Supplementary Information

Summary: 18 pages, Full methods, 5 tables, 5 figures.

Climate effects on belowground tea litter decomposition depend on ecosystem and organic matter types in global wetlands

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Supplementary Methods

2.1. TeaComposition H_2O initiative and site metadata

This work was performed within TeaComposition H_2O, a global initiative to collect long-term decomposition data from wetlands and aquatic ecosystems using standardised litter methods. The initiative comprises 226 sites across eight macroclimates [1]. The standardised litters are Lipton© (Unilever) green and rooibos teas representing labile (high water-soluble compound content) and recalcitrant/stable (high fibre/lignin content) forms of organic matter (OM), respectively [2]. Generally, the definitions of labile and recalcitrant are context dependent and sit along a spectrum depending on substrate type and chemical characteristics, spatiotemporal frame of observation, and microbial characteristics [3]. For the purpose of this study in which initial chemical characteristics are well-known [2,4], we will refer to the tea litter OM in terms of its inherent chemical characteristics [5], that is labile and recalcitrant OM for green and rooibos teas, respectively. The tea litter bags were the same batch from Lipton© as the terrestrial-focussed TeaComposition initiative, packaged in the original nylon mesh [6,7]. Since fine nylon mesh sizes used in litter bag-based decay studies like ours will exclude larger fauna, it is likely that decomposition may be underestimated if faunal grazers are common to the study area [8].

Since wetlands are globally diverse ecosystems, we used the Ramsar Convention wetland definition that is inclusive of freshwater/aquatic and coastal/marine marshes, peatlands and waters that do not exceed six metres depth at low tide [9]. Basic site information was collected for each site to help characterise features: latitude, longitude, ecosystem type, inundation type, macroclimate, and local monthly air temperature and precipitation. Ecosystem type included both coastal/saline and inland/freshwater ecosystems (Figure 1, Table S1). Coastal/marine ecosystems included macroalgal stands, seagrass meadows, mangrove forests, tidal marshes and supratidal forests. Inland ecosystems included freshwater wetlands (e.g.

peatlands, marshes), lotic ecosystems (e.g. creek, stream) and lentic ecosystems (e.g. shallow ponds, lakes). Inundation was roughly classified by the local site leaders as subtidal and intertidal for coastal/marine ecosystems and permanently or seasonally/intermittently inundated for inland ecosystems. The 'freshwater wetland' category comprised a diversity of wetland types, so we further categorised using the IUCN Ecosystem Typology 2.0 for additional statistical analysis (Table S1) [10]. The typologies incorporate both inundation regime and climate characteristics: boreal and temperate fens; boreal, temperate and montane peat bogs; permanent marshes; seasonal floodplain marshes; subtropical/temperate forested wetlands; tropical flooded forests and peat forests; and tundra.

Local climate data were compiled for each site using the closest weather station. Monthly mean temperature and total precipitation were calculated for each month from deployment to the final sampling. Monthly mean temperature variation was calculated as the standard deviations of the monthly temperatures during the incubation period. Subtidal sites used in the final analyses represented <10% of the sites, most of which were lagoonal/estuarine (e.g. near intertidal). Therefore, local air temperatures were used for all sites [11].

2.2. Decomposition experiment

For the present study, we focussed on vegetated sites that did not receive experimental manipulations, resulting in data from 196 sites (Figure 1). At each site green and rooibos tea litter bags were buried 10-15 cm deep in two plots at least 1 m apart. Within each plot there were two replicates for each tea type (i.e. n = 4 for each tea type at each sampling time and site) [8]. Deployment occurred in the summer of 2017 for the northern hemisphere (e.g. ~June-August) and summer of 2017/2018 for the southern hemisphere (e.g. ~December-February).

Tea bags were collected 3, 6, 12, 24 and 36 months after deployment. As sites varied in accessibility and time constraints, some sites ended the experiment earlier at 12 or 24 months. Further, the 3-year sampling in 2020 occurred during the global coronavirus pandemic, resulting in 19 sites with delayed final samplings between 36 and 48 months. Initial mass was calculated by weighing the tea in the bag and then subtracting the mean bag mass of 0.20 g (± 0.002 g S.E.M, averaged over 40 empty bags). Post-incubation samples were cleaned of soil and dried at 60-70 °C until constant weight. Contaminating root biomass (i.e. root in-growth) was removed before weighing the final dry tea mass without the bag.

