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Cover Page Footnote

Yang Kai Tang is a May 2020 graduate with an Environmental, Soil, and Water Science major in the Department of Crop, Soil, and Environmental Sciences. Mary C. Savin, faculty mentor, professor of microbial ecology, Department of Crop, Soil, and Environmental Sciences. Dirk Philipp, associate professor, Department of Animal Science. Ken Coffey, professor, Department of Animal Science. Jiangchao Zhao, associate professor, Department of Animal Science.

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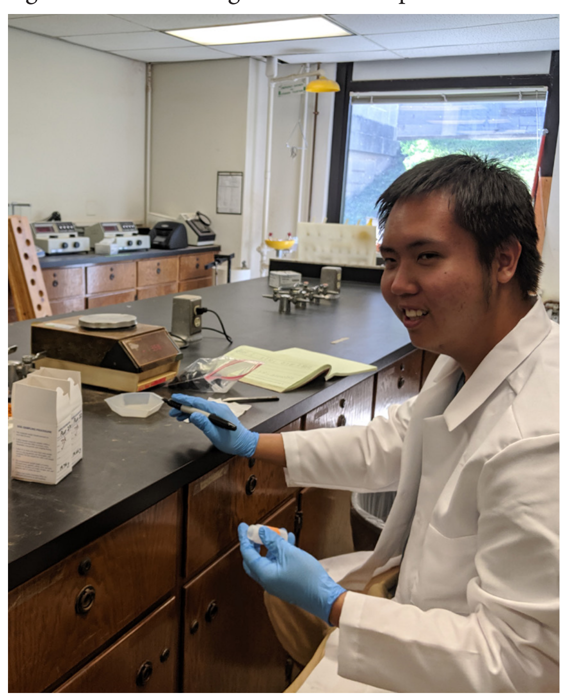
Meet the Student-Author

Yang Kai Tang

My country of origin, Malaysia, is situated near the equator where the Malaysian peninsular is protected from storms and tsunamis from the Indian Ocean by the Indonesian archipelago. The predictable tropical climate also allows for tropical rainforests and mangrove swamps to flourish. Malaysia's biodiversity of tropical rainforests is unparalleled, with wetlands being the only other biome that can compare in terms of biodiversity. Before attending the University of Arkansas, I had always thought of the soil as only being the ground that supports our weight and as a growth medium for plants and trees. Through specific classes that I have taken at the University of Arkansas, I learned how important soil is to sustain and support life on Earth, as food and water are essential to human survival. The professors at the University of Arkansas taught me the interconnectedness of soil with water quality, greenhouse gas emissions, and human survivability. These experiences, coupled with the opportunity to start an undergraduate research project under Dr. Mary Savin, allowed me to learn more about being an environmental and soil scientist and gain hands-on research experience. I would like to thank Dr. Mary Savin, my mentor, for guiding me throughout this research project, and Dr. Dirk Philipp for his help and support. I would also like to thank Samuel Park and Mason Downing for assisting me with collecting data for the experiment.



Yang in the soil lab of the Crop, Soil, and Environmental Sciences department preparing soil samples for pH and electrical conductivity analysis.

Research at a Glance

- Stabilization of organic matter helps build organic matter and contributes to healthy soil.
- The Tea Bag Index provides an easy and cost-effective method for evaluating the rate of decomposition and stabilization to compare treatments.
- Addition of a legume-containing polyphenolic compound into ruminant diets did not appear to have an impact on the decomposition or stabilization of organic matter in soil.

Decomposition in pasture soil receiving excreta from ruminants fed alfalfa forage diet supplemented with increasing proportions of Sericea Lespedeza legume

Yang Kai Tang, Mary C. Savin,† Dirk Philipp,§ Ken Coffey,‡ and Jiangchao Zhao¶*

Abstract

Healthy soil is fundamental to a productive pasture system as it will decompose labile organic matter and promote retention of carbon to build a stable, resistant pool of organic matter. An easy, standardized approach to measure decomposition and litter stabilization that is gaining popularity in both citizen science and research studies is the use of the Tea Bag Index. The Tea Bag Index is a relatively new method evaluating the loss of organic material in two different kinds of commercial tea bags (green tea and Rooibos tea) after burial in the soil for 90 days. The objective of this experiment was to use the Tea Bag Index to determine if decomposition rate and litter stabilization were affected by inputs of excreta from ruminants fed alfalfa forage diets modified with 0%, 9%, 18%, or 27% of the tannin-containing legume sericea lespedeza, urea, or an untreated negative control in soil plots (n = 4). There was no difference in decomposition rate or litter stabilization among any treatments measured in the 8 cm of surface soil during the first spring growing season after treatment application of excreta or urea to the soil. Results of this experiment indicated that animal amendments simulating urine and manure patches did not result in detectable changes in organic matter decomposition during the first spring season after application to silt loam pasture soil growing tall fescue grass in the mid-South.

