et al. (2013) graphic depiction of the discriminatory potential of the TBI within differing ecosystems (Figure 7). Most data points of the WRC fall within a decomposition rate of 0.01 and 0.02 and within a stabilization factor of 0.10 and 0.25. Upon comparison with other ecosystems, a large number of mowed/fertilized treatment samples grouped near the grassland-ambient ecosystem in Iceland (point #6 of Figure 7) and the forest ecosystem in the Netherlands (point #9 of Figure 7). Many mowed treatment samples, however, grouped near the peat-disturbed and peat-undisturbed ecosystems of Iceland and the peat ecosystem of the Netherlands (points #3, #4, and #12 in Figure 7).

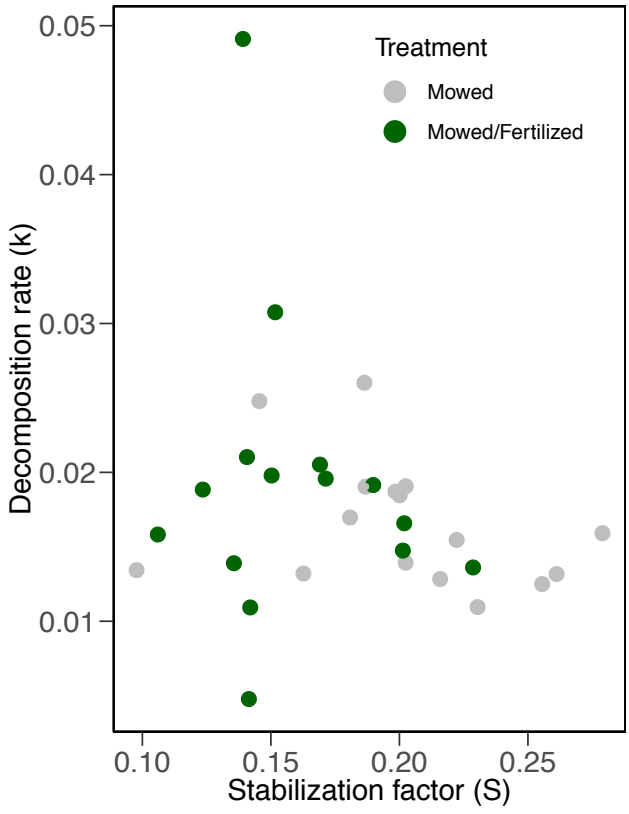

Figure 6. Initial decomposition rate *k* and stabilization factor *S* for different tea bags within the WRC. Calculations were based on a 111-day incubation period. Tea bags from mowed plots are indicated in grey, while tea bags from mowed/fertilized plots are indicated in green.

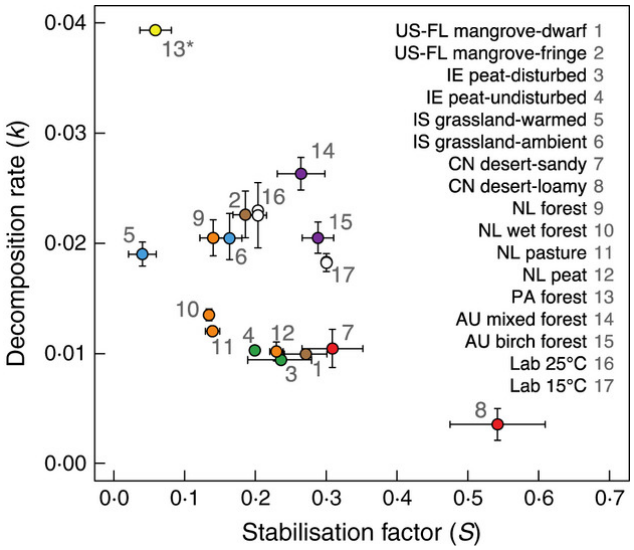

Figure 7. Initial decomposition rate *k* and stabilization factor *S* for different ecosystems. Graph was retrieved from the Keuskamp et al. Tea Bag Index. "Calculations were based on a single incubation time between 66 and 90days. Labels indicate country … followed by ecosystem and either soil type or temperature" (Keuskamp et al. 2013).

Discussion

The results obtained from the litter mass lost following the field incubation indicate that soil microbes are capable of decomposing rooibos tea litter (higher C:N ratio) more quickly in fertilized compared to unfertilized plots due to nutrient enrichment from the fertilizer treatment. However, green tea litter (lower C:N ratio) decomposition rates were similar among fertilized and unfertilized plots. This suggests that green tea itself is providing a N source to microbes regardless of external soil nutrient conditions, whereas rooibos tea has a much higher C:N ratio and is reliant on N solely from the fertilized soil in order to have increased rates of decomposition and litter mass lost. Overall, as expected, the low C:N ratio litter decomposed faster than the high C:N ratio litter.