2.3. Data cleaning and decay modelling

Data cleaning involved two key steps: filtering out samples with potential root or soil contamination, indicated by an increase in mass with time within a site; and removing extreme outliers (e.g. potential miscalculations or mass errors) that lied outside the 2.5-97.5 quantiles for each tea type at each time point. As the study focused on quantifying the long-term tea litter decay, we calculated decay parameters for sites that had incubations for a year or longer. Only sites with at least two data points across three sampling times (i.e. six data points over the first year) were included. After filtering, 181 sites remained for labile green tea and 184 sites for recalcitrant rooibos tea.

For our decay modelling approach, we fit the site-level data with single exponential and asymptotic decay functions following Gill et al. [12].

Asymptotic exponential decay:

Proportion mass remaining = $A + (1 - A)e^{-t\,k_a}$ (eq. 1)

Where A is the asymptote (A), t is time (days), k_a is the early decay rate. The asymptotic decay function uses a negative exponential function approaching a non-zero horizontal asymptote. This formulation partitions the tea litter between early- and late-stage decay. The early stage is characterised by the initial rapid decay (1-A, as a proportion) at rate k_a (day^{-1}). The later stage is characterised by very slow or negligible decay after reaching the asymptote, i.e. the proportion of stable OM (A).

Single exponential decay:

Proportion mass remaining = $e^{-t\,k_s}$ (eq. 2)

The single exponential decay function describes the tea litter as a single pool that decomposes at a constant rate (k_s, proportion day^{-1}) over time (t, days).

By using the parameters from both the asymptotic and the single exponential decay models, we were able to describe tea litter decay in the following ways: (1) the negative exponential rate before reaching the asymptote quantifies the early decay rate (k_a) and is linked to abiotic leaching of water soluble compounds (eq. 1), (2) the asymptote (A) is the proportion of stable mass remaining under a long-term decay rate and has the potential to contribute to soil carbon stocks (eq. 1), and (3) the overall negative exponential rate (k_s) quantifies the overall decay rate in each time series (eq.2)[12]. Preliminary exploration of the data showed that both asymptotic and single functions fit the labile green tea data well (mean $R^2 > 0.8$), with a tendency for the single exponential decay model to reach zero within the three years. The single exponential decay function fit less well with recalcitrant rooibos tea data (mean $R^2 = 0.65$) but improved under the asymptotic function (mean $R^2 = 0.8$), the former likely due to low early mass leaching. We attempted to fit double negative exponential decay functions to partition early and later decay rates for each tea type at a site. However, like Gill et al. [12], the function could not converge, making this approach not suitable for our global dataset.

2.4. Statistical analyses and prediction modelling

Using linear models, we tested the effects of ecosystem type and climate on litter decay parameters (i.e., A, k_a and k_s) for each OM type (Table S2). The number of days of incubation was included as a fixed factor in all models, using a second degree polynomial function. We log10-transformed k_a and k_s to meet the assumptions of linearity and homogeneity of variance. Ecosystem type and climate were important factors in previous shorter-term tea litter decay studies 3 to 12 months; [6,8]. Therefore, the first model included the following terms: precipitation, temperature, temperature variability (as standard deviation), ecosystem type, and two-way interactions between ecosystem type and each of the three local climate terms, to compare the sensitivity of the ecosystem types to the climatic factors. The macroalgal ecosystems were only represented by two sites each, so they were removed for this analysis. Model selection using Bayesian Information Criterion (BIC) was performed for each OM type and decay parameter combination [13]. The second and third models for macroclimate and freshwater wetland IUCN typologies were analysed in single factor models separately. The IUCN Global Ecosystem Typology 2.0 categories incorporate both inundation and climate characteristic, as well as vegetation [10]. For all models, significant interactions between factors were explored with Tukey posthoc pairwise comparisons using the emmeans package [14]. All analyses were performed using the lm() function in R version 4.1.3 [15].

