Using the Tea Bag Index to unravel how interactions between an antibiotic (Trimethoprim) and endocrine disruptor (17a-estradiol) affect aquatic microbial activity.

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Abstract

The constant release of complex mixture of pharmaceuticals, including antimicrobials and endocrine disruptors, into the aquatic environment. These have the potential to affect aquatic microbial metabolism and alter biogeochemical cycling of carbon and nutrients. Here we advance the Tea Bag Index (TBI) for decomposition by using it in a series of contaminant exposure experiments to test how interactions between an antibiotic (trimethoprim) and endocrine disruptor (17a-estradiol) affects microbial activity in an aquatic system. The TBI is a citizen science tool used to test microbial activity by measuring the differential degradation of green and rooibos tea as proxies for labile and recalcitrant organic matter decomposition. Exposure to trimethoprim and 17a-estradiol had significant independent negative effects upon decomposition of labile organic matter (green tea), suggesting additive effects upon microbial activity. Exposure to 17a-estradiol alone negatively affected the degradation of more recalcitrant organic matter (rooibos tea). Consequently, trimethoprim and 17a-estradiol stabilized labile organic matter against microbial degradation and restricted degradation rates. We propose that the method outlined could provide a powerful tool for testing the impacts of multiple interacting pollutants upon microbial activity, at a range of scales, across aquatic systems and over biogeochemically relevant time scales.

Main Text

Globally, pharmaceutical use has increased by ~ 3 % per annually since the year 2000 leading to a constant discharge of these compounds into the aquatic environment via both point (Waste Water Treatment Works and Septic Tanks) and diffuse sources (e.g. agricultural run-off) (Rosi-Marshall & Royer, 2012; Van Boeckel et al, 2014; Gros et al, 2007). Although this results in low concentrations of those elements, they could affect natural stream processes because pharmaceuticals are designed to be effective at micromolar or nanomolar concentrations. Pharmaceuticals have the potential to affect the microbial processes which control aquatic carbon and nitrogen cycling (Brodin et al, 2014; Rosi-Marshall & Royer, 2012). The specific effects of pharmaceuticals in the environment are likely to be complex as a consequence of the myriad of potentially interactions between these compounds (Hernando et al, 2006). As such, we need to understand how pharmaceuticals influence microrganism-mediated ecosystem processes.

Contaminant exposure experiment provides a powerful tool for testing the sensitivity of aquatic microbial communities to pharmaceuticals and other pollutants (Tank et al, 2006; Costello et al, 2015). A well-refined method is provided by Costello et al (2015) to test how pharmaceuticals affect microbial biofilm growth and community structure and ecophsysiological responses of the biofilm, such as community respiration (Rosi-Marshall et al, 2013; McClean & Hunter, 2019). However, this method cannot provide information on biogeochemical processes such as the degradation organic matter, which occurs over times-scales measured in weeks or months (Vannote et al, 1980; Raymond & Bauer, 2001). The Tea Bag Index (TBI) provides a powerful low-cost tool for investigating microbial activity in soils and aquatic systems, based upon the traditional use of leaf-litter bags in ecology (Keuskamp et al, 2013; Seelen et al, 2019). The key strength of TBI is its ability to provide a standardized method of quantifying microbial activity by comparing the relative degradation of a labile (green tea) and recalcitrant (rooibos tea) organic matter source. The use of TBI within contaminant exposure experiments will, therefore, allow the impacts of exposure to a pollutant to be quantified in terms of microbially-mediated organic matter degradation.