The comparative results from the decomposition rates (*k*) and stabilization (S) factors of the WRC and the TBI indicates that the tea litter bags buried in the mowed/unfertilized treatment had *k* and S values similar to that of peatland ecosystems (Keuskamp et al. 2013). These results reflect the coastal plain wetland environment of the West Research Campus offering additional evidence that wetland environments store a disproportionate amount of C compared to other ecosystems. As less decomposition was occurring in mowed plots, specifically with high C:N ratio litter, less C was being released providing evidence that C storage potential resembles that of peatlands (Hill et al. 2018). Natural wetland ecosystems that are disconnected and isolated from pulses of nutrients from agricultural or urban runoff are typically nutrient limited by N and P (Vitousek et al. 2010). The plant species that are adapted to these low-nutrient ecosystems can maintain positive population growth and contribute to organic C additions to soils. Further, flooded environments, such as wetlands, are also known to support anaerobic microbial processes, which result in slower rates of decomposition (Collins et al. 2015). Taken together,

low nutrient environments can be sites of high plant biodiversity leading to organic C inputs and balanced with slower decomposition rates, which contribute to long-term C storage in soils (Kleber et al. 2011; Hooper et al. 2005).

The mowed/fertilized plots, however, had *k* and *S* values that grouped closer towards grassland and forest ecosystems which have lower C storage potential because more available nutrients and oxic environments support aerobic respiration and higher decomposition rates. This suggests that due to fertilizer additions at the WRC, more decomposition is taking place despite more plant biomass due to fertilization. Thus, the mowed/fertilizer environment decomposition status is more similar to that of a grassland or forest when using the TBI (Riggs et al. 2015). Results from this study provide support that nutrient enrichment can have a lasting influence on plant-soil-microbe interactions that affect C storage potential of wetland ecosystems (Hartman et al. 2017; Lambers et al. 2009; Allison et al. 2014).

The decomposition results of this study demonstrate that single species plant litter stoichiometry affects microbial function, specifically changes in the C:N ratio of belowground litter and in the C:N ratio of surrounding soils. The results of this study will offer important insight for development of future studies to take place that compare decomposition rates throughout the entire WRC. As data are collected annually on plant and microbial community composition, there is now decomposition data that shows it pertinent to begin to study how long-term fertilization influences C:N ratios within belowground plant biomass. If generalizable patterns between plant-community wide root C:N ratios reflect species-specific litter type decomposition from this study, then plant trait nutrient stoichiometry can then be used to study C flux at the ecosystem scale. By applying this single species tea result as a model study, future

studies can be conducted on a larger scale that can be used to begin to disentangle the causal factors of climate change and its potential mitigation.

FUTURE DIRECTIONS

This single species study of fertilized and unfertilized environment will be submitted to the global Tea Bag Index. Within that study, these results are aided in collecting largescale decomposition data that are well-standardized and cost-effective. By taking part in a largescale comparative litter decomposition study, potential drivers of carbon release through microbial processes are closer to being revealed. The TBI will also allow for comparison of results alongside field decomposition experiments as the results of this small-scale study are taken and applied within the ecosystem-wide WRC.

We are currently in the process of bacterial sequencing tea-associated microbial communities in order to visualize the community responses to fertilization and litter type. We will use principal coordinates analysis (PCoA) of bacterial community composition based on the Bray-Curtis dissimilarity and use permutational multivariate analysis of variance (PERMANOVA) to examine among-treatment differences in bacterial communities. With these bacterial community results, we expect to see that differences in decomposition rates will be associated with shifts in soil microbial communities. We will also be able to compare tea-associated microbial community data to microbial composition data of the WRC since 2014. Taken together with the decomposition data collected, these comparisons will allow us to discover relationships between nutrient addition, soil and plant stoichiometry, microbial community composition, and C emissions.

Literature Cited

Allison, S. D., S. S. Chacon, & D. P. German. 2014. Substrate concentration constraints on microbial decomposition. *Soil Biology and Biochemistry*, 79, 43–49.

Cherif, M. and Loreau, M. (2008). When microbes and consumers determine the limiting nutrient of autotrophs: a theoretical analysis. *Proceedings of the Royal Society B*, 276(1656), 487–497.

Collins D. P., Conway, W. C., Mason, C. D., & Gunnels, J. W. (2015). Decomposition of three common moist-soil managed wetland plant species. *Journal of Fish and Wildlife Management*, 6(1), 102–111.

Guignard, M. S., Leitch, A. R., Acquisti, C., Eizaguirre, C., Elser, J. J., Hessen, D. O., Jeyasingh, P. D., Neiman, M., Richardson, A. E., Soltis, P. S., Soltis, D. E., Stevens, C. J., Trimmer, M., Weider, L. J., Woodward, G., & Leitch, I. J. (2017). Impacts of nitrogen and phosphorus: from genomes to natural ecosystems and agriculture. *Frontiers in Ecology and Evolution*, 5(70).