We generated worldwide spatial predictions of decay parameters (i.e., *A*, k_a, and k_s) based on linear models using only local climate parameters (precipitation, temperature and temperature variability, days of incubation as a second degree polynomial function), without accounting for ecosystem type due to incomplete geospatial coverage of each ecosystem type in this study. We sourced from Copernicus Climate Data Store spatially-explicit climate factors for temperature, precipitation and temperature variability using eight IPCC global climate models from the IPCC's Fifth Assessment Report (i.e., Coupled Model Intercomparison Project Phase 5; CMIP5). The models (with affiliations and countries) were: ACCESS1-0 (BoM-CSIRO, Australia), ACCESS1-3 (BoM-CSIRO, Australia), BNU-ESM (BNU, China), CESM1-BGC (NCAR, USA), CSIRO-Mk3-6-0 (CSIRO, Australia), IPSL-CM5A-MR (IPSL, France), MPI-ESM-LR (MPI, Germany), and the NorESM1-M (NCC, Norway) (see Table S3 for a basic description). This approach allowed us to account for different assumptions from independent research groups worldwide. Each model simulates grids of mean monthly data for rainfall (i.e., amount of water per unit area and time; mm $month^{-1}$) and temperature (i.e., temperature of the air at 2 m height; degree Celsius) at a latitude-longitude resolution from 1.87 x 1.25 to 2.81 x 2.79 (e.g. each pixel is roughly 200 x 200 km), depending on the model. While we are mostly interested in wetland projections for inland and coastal regions of the global, we kept open ocean in the scope of the projections to capture changes in decomposition parameters for wetlands of small islands. All models assumed a Representative Concentration Pathway of 4.5 (RCP 4.5, a greenhouse gas concentration trajectory adopted by the IPCC). Each climate model was used to estimate yearly averages for present (January 2018 to December 2021) and future (January 2048 to December 2051) conditions, allowing us to take into consideration seasonal and interannual climate cycles. The best-fitting model was used to generate spatial predictions on decay rate parameters for 2020 and 2050 based on simulated conditions of rainfall and temperature extracted from each CMIP5 (N = 8). Finally, the mean and 95% confidence intervals were calculated among the predictions based on the eight CMIP5s for 2020 and 2050.

Table S1: Distribution of the 196 vegetated, unmanipulated TeaComposition H_2O sites in this study by macroclimate and ecosystem type. Macroclimatic zones are from Walter and Breckle (1999). Subcategories for the freshwater wetland sites are from the IUCN Ecosystem Typology 2.0. Inundation category information was provided by site owners. Note, 12 and 15 sites for rooibos and green tea datasets, respectively, did not pass data QA/QC or were remove from analyses because of low ecosystem-level replication.

Macroclimate	Freshwater wetlands	Lentic ecosystem	Lotic ecosystem	Mangrove forests	Marine macroalgal	Seagrass meadow	Supratidal forests	Tidal marshes
Arctic climate	2	0	0	0	0	0	0	0
Boreal climate	0	0	0	0	0	1	0	0
Temperate climate	15	5	12	1	0	11	0	21
Warm-temperate climate	5	1	1	15	0	6	2	11
Mediterranean climate	13	1	2	1	1	6	0	5
Subtropical arid climate	2	0	0	4	0	1	0	0
Equatorial humid climate	11	0	1	22	1	3	2	0
Semi-arid tropical climate	8	1	0	1	0	0	0	1
Total	*56*	*8*	*16*	*44*	*2*	*28*	*4*	*38*

Freshwater wetland subcategories	
Boreal and temperate fens	5
Boreal, temperate and montane peat bogs	5
Permanent marshes	3
Seasonal floodplain marshes	24
Subtropical/temperate forested wetlands	4
Tropical flooded forests and peat forests	13
Tundra	2
Total	*56*

Table S2: Sample sizes for the three linear models. OM = organic matter.

Model	Observations: Labile OM (Green Tea)	Observations: Recalcitrant OM (Rooibos Tea)
Ecosystem & Climate	171	174
Macroclimate	179	182
Freshwater wetland	50	49

Table S3: Temperature and precipitation descriptions for the eight IPCC global climate models from Coupled Model Intercomparison Project Phase 5. All values are based on Representative Concentration Pathway (RCP) of 4.5.

IPCC global climate model	Temperature Mean (°C)	Temperature Variation (°C)	Precipitation Mean (mm^2)
ACCESS1-0 (BoM-CSIRO, Australia)	6.85	5.97	78.71
ACCESS1-3 (BoM-CSIRO, Australia)	7.05	5.28	82.07
BNU-ESM (BNU, China)	7.13	6.41	82.08
CESM1-BGC (NCAR, USA)	6.69	5.35	76.70
CSIRO-Mk3-6-0 (CSIRO, Australia)	5.15	6.38	73.92
IPSL-CM5A-MR (IPSL, France)	7.18	5.10	73.85
MPI-ESM-LR (MPI, Germany)	6.75	4.91	75.79
NorESM1-M (NCC, Norway)	5.96	4.98	71.89

Table S4: Proportion of mass remaining of standardised litters between 3 and 36+ month incubations. Values represent means and standard error. OM = organic matter.