Antibiotics and endocrine disruptors represent some of the most widely detected pharmaceuticals in the environment (Álvarez-Muñoz et al, 2015; Archer et al, 2017; Rosi-Marshall & Royer, 2012), with both known to have significant effects upon the structure of aquatic microbial communities (Wieser et al 2016;Yuan et al 2017). Consequently, interactions between antibiotics and endocrine disruptors are of potential environmental relevance. We tested interactions between a broad-spectrum antibiotic (Trimethoprim) and the endocrine disruptor (17a-estradiol) affect in-stream microbial activity, using a modified contaminant exposure experiments (see supplementary methods). Briefly, we constructed contaminant exposure experiments out of 120 ml screw-cap vials with a 3.5 cm diameter hole bored into the lid. We prepared 40 vials containing 100 ml of 2 % agar gel of which ten where spiked with either a 688 $\mu mol.l^{-1}$ dose of Trimethoprim, or 688 $\mu mol.l^{-1}$ dose of 17a-estradiol. Ten others were spiked with a 688 $\mu mol.l^{-1}$ dose of both Trimethoprim and 17a-estradiol, and ten controls containing no pharmaceuticals, only agar. We placed one pre-weighed Lipton Green Teabag (EAN 87 22700 05552 5) and one pre-weighed Lipton Rooibos Teabag (EAN 87 22700 18843 8) in non-woven bags in the headspace of each vial. The experiments were placed into a suburban stream and incubated for 83 days between March and June 2019. We quantified mass loss of the green and rooibos tea bags after drying the bags (72 h at 65°C), and calculated the stabilization factor (S) and initial decomposition rate (k) of the labile organic material (following Keuskamp et al, 2013). We tested for significant treatment effects using two-way analysis of variance.

Over the course of the experiment, dissolution of the agar delivered estimated daily doses of approximately 275 $nmol\ .d^{-1}$ of trimethoprim and 17a-estradiol in both the single and combined pharmaceutical treatments (see supplementary methods). Rooibos Tea mass loss decreased upon 17a-estradiol exposure, with no additional/significant effect of Trimethoprim (Figure 1 A). By contrast for green tea, we observed additive and inhibiting effects of both trimethoprim and 17a-estradiol (Figure 1 B). Based on these data we can demonstrate that pharmaceutical pollution by 17a-estradiol and trimethoprim increased both stabilization factor (Figure 2 A), initial decomposition rate (Figure 2 B) of the labile organic matter within the teabags. Although, the difference in stabilization between treatments with and without 17a-estradiol were not as big as when trimethoprim was present, the interaction was not

94 significant (see supplementary results). This indicates significant independent effects
95 of trimethoprim and 17a-estradiol upon microbial activity.

96 Our results demonstrate that chronic exposure to low doses of both an antibiotic
97 (trimethoprim) and an endocrine disruptor (17a-estradiol) inhibits microbial
98 degradation, with differential effects on different phases of the decomposition
99 process. Chronic exposure to both an antibiotic and endocrine disruptor have
100 significant additive positive effects upon both the initial decompostiion rate (k) and
101 stabilization factor (s) of labile organic matter. The latter may increase the residence
102 time for organic matter in aquatic systems. Inland waters (rivers, lakes and streams)
103 typically receive large inputs of terrestrial organic matter, which is then partially
104 metabolized, temporarily buried within the sediment or transported to the ocean
105 (Battin et al, 2009). Thus, the combined effects of multiple pharmaceutical
106 contaminants are likely to have important implications for the global carbon and
107 nutrient cycles.

108 By integrating TBI into contaminant exposure experiments we have developed a low-
109 cost tool to quantify how chronic pollutant exposure affects instream decomposition
110 over biogeochemically relevant time-scales. Whilst our study was restricted to one
111 stream, this method can easily be replicated at multiple sites. We therefore foresee
112 two important applications as it would allow sensitivity analysis of microbial
113 responses to multiple pollutants to be made (i) across large spatial and temporal
114 scales and (ii) in increasingly complex experimental designs testing the interactions
115 between multiple chemical pollutants on decomposition in both freshwater and
116 marine systems. [992 words]

117 **Author Contributions**

118 Experiments were designed by WRH following discussions with JS, and were
119 conducted by WRH and AW. All authors contributed to the writing of the manuscript.