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† Mary C. Savin, the faculty mentor, is a professor of microbial ecology in the Department of Crop, Soil, and Environmental Sciences.
§ Dirk Philipp is an associate professor in the Department of Animal Science.
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Introduction

Organic matter comprises a variety of compounds of unspecific chemical composition that are made up of the partial breakdown products of plant and animal materials that are re-synthesized into new compounds of varying stability (Brady and Weil, 2017). Decomposition is carried out primarily by the biological community. Partial decomposition can be followed by humus synthesis to stabilize and accumulate organic matter contributing to soil's ability to sequester carbon. Carbon sequestration is a viable and cost-effective option to reduce the impacts of rising levels of greenhouse gas emissions on the global climate (Minasny et al., 2017). Organic matter is essential for healthy soil as organic matter provides many benefits to the soil. Organic matter reduces bulk density by providing increased porosity, stores water, increases the cation exchange capacity, serves as a nutrient reservoir, improves soil structure, and acts as a carbon sink.

Decomposition is an integral part of the carbon cycle. The Tea Bag Index was developed as a cost-effective, simple-to-execute, practical, and standardized method of measuring decomposition rate and litter stabilization in soil (Keuskamp et al., 2013). The Tea Bag Index uses green (*Camellia sinensis* [Sweet.] Robert) and Rooibos (*Aspalathus linearis* [Burm.f.] R. Dahlgren) teas that differ in composition and thus decomposition rate in order to measure decomposition rate and a stabilization factor and to function as an indicator for litter decomposition in soil. Green tea decomposes rapidly and can be used to calculate stabilization as it represents the simpler organic compounds of the two teas (Keuskamp et al., 2013). Rooibos tea has a slower decomposition rate and will continue decomposing over the length of the proposed study and can be used to determine the decomposition rate of organic matter due to the increased complex organic compound content that will be humified (90 days, Keuskamp et al., 2013). The Tea Bag Index method is used as a substitute for litterbag studies. The commercially sold products consist of tea leaves wrapped in a porous bag with a mesh size of 0.25 mm that is made out of non-degradable material (Keuskamp et al., 2013). The mesh size allows microorganisms, microfauna, and small mesofauna but prevents macrofauna from entering the teabag (Setälä et al., 1996). Tea leaves are a good substitute for plant litter because the sources for much of the organic inputs into the soil are from plants.

The objective of this research project was to determine if inputs of urine or manure from sheep fed diets of alfalfa forage containing 0%, 9%, 18%, or 27% polyphenolic compound containing legume sericea lespedeza [*Lespedeza cuneata* (Dum. Cours.) G.] changed decomposition rate and organic matter stabilization in pasture soil growing tall fescue (*Lolium arundinaceum* [Schreb.] Darbysh). The decomposition rate was hypoth-esized to increase in the treatments receiving sheep urine and manure compared to the negative control. The treatments receiving manure from sheep fed with a diet containing increasing concentration of the tannin-containing legume were expected to decompose at a slower rate with greater litter stabilization compared to soil with manure input from sheep fed with alfalfa or compared to the urea positive control. The tannin and other polyphenolic compounds in the alternative legume diet were anticipated to result in greater retention of nitrogen in the animal and in organic compounds in manure rather than being in urine, thus slowing decomposition rates and facilitating retention (i.e., stabilization) into soil organic matter.

Materials and Methods

Treatments were applied to the soil surface of 40 1-m^2 pasture plots growing tall fescue on 6 November 2018. The 10 treatments were applied in a randomized block design with each treatment replicated 4 times. Treatments consisted of either urine or manure from sheep fed one of four diets: 100% alfalfa silage, or alfalfa with 9%, 18%, or 27% sericea lespedeza on a dry matter basis. Urea fertilizer was the positive control, and the negative control was untreated. Amendments applied were equivalent to 60 g N/m^2 for urea and urine treatments. The soil application rates for the respective 0%, 9%, 18%, and 27% sericea lespedeza percentages in the diet were equivalent to 50.7, 40.8, 44.7 g, or 45.4 g N/m^2, respectively, for the four manure treatments.