Labile OM (Green Tea)

Ecosystem type	3 months	12 months	24 months	36+ months
Freshwater wetland	0.363 ± 0.011	0.240 ± 0.009	0.208 ± 0.009	0.188 ± 0.014
Lentic ecosystem	0.189 ± 0.016	0.147 ± 0.009	0.09 ± 0.007	0.084 ± 0.003
Lotic ecosystem	0.324 ± 0.027	0.14 ± 0.012	0.107 ± 0.011	0.062 ± 0.013
Mangrove forest	0.272 ± 0.011	0.228 ± 0.009	0.204 ± 0.008	0.155 ± 0.007
Marine macroalgal	0.17 ± 0.023	0.163 ± 0.011	0.133 ± 0.013	
Seagrass meadow	0.166 ± 0.009	0.124 ± 0.008	0.109 ± 0.006	0.055 ± 0.004
Supratidal forest	0.295 ± 0.019	0.235 ± 0.017	0.173 ± 0.021	0.105 ± 0.031
Tidal marsh	0.248 ± 0.015	0.162 ± 0.006	0.155 ± 0.008	0.138 ± 0.01

Recalcitrant OM (Rooibos Tea)

Ecosystem type	3 months	12 months	24 months	36+ months
Freshwater wetland	0.749± 0.009	0.602 ± 0.011	0.523 ± 0.01	0.442 ± 0.022
Lentic ecosystem	0.694 ± 0.012	0.591 ± 0.025	0.478 ± 0.03	0.505 ± 0.028
Lotic ecosystem	0.73 ± 0.01	0.546 ± 0.012	0.359 ± 0.023	0.399 ± 0.04
Mangrove forest	0.691 ± 0.013	0.595 ± 0.008	0.512 ± 0.009	0.381 ± 0.006
Marine macroalgal	0.711 ± 0.001	0.594 ± 0.018	0.449 ± 0.031	
Seagrass meadow	0.645 ± 0.012	0.558 ± 0.012	0.476 ± 0.016	0.368 ± 0.02
Supratidal forest	0.761 ± 0.01	0.543 ± 0.009	0.408 ± 0.015	0.351 ± 0.009
Tidal marsh	0.717 ± 0.011	0.612 ± 0.009	0.551 ± 0.011	0.503 ± 0.019

Table S5: Effect of local temperature and precipitation on decay parameters. Values represent estimates for significant effects, with asterisks representing p-values. Decay parameter asymptotic *A* represents the proportion of stable mass remaining (eq. 1) and k_a represents early decay rate (d^{-1}; eq. 1); single exponential k_s overall decay rate (d^{-1}; eq. 2). Precipitation and temperature variables are annual means/error from monthly averages obtained from local weather stations. OM = organic matter.

	Labile OM (Green Tea)			Recalcitrant OM (Rooibos Tea)		
Explanatory Variables	*A*	k_a	k_s	*A*	k_a	k_s
Precipitation Mean (mm^2)	-0.00027*	n.s.	n.s.	-0.000497***	n.s.	0.00036*
Temperature Mean (°C)	n.s.	n.s.	n.s.	-0.00457**	n.s.	0.01434***
Temperature Variation (°C)	-0.0113***	0.0209*	0.0229***	n.s.	0.0324*	0.01992***

p-values: *** < 0.001, ** 0.001 <= to < =0.01, * 0.01 < to <= 0.05, n.s. = not significant