Acknowledgements

This work was funded through start-up funds provided to WRH by the University of Ulster School of Geography and Environmental Science. JMS acknowledges the Swedish Research Council (Vetenskapsrådet) for funding.

References

Álvarez-Muñoz D, Rodríguez-Mozaz S, Maulvault AL, Tediosi A, Fernández-Tejedor M, Van den Heuvel F*, et al.* (2015). Occurrence of pharmaceuticals and endocrine disrupting compounds in macroalgaes, bivalves, and fish from coastal areas in Europe. Environmental Research 143:56-64.

Archer E, Petrie B, Kasprzyk-Hordern B, Wolfaardt GM (2017). The fate of pharmaceuticals and personal care products (PPCPs), endocrine disrupting contaminants (EDCs), metabolites and illicit drugs in a WWTW and environmental waters. Chemosphere 174:437-446.

Battin TJ, Luyssaert S, Kaplan LA, Aufdenkampe AK, Richter A, Tranvik LJ (2009). The boundless carbon cycle. Nature Geoscience 2:598.

Brodin T, Piovano S, Fick J, Klaminder J, Heynen M, Jonsson M (2014). Ecological effects of pharmaceuticals in aquatic systems: impacts through behavioural alterations. Philosophical Transactions of the Royal Society of London B: Biological Sciences 369:20130580.

Costello DM, Rosi-Marshall EJ, Shaw LE, Grace M, Kelly JJ (2015). A novel method to assess effects of chemical stressors on natural biofilm structure and function. Freshwater Biology 61:2129-2140.

Gros M, Petrović M, Barcelo D (2007). Wastewater treatment plants as a pathway for aquatic contamination by pharmaceuticals in the Ebro river basin (northeast Spain). Environmental Toxicology and Chemistry 26:1553-1562.

Hernando MD, Mezcua M, Fernández-Alba AR, Barceló D (2006). Environmental risk assessment of pharmaceutical residues in wastewater effluents, surface waters and sediments. Talanta 69:334-342.

Keuskamp JA, Dingemans BJJ, Lehtinen T, Sarneel JM, Hefting MM (2013). Tea Bag Index: a novel approach to collect uniform decomposition data across ecosystems. Methods Ecol Evol 4:1070-1075.

McClean P, Hunter WR (2019). 17a-Estradiol limits the impact of ibuprofen upon community respiration by streambed biofilms in a sub-urban stream. bioRxiv :718924, doi:10.1101/718924.

Raymond PA, Bauer JE (2001). Riverine export of aged terrestrial organic matter to the North Atlantic Ocean. Nature 409:497-500.

Rosi-Marshall EJ, Kincaid DWL, Bechtold H, Royer TV, Rojas M, Kelly JJ (2013). Pharmaceuticals suppress algal growth and microbial respiration and alter bacterial communities in stream biofilms. Ecological Applications 23:583-593.

Rosi-Marshall EJ, Royer TV (2012). Pharmaceutical compounds and ecosystem function: an emerging research challenge for aquatic ecologists. Ecosystems. 15:867-880.

Seelen LMS, Flaim G, Keuskamp J, Teurlincx S, Arias Font R, Tolunay D*, et al.* (2019). An affordable and reliable assessment of aquatic decomposition: Tailoring the Tea Bag Index to surface waters. Water Research 151:31-43, doi:https://doi.org/10.1016/j.watres.2018.11.081.

Tank JL, Bernot MJ, Rosi-Marshall EJ (2006). Nitrogen limitation and uptake. In: Methods in Stream Ecology. Academic Press: San Diego, CA., pp 213-238.

Van Boeckel TP, Gandra S, Ashok A, Caudron Q, Grenfell BT, Levin SA*, et al.* (2014). Global antibiotic consumption 2000 to 2010: an analysis of national pharmaceutical sales data. Lancet Infectious Diseases 14:742-750.