Litter decomposition rate and stabilization factor were calculated based on loss of mass in green and Roobios tea bags over 90 days following the protocol for the Tea Bag Index from Keuskamp et al. (2013). Four pairs of tea bags per plot were buried on 12 April 2019 to decompose for 90 days. In this experiment, decomposition was measured from April to July 2019, 5 to 8 months following the application of animal excreta or urea to the soil surface.

A soil sample was collected at the end of the study from the top 5 cm of each plot using a 6-cm diameter bulk density core, dried at 55 °C for 7 days, and used to determine bulk density. A pH and electrical conductivity (EC) probe and calibrated meters were used to measure pH and EC on 1:2 soil water (wt:vol) ratio subsamples from the bulk density soils. Soil organic matter (OM) was determined by loss-on- loss-on-ignition after grinding a subsample with a pestle and mortar, sieving through a mesh with a pore size of 2 mm, and further drying at 105 °C for 24 hours before combusting in a furnace at 450 °C for 4 hours.

Averages and standard errors for pH, EC, OM content, stabilization factor, and decomposition rate were calculated per treatment. Treatment effects were analyzed by analysis of variance (ANOVA) or Kruskal-Walis ANOVA on Ranks if data were not normally distributed. Significance was evaluated at an alpha value of 0.05.

Results and Discussion

Decomposition rate (k d^{-1}) was not different among treatments (*P* = 0.23, Fig. 1). The average decomposition rate at the study site was 0.018 d^{-1}, which was the same decomposition rate measured from 5 February to 18 April 2019 in a green roof in Fayetteville, Arkansas using the same method (LaSalle, 2019). Another tea bag index study conducted on cattle grazed grasslands in the Netherlands measured decomposition rates of 0.0158 and 0.0165 d^{-1}, which were similar to the average rate of this study (Iepema et al., 2015). The stabilization fractions of the 10 treatments were also not statistically different (*P* = 0.32, Fig. 2).

Although the ANOVA results indicated that there was a significant difference, soil pH was not different between any treatments after a Tukey's honestly significant difference test (Table 1). Microbial decomposers break down organic material and produce enzymes important in nutrient cycle processes. Soil pH is an important factor in microbial activity as all microbes have an optimum pH range at which they are most active. The ratio of bacteria-to-fungi may become unbalanced if soil pH either decreases or increases outside of optimal ranges. Bacteria tend to favor neutral soil environments, while fungi prefer more acidic soil environments. However, the treatments did not have an impact on decomposition as rate and stabilization factors were not different between treatments.

Organic matter (*P* = 0.38; Table 1), total nitrogen (*P* = 0.41; data not shown) and total carbon (*P* = 0.2; data not shown), EC (*P* = 0.56; Table 1), and bulk density (*P* = 0.46; Table 1) also were not significantly different between treatments. Inputs of OM will provide soil microbes with a fresh supply of organic carbon. Greater amounts of simple organic compounds will decompose rapidly as microbial enzymes act quickly and contribute to increased decomposition rates of OM. However, decomposition and stabilization were not affected by total OM. In future studies, particulate OM should be measured as it represents the labile fraction of total OM and may assist in determining a relationship with decomposition and stabilization rate. The carbon-to-nitrogen (C:N) ratio is another important factor in the decomposition rate because nitrogen is required by microbes to synthesize proteins and grow, and carbon serves as a food source. Although the sheep excreta and urea amendments served as a source