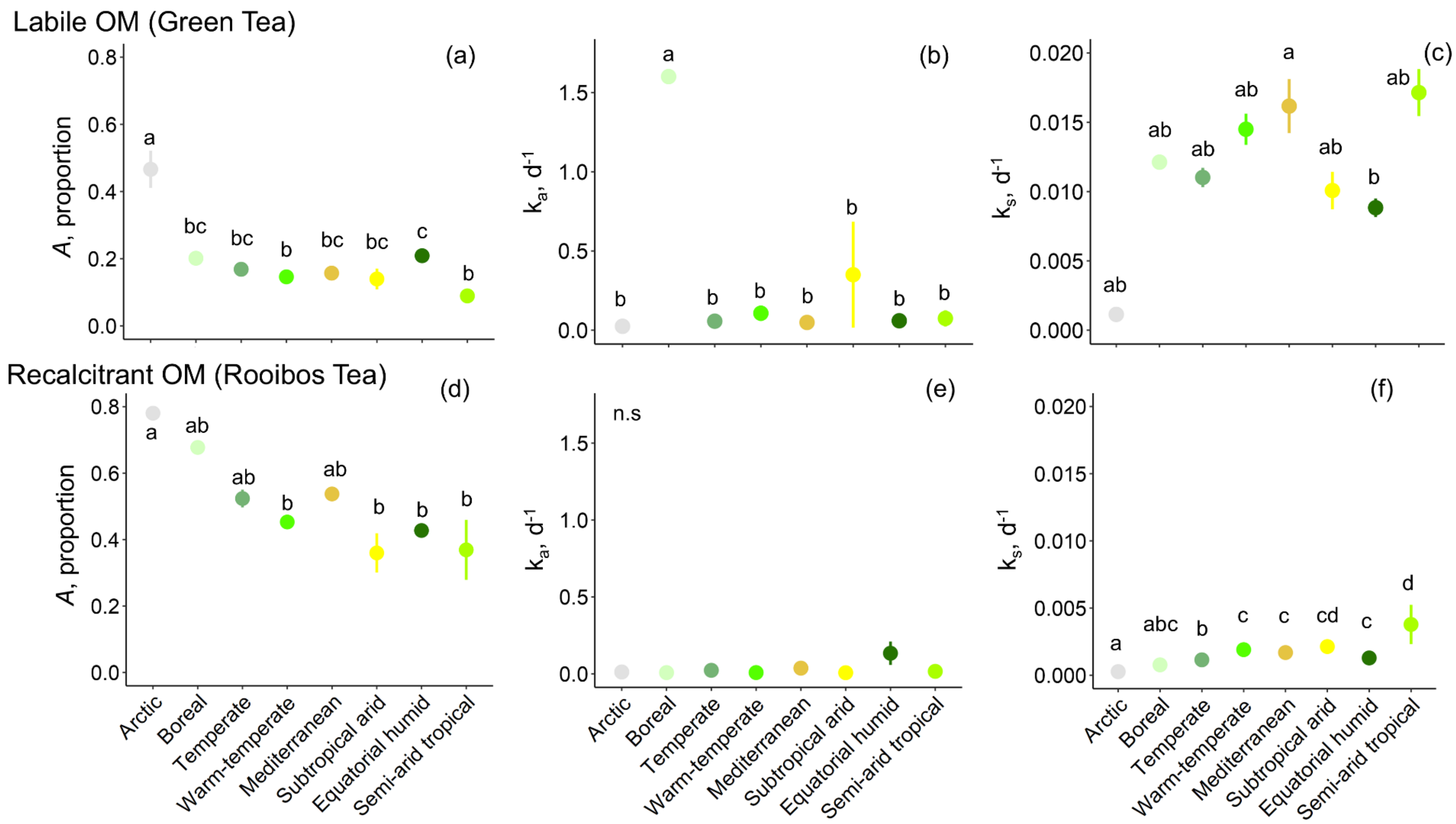

Figure S1: Variation in decay parameters across macroclimate categories. Decay parameter asymptotic *A* (a, d) represents proportion of stable mass remaining (eq. 1), k_a (b,e) represents early decay rate (d^{-1}; eq. 1), and single exponential k_s (c, f) represents the overall decay rate (d^{-1}; eq. 2). Data presented are means ± standard errors. Boreal climate data represent two sites. Colours match the macroclimates in the global map (Figure 1). Different letters represent statistically significant differences among groups (post-hoc analyses, $p < 0.05$). Non-significant effects of macroclimate are denoted by the letters n.s. ($p > 0.05$) (Table 1).