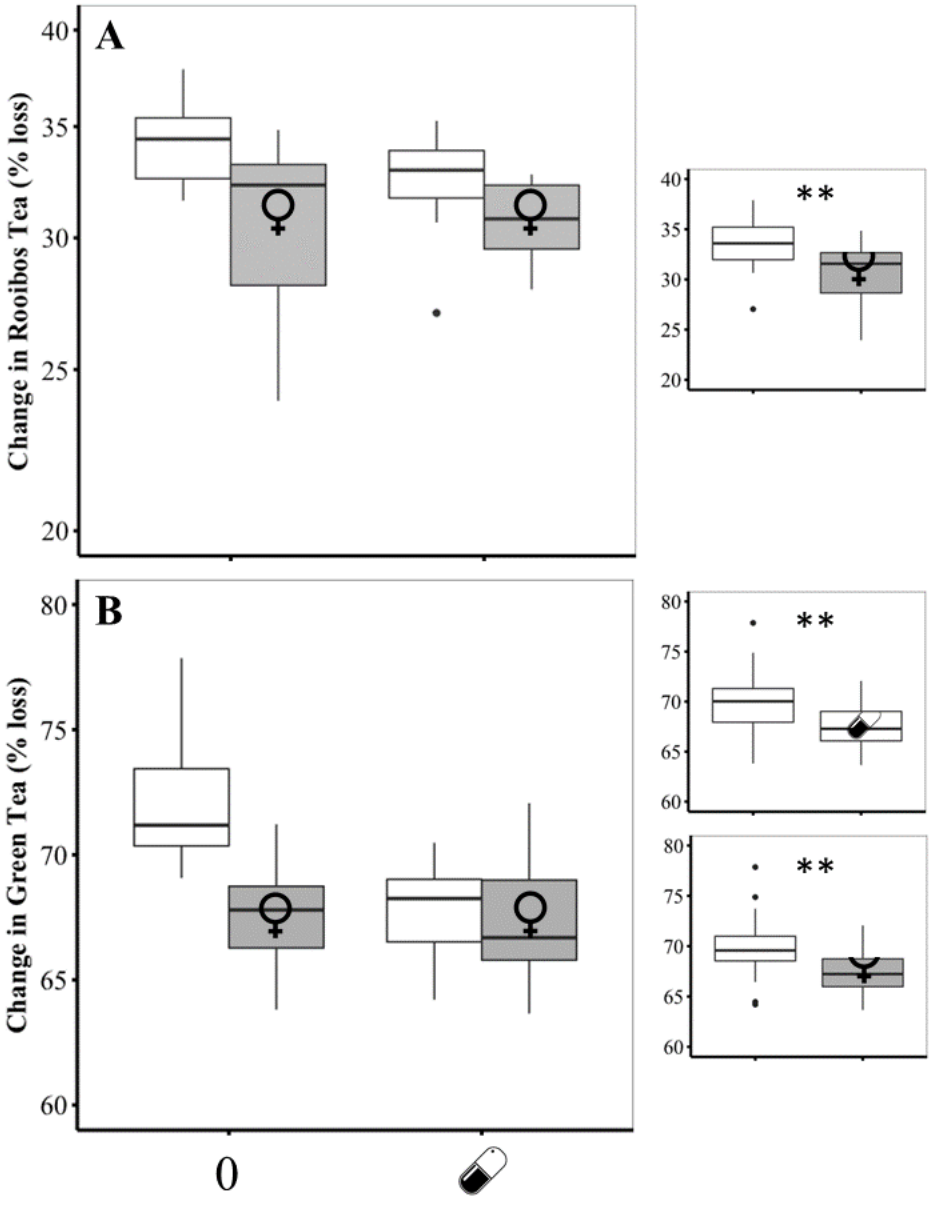
Vannote RL, Minshall GW, Cummins KW, Sedell JR, Cushing CE (1980). The River Continuum Concept. Can J Fish Aquat Sci 37:130-137.

