Ecosystem type drives tea litter decomposition and associated prokaryotic microbiome communities in freshwater and coastal wetlands at a continental scale

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Abstract

Wetland ecosystems are critical to the regulation of the global carbon cycle, and there is a high demand for data to improve carbon sequestration and emission models and predictions. Decomposition of plant litter is an important component of ecosystem carbon cycling, yet a lack of knowledge on decay rates in wetlands is an impediment to predicting carbon preservation. Here, we aim to fill this knowledge gap by quantifying the decomposition of standardised green and rooibos tea litter over one year within freshwater and coastal wetland soils across four climates in Australia. We also captured changes in the prokaryotic members of the tea-associated microbiome during this process. Ecosystem type drove differences in tea decay rates and prokaryotic microbiome community composition. Decomposition rates were up to 2-fold higher in mangrove and seagrass soils compared to freshwater wetlands and tidal marshes, in part due to greater leaching-related mass loss. For tidal marshes and freshwater wetlands, the warmer climates had 7-16% less mass remaining compared to temperate climates after a year of decomposition. The prokaryotic microbiome community composition was significantly different between substrate types and sampling times within and across ecosystem types. Microbial indicator analyses suggested putative metabolic pathways common across ecosystems were used to breakdown the tea litter, including increased presence of putative methylotrophs and sulphur oxidisers linked to the introduction of oxygen by root in-growth over the incubation period. Structural equation modelling analyses further highlighted the importance of incubation time on tea decomposition and prokaryotic microbiome community succession, particularly for rooibos tea that experienced a greater proportion of mass loss after the first three months compared to green tea. These results provide insights into ecosystem-level attributes that affect both the abiotic and biotic controls of belowground wetland carbon turnover at a continental scale, while also highlighting new decay dynamics for tea litter decomposing under longer incubations.

68

69 Keywords: 16S amplicon sequencing, carbon cycling, indicator analysis, labile, recalcitrant,

70 TeaComposition H_2O

Introduction

Decomposition, or the breakdown of plant detritus, is a fundamental ecosystem process. It is essential for nutrient regeneration and the transformation of living biomass into soil organic matter, and therefore critical to rate and magnitude of carbon accumulation and mineralisation (Berg and McClaugherty 2008, Wieder et al. 2013). In terrestrial ecosystems, decomposition metrics, such as litter mass, carbon and nitrogen loss, and microbial biomass and activity, are used to inform biogeochemical cycling in broad-scale earth system models (Bonan et al. 2013, Wieder et al. 2013, Bradford et al. 2017). As wetlands are critical to regulating atmospheric carbon concentrations, there is a need for analogous estimates of wetland carbon inputs and losses across a range of spatial scales to inform carbon cycling and climate models (Mitsch et al. 2013), and ecosystem management actions (Moomaw et al. 2018, Kelleway et al. 2020). Yet for wetland ecosystems, particularly for coastal vegetated ecosystems, there is a gap or a disparity in the types of observational decomposition data collected (Baird et al. 2020, Trevathan-Tackett et al. 2020b).

The environmental conditions unique to freshwater and tidal wetland ecosystems, such as hydroperiod, inundation, and allochthonous soil accumulation, shape the biogeochemistry and microbial ecology of the soils (Macreadie et al. 2017, Yarwood 2018, Cragg et al. 2020). In the belowground compartment, water-logged soils are an important wetland attribute that enhances sequestration and preservation potential through reduced carbon remineralisation rates via anaerobic metabolic pathways (e.g. sulphate reduction, methanogenesis and fermentation performed by prokaryotic soil microbiota; Yarwood 2018, Cragg et al. 2020). In coastal soils, sulphate reducers dominate organic matter turnover; however, through greenhouse gas flux measurements, methane cyclers have recently been shown to contribute to carbon emissions in tidally-influenced ecosystems (Al-Haj and Fulweiler 2020). In