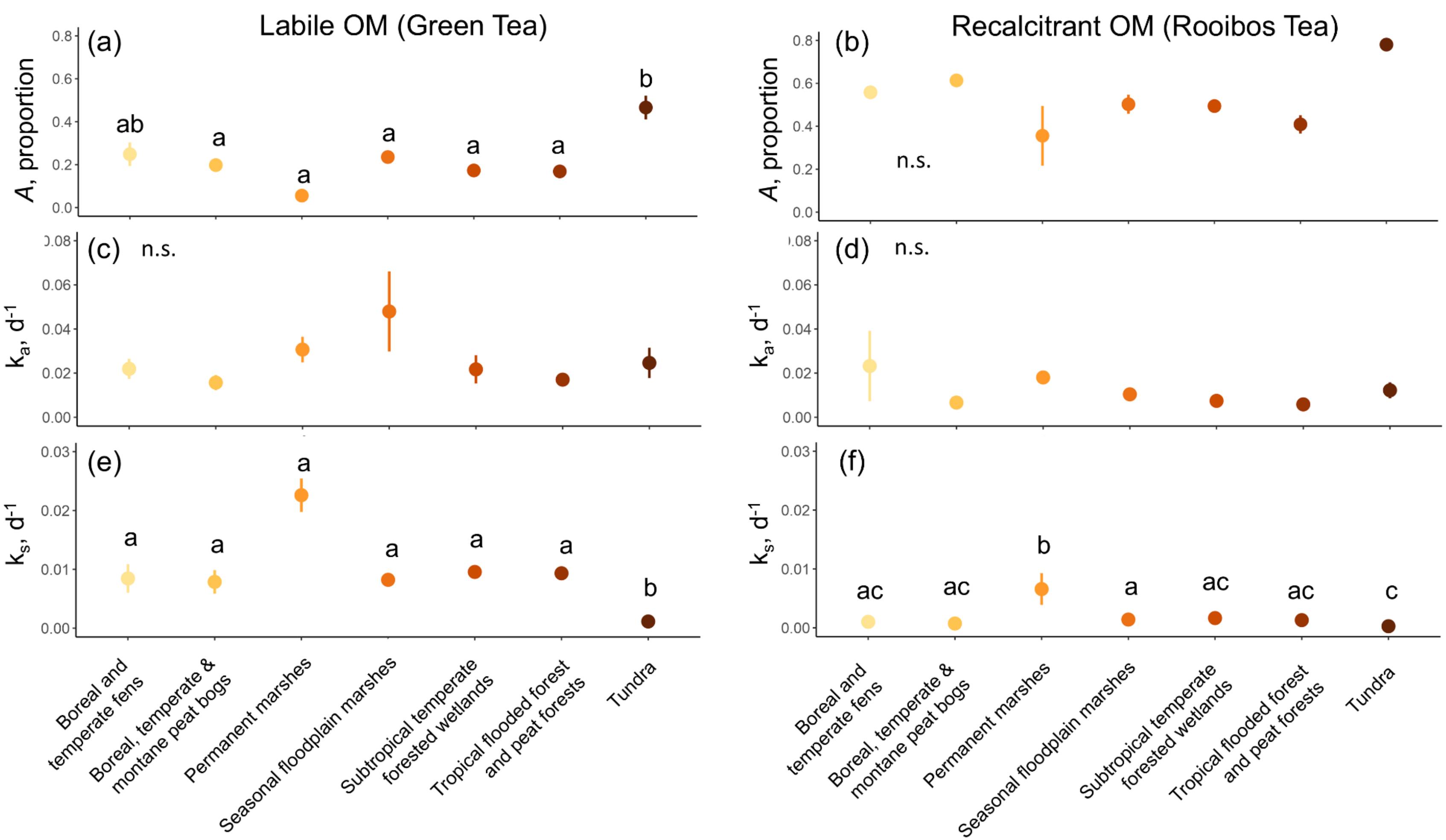

Figure S2: Variation in decay parameters across freshwater wetland IUCN typologies. Decay parameter asymptotic *A* (a, b) represents the proportion of stable mass remaining (eq. 1), k_a (c,d) represents early decay rate (d^{-1}; eq. 1), and single exponential k_s (e, f) represents overall decay rate (d^{-1}; eq. 2). Data presented are means and standard error of means. Typologies with different letters were significantly different in post-hoc pair-wise comparisons. Non-significant effects of typology are denoted by n.s. (Table 1).