Waiser MJ, Swerhone GD, Roy J, Tumber V, Lawrence JR (2016). Effects of erythromycin, trimethoprim and clindamycin on attached microbial communities from an effluent dominated prairie stream. Ecotoxicol Environ Saf 132: 31-39. doi: 10.1016/j.ecoenv.2016.05.026.

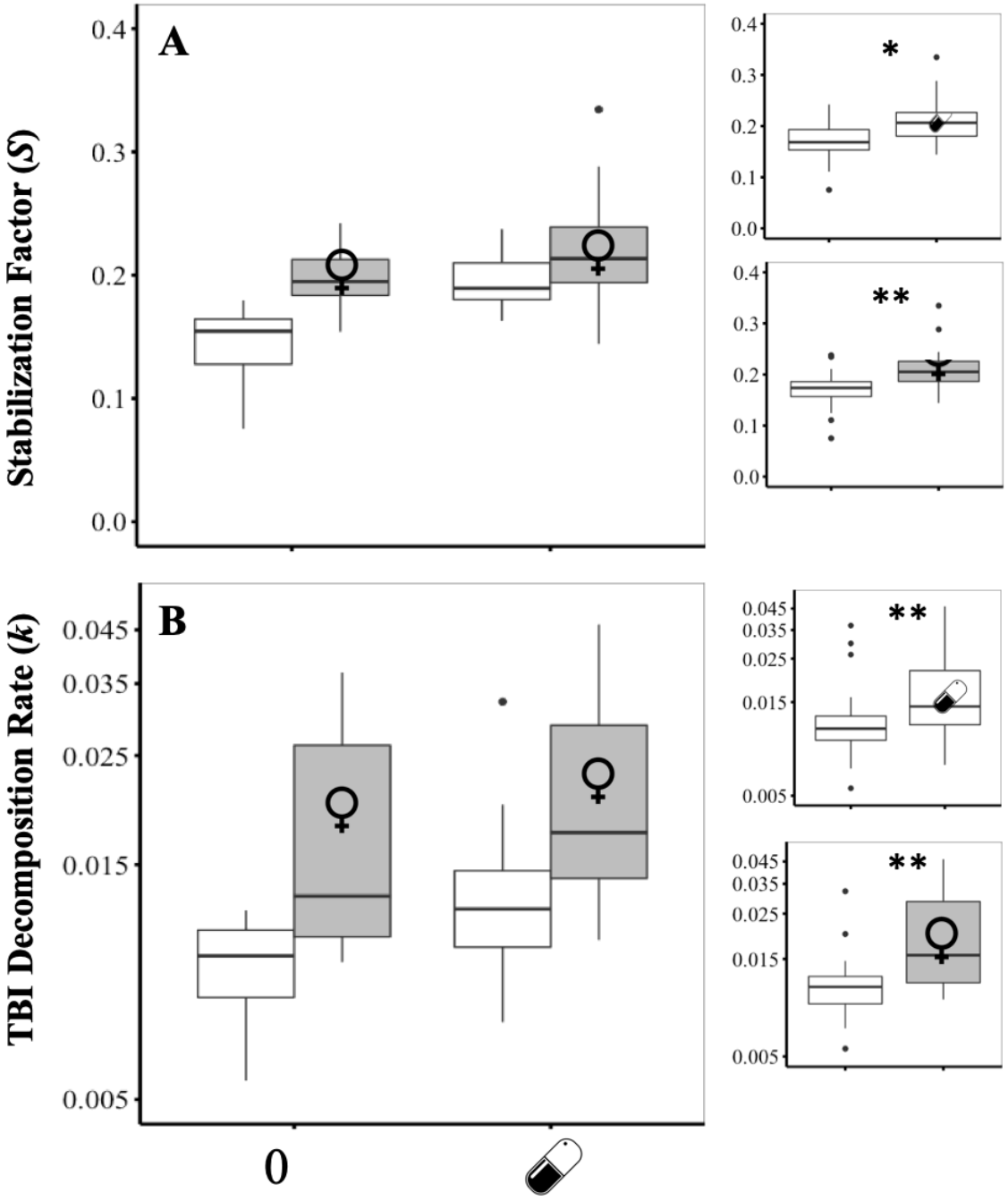
Yuan K, Xiao S, Jiang X, Yang L, Chen B, Luan T, Lin L, Tam NFY (2017). Effects of endocrine disrupting chemicals (EDCs) on bacterial communities in mangrove sediments. Mar Pollut Bull 122: 122-128