96 contrast, methanogenesis is typically the prevalent carbon mineralisation pathway in
97 freshwater wetland soils. Yet, methanogens co-exists with a smaller consortia of sulphate
98 reducers that may divert carbon flow from methane to carbon dioxide (Pester et al. 2010).
99 Additionally, salinity category, i.e. saline versus non-saline, is the strongest selector for
100 prokaryotic microbiome structure on a global scale (Lozupone and Knight 2007). The
101 influence of salinity on community structure may be due to osmotic or ionic tolerance of
102 marine taxa, as well as sulphate availability (Lozupone and Knight 2007) that would
103 influence the predominant metabolic pathways under anoxic conditions.
104
105 In addition to the belowground physicochemical conditions, belowground biomass (roots,
106 rhizomes) plays a key role in the accumulation of organic carbon stocks and peat formation.
107 Belowground biomass directly contributes to the carbon stocks and soil stability (Kirwan and
108 Megonigal 2013, Lamont et al. 2020), with soil carbon accumulation enhanced through slow
109 decomposition rates and the generally more recalcitrant organic matter quality of the detritus
110 than other biomass types (Chimner and Ewel 2005, Ouyang et al. 2017, Trevathan-Tackett et
111 al. 2017). Further, compared to their aboveground counterparts, belowground biomass is
112 subject to lower chances of herbivory, export and aerobic decay, all of which can enhance the
113 amount of carbon available for sequestration *in situ* (Cebrian 2002). Despite the importance
114 of belowground biomass to carbon cycling dynamics, the variables affecting its
115 decomposition in wetlands generally receives less attention than leaf litter breakdown,
116 particularly in coastal wetlands (Ouyang et al. 2017, Trevathan-Tackett et al. 2020b).
117 Consequently, there is a fundamental need to resolve the mechanisms underlying
118 belowground decomposition processes, including the interaction between detrital organic
119 matter, the microbial community involved in the decomposition process and the

120 environmental conditions across wetland ecosystem types that are conducive or limiting to
121 litter breakdown (Yarwood 2018, Spivak et al. 2019).
122
123 Cross-ecosystem studies using standardised tea litter proxies have progressed our
124 understanding of belowground organic matter cycling dynamics at regional-to-global scales.
125 Tea litter decomposition has shown to be sensitive to ecosystem type and climate at a global
126 scale, whereby warmer and more humid climates enhance decomposition in the first three
127 months of incubation compared to cooler or drier climates (Keuskamp et al. 2013, Djukic et
128 al. 2018). However, in some cases, the temperature-enhancing effects on organic matter
129 breakdown may only be observed when also accounting for local- or microsite-based factors
130 like inundation (Mueller et al. 2018) or vegetation (Petraglia et al. 2019) or when the data are
131 aggregated at a biome or regional level (Djukic et al. 2018, Mueller et al. 2018). In addition
132 to temperature effects on tea litter decay, a cross-ecosystem study of tidally-influenced
133 freshwater and tidal marshes and mangrove ecosystems showed that lower elevations, and
134 thereby zones flooded more frequently, decreased the tea organic matter stability (Mueller et
135 al. 2018). This reduced stability could have potential consequences for soil formation under
136 sea level rise scenarios (Mueller et al. 2018). Furthermore, resolving the drivers of
137 belowground decomposition using cross-ecosystem experiments may complement large-scale
138 datasets that measure and model the drivers of belowground carbon stocks and sequestration
139 rates, in order to identify opportunities for conservation, restoration and climate change
140 mitigation efforts (for example, Nahlik and Fennessy 2016, Serrano et al. 2019, Ewers Lewis
141 et al. 2020).
142
143 The aim of this study is to assess belowground wetland decomposition using a cross-
144 ecosystem design at a continental scale. Using standardised tea litter, we quantify the

145 breakdown of organic matter in seagrass meadows, mangrove forests, tidal marshes and
146 freshwater wetlands from 37 sites and four climate biomes across Australia. As with previous
147 large-scale tea litter studies, we expect to show strong ecosystems differences in
148 decomposition dynamics among the wetland types. Where possible, we tested the effects of
149 climate biome within ecosystem type. We also described the tea-associated microbiota for
150 each ecosystem type across a subset of these wetlands. We expect to find key differences in
151 the prokaryote microbiome community structure and putative functional pathways between
152 the saline and non-saline ecosystems. Our multidisciplinary approach combining ecological
153 and microbiological techniques in a designed *in situ* experiment using standardised litter
154 assays will advance our understanding of the belowground decomposition process in wetland
155 ecosystems and contribute to improvements of integrative biogeochemical conceptual
156 models.

157

158 **Materials and Methods**

159 *Tea litter, study area, deployment and sampling*

160 We used nylon mesh tea litterbags with green tea and rooibos tea (Keuskamp et al. 2013).
161 The teas represented two types of chemical recalcitrance: green tea as a relatively labile form
162 of organic matter (OM) with higher water soluble content, and rooibos tea as a more
163 recalcitrant or stable form of OM because of its higher lignin (acid insoluble) content
164 (Keuskamp et al. 2013). These 'alien' litters are biochemically representative of local litter
165 chemistries (Didion et al. 2016, Duddigan et al. 2020, Trevathan-Tackett et al. 2020a), and
166 meant to be used at litter proxies and reference to understand decomposition and organic
167 matter cycling (Keuskamp et al. 2013). Generally, the litter bag technique tends to exclude
168 larger grazers from entering the mesh bag at the cost of retaining smaller particulate litter

169 with the finer mesh sizes. Because of this, litter bag methods that use small mesh size need to
170 keep in mind that decomposition may be underestimated if faunal grazers are common to the
171 study area (Bradford et al. 2002).