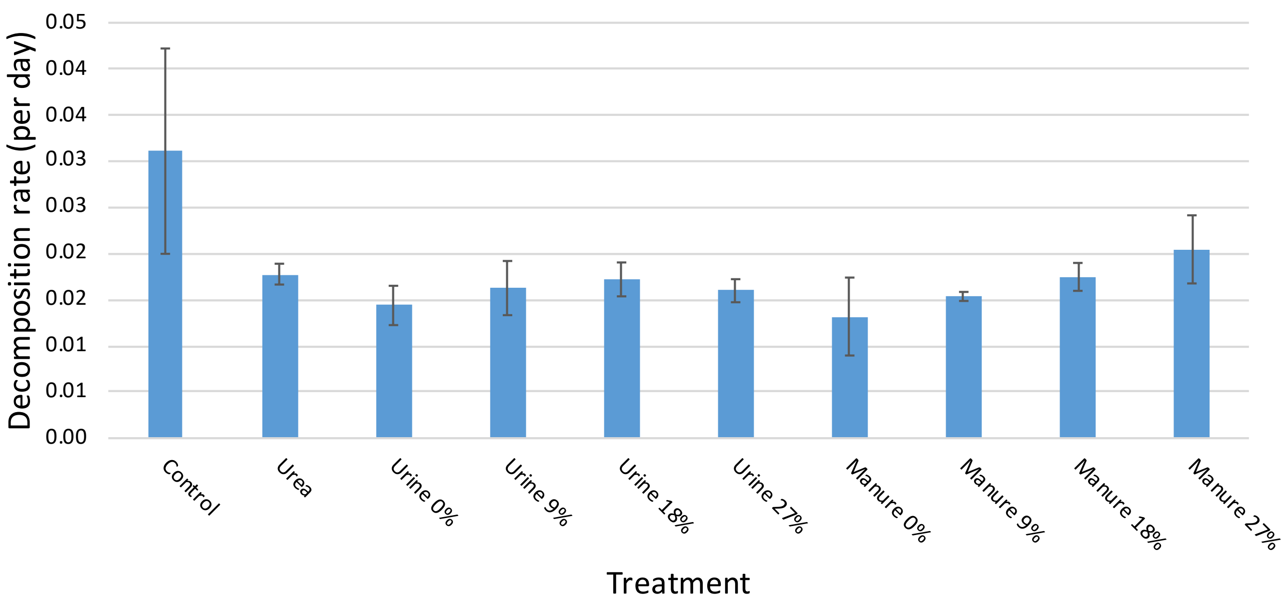

Fig. 1. Decomposition rate in soil pasture plots growing tall fescue five to eight months following application of urine or manure treatments from sheep fed a modified alfalfa-based diet or following application of a control (n = 4). The negative control was unamended, and urea was the positive control. Urine was from sheep fed alfalfa supplemented with 0%, 9%, 18%, and 27% sericea lespedeza, and manure was from sheep fed alfalfa supplemented with 0%, 9%, 18%, and 27% sericea lespedeza. The % behind the treatments represents the content of polyphenolic compounds in sheep diet.

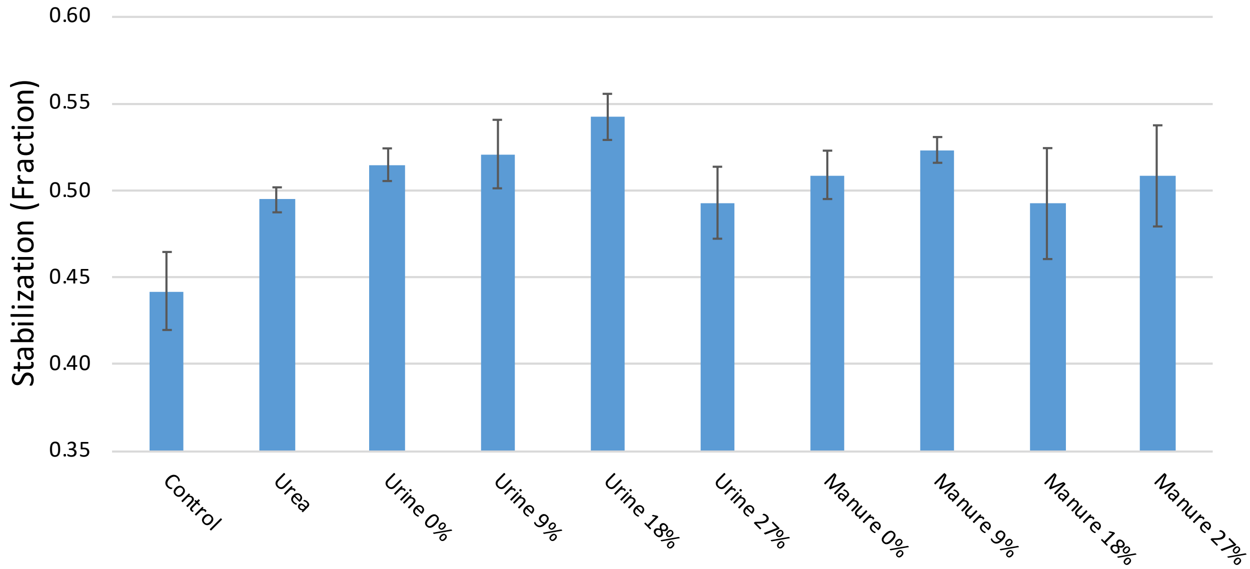

Fig. 2. Stabilization fraction in soil pasture plots growing tall fescue five to eight months following application of urine or manure treatments from sheep fed a modified alfalfa-based diet or following application of a control (n = 4). The negative control was unamended, and urea was the positive control. Urine was from sheep fed alfalfa supplemented with 0%, 9%, 18%, and 27% sericea lespedeza, and manure was from sheep fed alfalfa supplemented with 0%, 9%, 18%, and 27% sericea lespedeza. The % behind the treatments represents the content of polyphenolic compounds in sheep diet.