Harpole, W. S., Sullivan, L. L., Lind, E. M., Firn, J., Adler, P. B., Borer, E. T., Chase, J., Fay, P. A., Hautier, Y., Hillebrand, H., MacDougall, A. S., Seabloom, E. W., Williams, R., Bakker, J. D., Cadotte, M. W., Chaneton, E. J., Chu, C., Cleland, E. E., D'Antonio, C., Davies, K. F., Gruner, D. S., Hagenah, N., Kirkman, K., Knops, J. M. H., Pierre, K. J. L., McCulley, R. L., Moore, J. L., Morgan, J. W., Prober, S. M., Risch, A. C., Schuetz, M., Stevens, C. J., & Wragg, P. D. (2016). Addition of multiple limiting resources reduces grassland diversity. *Nature,* 537, 93–96.

Hartman, W. H., R. Ye, W. R. Horwath, & S. G. Tringe (2017). A genomic perspective on stoichiometric regulation of soil carbon cycling. *The ISME Journal,* 11, 2652–2665.

Hill, B. H., C. M. Elonen, A. T. Herlihy, T. M. Jicha, & G. Serenbetz 2018. Microbial ecoenzyme stoichiometry, nutrient limitation, and organic matter decomposition in wetlands of the conterminous United States. *Wetlands Ecology and Management*, 26, 425–439.

Hooper, D. U., F. S. Chapin, J. J. Ewel, A. Hector, P. Inchausti, S. Lavorel, J. H. Lawton, D. M. Lodge, M. Loreau, S. Naeem, B. Schmid, H. Setälä, A. J. Symstad, J. Vandermeer, and D. A. Wardle 2005. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecological Monographs,* 75, 3–35.

Hoosbeek M. R., Lukac M., van Dam D., Godbold D. L., Velthorst E. J., Biondi F. A., Peressotti A., Cotrufo M. F., de Angelis P., & Scarascia-Mugnozza G. (2004). More new carbon in the mineral soil of a poplar plantation under Free Air Carbon Enrichment (POPFACE): Cause of increased priming effect? *Global Biogeochemical Cycles*, 18(1).

Huisingh, D., Zhang, Z., Moore, J., Qiao, Q., & Li, Q. (2015). Recent advances in carbon emissions reduction: policies, technologies, monitoring, assessment and modeling. *Journal of Cleaner Production,* 103(15), 1–12.

Keuskamp, J. A., Dingemans, B. J., Lehtinen, T., Sarneel, J. M., & Hefting, M. M. (2013). Tea Bag Index: a novel approach to collect uniform decomposition data across ecosystems. *Methods in Ecology and Evolution*, 4(11), 1070–1075.

Kleber, M., P. S. Nico, A. Plante, T. Filley, M. Kramer, C. Swanston, & P. Sollins 2011. Old and stable soil organic matter is not necessarily chemically recalcitrant: implications for modeling concepts and temperature sensitivity. *Global Change Biology*, 17, 1097–1107.

Lambers, H., C. Mougel, B. Jaillard, & P. Hinsinger (2009). Plant-microbe-soil interactions in the rhizosphere: an evolutionary perspective. *Plant and Soil,* 321, 83–115.

Lange, M., Eisenhauer, N., Sierra, C. A., Bessler, H., Engels, C., Griffiths, R. I., Mellado-Vázquez, P. G., Malik, A. M., Roy, J., Scheu, S., Steinbeiss, S., Thomson, B. C., Trumbore, S. E., & Gleixner, G. (2015). Plant diversity increases soil microbial activity and soil carbon storage. *Nature Communications*, 6(6707), 1–8.

Leff, J.W., S. E. Jones, S. M. Prober, A. Barberán, E.T. Borer, J.L. Firn, W.S. Harpole, S.E. Hobbie, K.S. Hofmockel, & J.M. Knops (2015). Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe. *Proceedings of the National Academy of Sciences*, 112, 10967–10972.

Leroux, S. J., Hawlena, D., & Schmitz, O. J. (2012). Predation risk, stoichiometric plasticity and ecosystem elemental cycling. *Proceedings of the Royal Society: Biological Sciences*, 279(1745), 4183–4191.

Rappaport, D. I., Morton, D. C., Longo, M., Keller, M., Dubayah, R., & Dos-Santos, M. N. (2018). Quantifying long-term changes in carbon stocks and forest structure from Amazon forest degradation. *Environmental Research Letters*, 13(6).

Riggs, C. E., S. E. Hobbie, E. M. Bach, K. S. Hofmockel, & C. E. Kazanski. 2015. Nitrogen addition changes grassland soil organic matter decomposition. *Biogeochemistry,* 125, 203–219.

Su, R., Kuehn, K. A., & Phipps, S. W. (2015), Fungal contributions to carbon flow and nutrient cycling during decomposition of standing Typha domingensis leaves in a subtropical freshwater marsh. *Freshwater Biology*, 60, 2100–2112.

Van Der Heijden, M.G., R.D. Bardgett, & N.M. Van Straalen (2008). The unseen majority: Soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters*, 11, 296–310.

Vitousek, P. M., S. Porder, B. Z. Houlton, & O. A. Chadwick. 2010. Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen–phosphorus interactions. *Ecological Applications,* 20, 5–15.