Figures

Figure 1. Effects of Trimethprim (💊) and 17a-estradiol (♀) on the degradation (% mass loss) of A) Green Tea and B) Rooibos Tea, as labile and refractory organic matter sources. Inserts show pooled data where significant independent effects of either Trimethoprim or 17a-estradiol where detected. Significance levels: *** p < 0.001; ** p < 0.01; * p < 0.05.



189 Figure 2. Effects of Trimethprim (💊) and 17a-estradiol(♀) on the A) labile organic
190 matter stabilization factor and B) organic matter degradation rate, calculated
191 following Keuskamp et al (2013). Inserts show pooled data where significant
192 independent effects of either Trimethoprim or 17a-estradiol where detected.
193 Significance levels: *** p < 0.001; ** p < 0.01; * p < 0.05.



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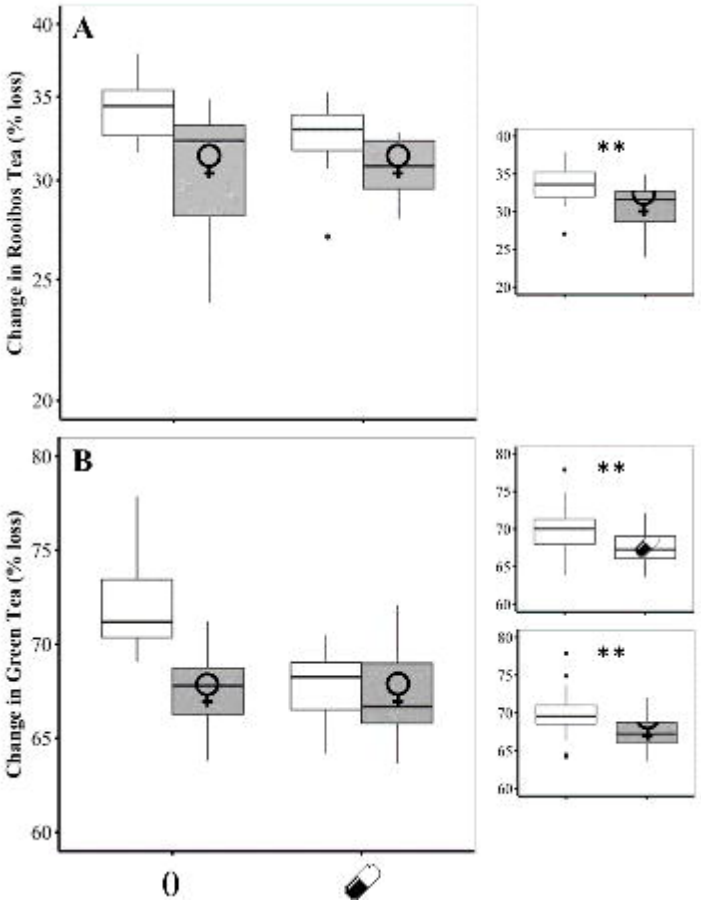
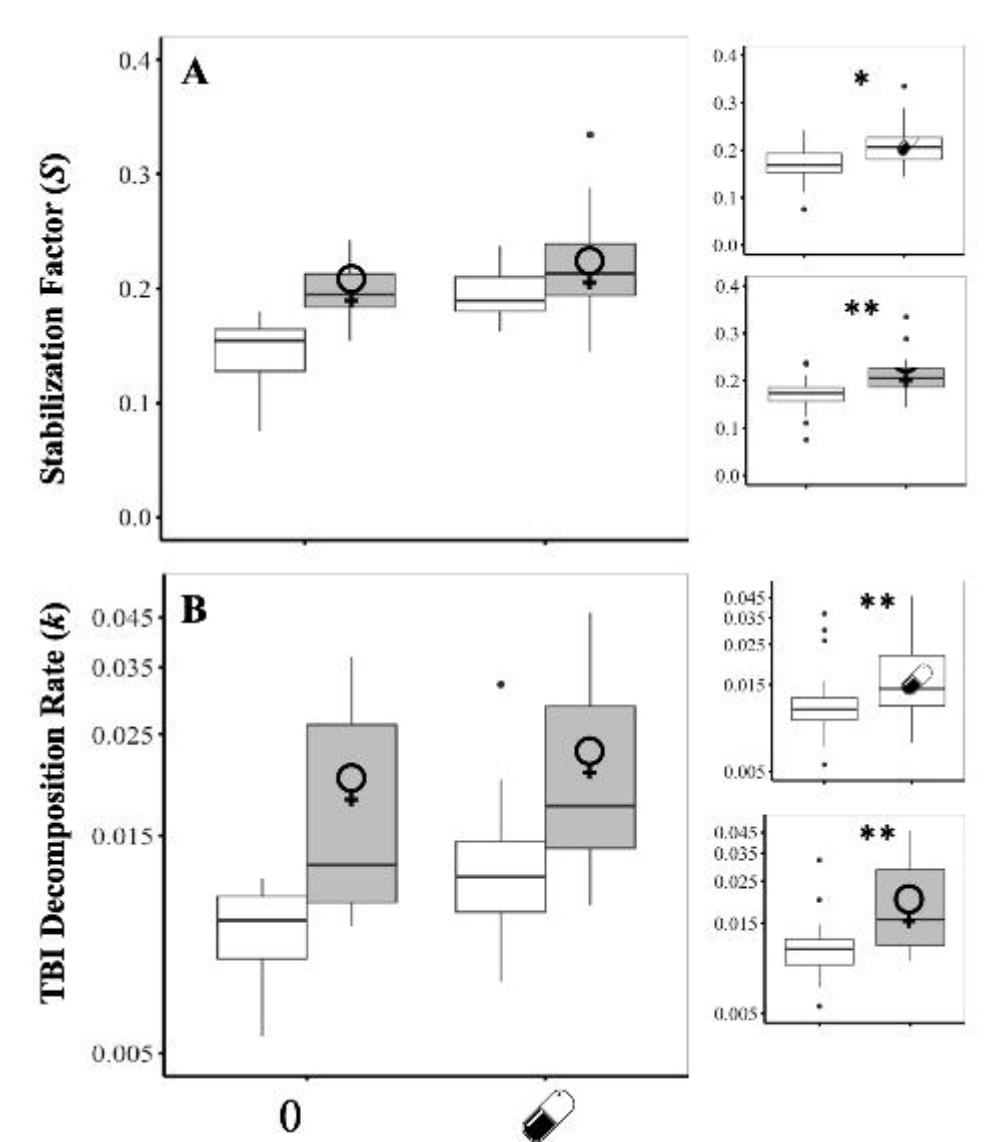

A
Change in Rooibos Tea (% loss)
40
35
30
25
20
**
B
Change in Green Tea (% loss)
80
75
70
65
60
**
**
0


A
Stabilization Factor (S)
0.4
0.3
0.2
0.1
0.0
*
**
B
TBI Decomposition Rate (k)
0.045
0.035
0.025
0.015
0.005
0