Table 1. Average (standard error) electrical conductivity, soil pH, total organic matter, and bulk density from soil in tall fescue pasture plots following application of urine or manure from sheep fed a modified alfalfa-based diet or in a control (n = 4).

Treatment	Electrical conductivity (μS cm^{-1})	Soil pH	Organic matter (%)	Bulk density (g/cm^3)
Negative Control[a]	233.0 (69.5)	5.5 (0.2)	3.73 (0.15)	1.62 (0.04)
Urea	218.1 (33.7)	6.1 (0.2)	4.00 (0.31)	1.54 (0.04)
Urine, 0%[b]	192.2 (19.6)	6.1 (0.0)	4.23 (0.19)	1.51 (0.06)
Urine, 9%	186.7 (26.7)	5.9 (0.1)	3.95 (0.39)	1.53 (0.04)
Urine, 18%	279.5 (44.8)	6.3 (0.2)	3.68 (0.14)	1.61 (0.07)
Urine, 27%	157.9 (30.6)	5.3 (0.1)	3.75 (0.34)	1.61 (0.08)
Manure, 0%	228.7 (26.4)	6.3 (0.2)	4.00 (0.29)	1.52 (0.04)
Manure, 9%	223.9 (47.8)	6.3 (0.2)	4.00 (0.33)	1.63 (0.09)
Manure, 18%	191.2 (11.5)	6.3 (0.3)	4.03 (0.40)	1.48 (0.04)
Manure, 27%	182.4 (22.2)	6.0 (0.3)	4.75 (0.24)	1.47 (0.09)

[a] The negative control was unamended, urea was the positive control, urine was from sheep fed alfalfa supplemented with 0%, 9%, 18%, and 27% *sericea lespedeza*, and manure was from sheep fed alfalfa supplemented with 0%, 9%, 18%, and 27% *sericea lespedeza*.

[b] The % behind the treatments represents the content of polyphenolic compounds in sheep diet.

of nitrogen, decomposition rate, and stabilization of the teas were not affected. This could be due to the rapid usage of nitrogen immediately after the amendments were added to the soil, whereas this study was conducted five to eight months after the addition of excreta and urea. Thus, it is possible that the timing of measurements did not capture alterations in the decomposition and changes in the soil properties. Alternatively, the use of tea bags may not have captured the impact inputs had on soil properties and processes.

Electrical conductivity provides a general measurement of salinity that relates to nutrient availability in soil. Salts are important for biochemicals and processes; however, salinity that is too high can hinder activity, which then affects OM decomposition rate and stabilization (NRCS, 2014). Although EC did not affect OM decomposition rate and stabilization, in future studies, further analysis of specific ion contents in the soil may reveal more definitive relationships with OM decomposition rate and stabilization, since EC provides only a general overview of salinity in soil. Soil bulk density in the surface 5 cm of the study site was slightly greater than that of a "typical" bulk density of 1.33 g/cm^3 in a medium-textured soil with 50% pore space, but this was not unexpected given that the plots were in a pasture impacted previously by cattle grazing and machinery (NRCS, 2008).

Conclusions

There were no significant differences in decomposition rate and stabilization of teabag litter buried in the soil of pasture plots receiving excreta of sheep fed diets modified to increase N retention in the animal and alter N decomposition and forms present in the urine and manure. If the diets had an effect on ruminant digestion and excretion of compounds, this did not impact decomposition rate or litter stabilization in the soil five to eight months after receiving input of excreta. These results indicate that diet modification incorporating sericea lespedeza may not alter soil carbon cycling in the first spring season after application to the silt loam soil surface growing tall fescue. Other soil processes and properties related to soil quality, such as N retention within the soil profile, may deserve further investigation to determine the impacts of excreta inputs from the animals fed diets modified with tannin-containing compounds.

Acknowledgments

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