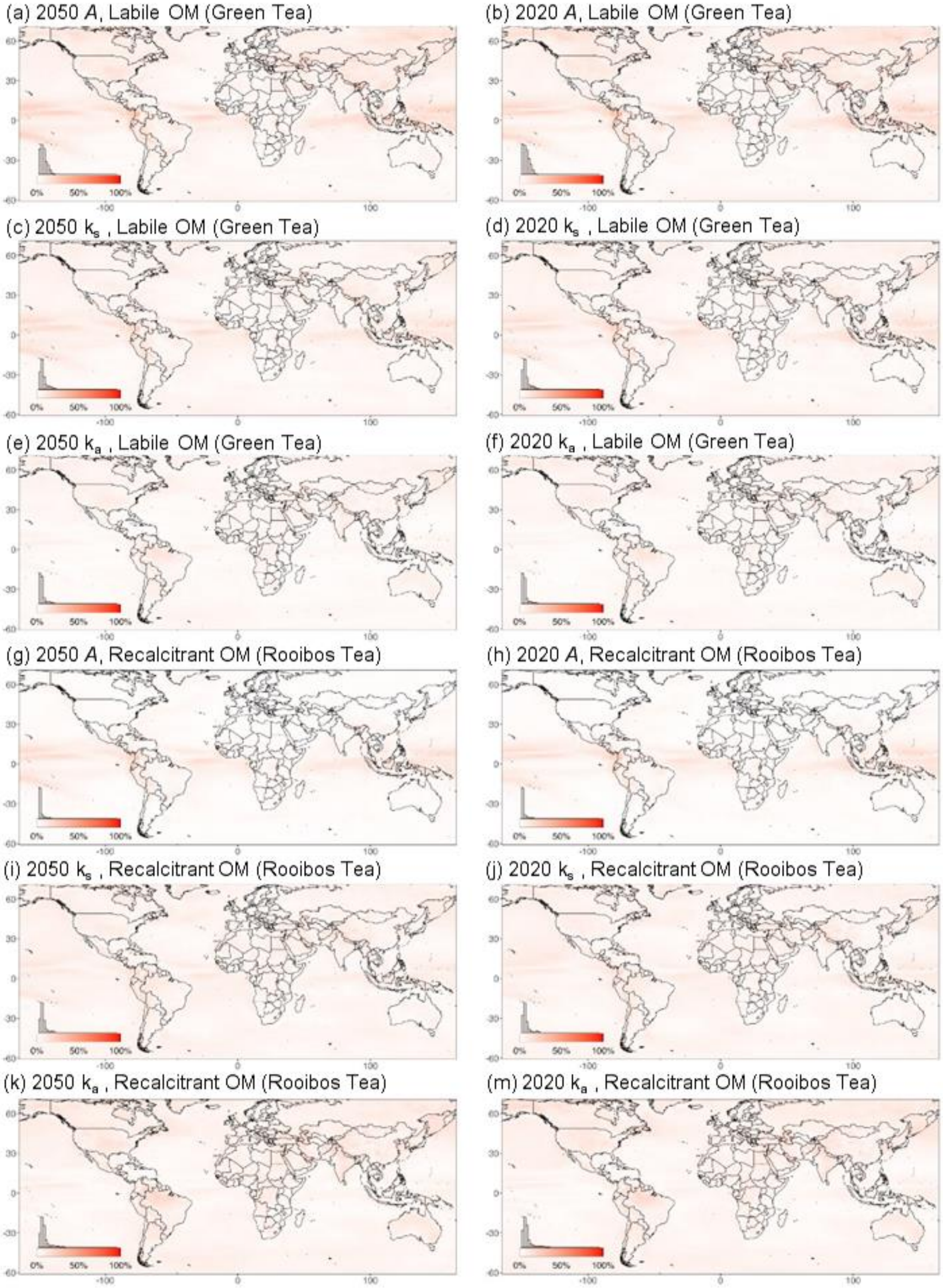

Figure S3: Coefficient of variation for predicted percent change for each organic matter type and parameter combination. Decay parameter asymptotic *A* represents recalcitrant mass remaining (eq. 1), k_a represents early decay rate (d^{-1}; eq. 1), and single exponential k_s represents overall decay rate (d^{-1}; eq. 2).