172 Tea litter bags were deployed as part of the global *TeaComposition* H_2O initiative
173 investigating decomposition in wetland and marine ecosystems, and is an extension and co-
174 initiative to the terrestrial-focussed *TeaComposition* (Djukic et al. 2018). Tea litter bags from
175 the same batch as *TeaComposition* were deployed using a parallel methodology, with slight
176 modification (Djukic et al. 2018). Tea litter bags were deployed in the summer between
177 November 2017 and February 2018 to a total of 37 study sites encompassing four wetland
178 ecosystems (seagrass meadow, mangrove forest, tidal marsh and freshwater wetlands),
179 spanning 25-27° of latitude and four climates in eastern Australian (Walter and Breckle 1999)
180 (Figure 1). The seagrass meadows were comprised of genera that have small to medium
181 morphologies (i.e. *Zostera*, *Halodule*, *Cymodocea* and *Syringodium*). Most of the seagrass
182 sites were subtidal, with the exception of a *Zostera muelleri* site in Gladstone (Table S1).
183 Most of the mangrove sites were *Avicennia marina* forests, with some sites having
184 *Rhizophora* spp. (sites 320, 327, 334, see Table S1). Both tidal marsh and freshwater marshes
185 included a range of vegetation types from succulent-dominant (3, 364-369) to tree-dominant
186 (355, 356, 358-360, 372, 375). Other sites were grass- or rushes-dominant (335, 374, 382,
187 385, 389, 390), while several sites were comprised of a mix of trees, grasses and rushes (376,
188 377, 401).

189 Initial mass was calculated by weighing the tea in the bag then subtracting the mean bag mass
190 of 0.2000 g (averaged over 40 empty bags; 4-decimal balance). Within each site, litter bags
191 were buried at 10-15 cm soil depth, ideally below the rhizosphere, and across two plots at
192 least 1 m apart. This not only simulates the decomposition of belowground plant detritus, but
193 protects the litter bags from excessive plant in-growth and disturbances at the soil surface.

Within each plot there were two replicates for each tea type (n = 4 for each tea at each sampling time and site) (Djukic et al. 2018). For sites that assessed both mass loss (decay) and microbiome sampling, an extra set of tea bags was deployed for each site (n = 4 for each tea type), whereby the tea bags earmarked for decay and microbiome sampling were alternated in order to capture the potential heterogeneity within the plot.

Tea bags for decay analyses were collected at 3, 6 and 12 months for all sites. The tea- and soil-associated prokaryotic microbiome communities were analysed for eight of these sites (Figure 1). Decay 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 tea mass without the bag. For the microbiome samples, the tea litter bags were opened on-site and approximately 1 ml volume of tea litter was transferred to a 2 ml vial containing Zymo DNA/RNA Shield. Using a cut-off 3 mL syringe core, approximately 1 cm^3 of bulk soils were sampled adjacent to and at the depth where the tea bags were incubating for comparative microbiome analyses. Microbiome samples were stored frozen until analysis.

DNA extraction, sequencing and bioinformatics

A subset of the microbiome samples from eight sites was selected for 16S rRNA gene amplicon analysis, i.e. the prokaryotic members of the tea- and soil-associated microbiome community. We targeted the 3- and 12-month sampling to capture the early and later stages of decay (Trevathan-Tackett et al. 2020b) from each ecosystem type representing two of the climates, temperate and warm temperate (Figure 1). Bead-beating of the tea litter and soil samples (0.2-0.4 g fresh weight sample; 60 s and 5.5 m s^{-1}; FastPrep-24) preceded DNA extraction with the Zymo Mini Kit as per manufacturer instructions for soil (ZymoResearch). DNA was quantified using Qubit Broad Range before normalising to 5 ng μl^{-1}. Normalised gDNA template and Zymo Microbial DNA Standard were amplified using the V4 variable

219 region of the 16S rRNA gene with the modified Eubacterial primers 515F (5′-
220 GTGCCAGCMGCCGCGG- 3′) and 806R (5′- GGACTACHVGGGTWTCTAAT- 3′)
221 (Walters et al. 2016). Triplicate PCR cycles were performed before pooling and cleaning with
222 Zymo Clean and Concentrate Kit with modified elution using 10 mM Tris buffer (94 °C at 3
223 min, 25 cycles of 94 °C at 30 s – 50 °C at 30 s – 72 °C at 30 s, then 72 °C at 5 min). PCR
224 products were indexed and sequenced on an Illumina MiSeq by Deakin University's
225 Genomic Centre.