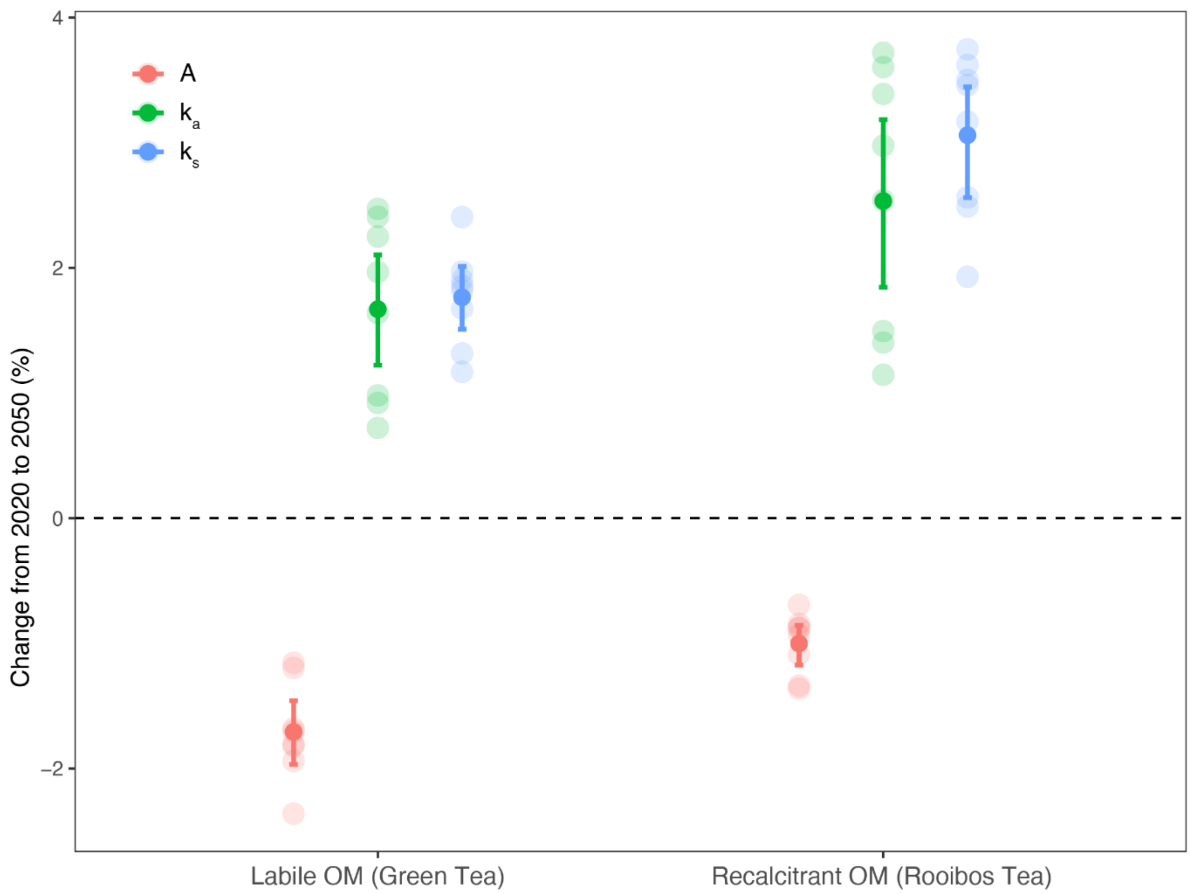

Figure S4: Global predicted percent change in litter decay between 2020 and 2050. Mean conditions (±95% CI) for 2020 and 2050 for three decay parameters of labile and recalcitrant OM based on climatic projections from eight CMIP5 models at RCP 4.5 scenario (see Fig. 6). Each point represents the overall mean from a global projection using a CMIP5 model. Decay parameter asymptotic *A* represents the proportion of stable mass remaining (eq. 1), k_a represents early decay rate (d^{-1}; eq. 1), and single exponential k_s represents overall decay rate (d^{-1}; eq. 2).

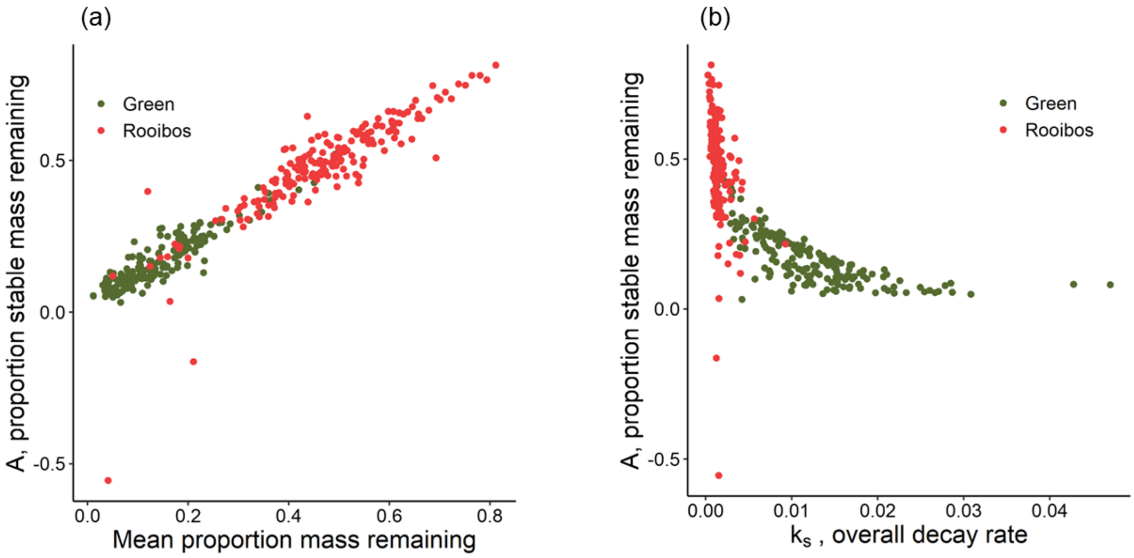

Figure S5: Relationships between decay parameters. (a) Final mean proportion mass remaining and asymptotic *A*, where Pearson's correlations were significant ($p < 2.2 \times 10^{-16}$) for both labile OM (green tea; $R^2 = 0.91$) and recalcitrant OM (rooibos tea; $R^2 = 0.88$) litters. (b) Overall decay rates and asymptotic *A*, where Pearson's correlations were significant for both labile ($p < 2.2 \times 10^{-16}$, $R^2 = -0.71$) and recalcitrant ($p = 1.6 \times 10^{-9}$, $R^2 = -0.43$) OM types. *A* is the proportion of stable mass remaining and was calculated as the asymptote of the exponential asymptotic decay model (eq. 1). The few low *A* values for recalcitrant OM is likely due to the asymptote not being reached at those sites. k_s represents overall decay rate (d^{-1}) calculated from a single exponential decay model (eq. 2).

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