226 Amplicon sequences were analysed in the QIIME2 DADA2 pipeline (Callahan et al. 2016,
227 Bolyen et al. 2018). The resulting amplicon sequence variants (ASVs) were normalised to
228 54,200 reads per sample before alpha diversity (observed ASVs, Pielou's Eveness) and beta
229 diversity (weighted UniFrac) metrics were calculated. This resulted in the removal of three
230 samples: sediment sampled at 3 months in a temperate tidal marsh and two sediments
231 sampled at a tropical mangrove at 3 and 12 months. Sequences were classified using the Silva
232 v132 database at a threshold of 99% homology (Quast et al. 2012).

233

234 *Data processing and statistical analyses*

235 Decomposition analyses. Initial and final tea mass was used to calculate the proportion mass
236 remaining for each of the time points. These data were used to assess the effect of time,
237 substrate, ecosystem and climate on decomposition in two ways: mixed linear models (MLM)
238 and exponential decay models. We used mixed effect models to assess the effects of tea type,
239 ecosystem type and incubation time on the proportion mass remaining using the lmer package
240 (Bates et al. 2015) in R version 3.6.2 (R Core Team 2019). To test if the temporal trends in
241 remaining mass were different among ecosystems and whether these depended on tea type,
242 we fitted a full model including a 3-way interaction, all 2-way interactions and single terms

as fixed factors. To account for the repeated sampling and for the spatial structure of the data, we included plot as a random factor nested within site. The model was reduced by removing non-significant terms, using backward model selection. Selection starting with the 3-way interactions, followed by 2-way and single terms. In cases where two 2-way non-significant interactions or main terms were present, the term with the highest p-values were removed first. Only significant factors ($p < 0.05$) were included in the final model. Interactions between factors were explored with posthoc pairwise comparisons using the emmeans package (Lenth et al. 2018). For freshwater and tidal marsh ecosystems that had climate-level replication, we also tested whether litter decomposition within an ecosystem type depended on the climatic conditions of the site by comparing sites of the same ecosystem but from different climates. We used a model including climate, incubation time and tea type and the same random structure as previously stated. Similarly, we started with a full model and reduced until only significant terms remained.

Exponential decay rates are often used to describe the decomposition process over the entire observed period of time (Wieder and Lang 1982), an approach used to calculate decay rates of natural litter. Single exponential decay rates were calculated using $W_0/W_t = e^{-kt}$, whereby W_0 was the initial mass, W_t was the mass at time t and k is the decay rate in proportion per day (d^{-1}), with the model inclusive of 100% mass at day 0. k was calculated separately for each tea type for each site, each ecosystem type, and each ecosystem*climate combination. Sites sampled 3-6 months after the ideal sampling time were included in the initial visualisation and site-level calculations, but these data points, or in some cases whole sites, were excluded from further the statistical analyses in order to remove time-related biases on the mass loss data as a result of the longer incubation (see Table S1 for incubation time in days).

Microbiome analyses. Prokaryotic microbiome alpha diversity was analysed using the MLM approach described above to assess differences in substrate type (green tea, rooibos tea, soil). To assess alpha diversity of the prokaryotic members of the tea-associated microbiome during decomposition, a three-way model was used to assess the differences across tea type, ecosystem (seagrass, mangrove, tidal marsh, and freshwater wetland) and time (3 months and 12 months). Beta diversity was analysed with a three-way multivariate PERMANOVA, with ecosystem, substrate and time as fixed factors. Limited site-level replication for each ecosystem precluded the inclusion of a random plot factor in the design. The PERMANOVA test was repeated with only the tea samples to investigate the microbial community succession as decomposition progressed. For the PERMANOVA analyses, a Monte Carlo correction (P(MC)) was applied in cases where permutations were <200 (PRIMER + PERMANOVA v 7) (Anderson et al. 2008). Indicator analyses on the filtered ASV taxonomy table (thereby removing rare ASVs) were performed using the indicspecies package (Dufrêne and Legendre 1997, Cáceres and Legendre 2009) to measure the association between the ASVs for the significant beta diversity results from the PERMANOVA analyses. An alpha of 0.05 was used for all statistical tests, except the indicator analyses which focussed on the most significant ASVs ($p < 0.001$).

Structural equation model. We used a structural equation model (SEM) with the piecewiseSEM package (Lefcheck and Freckleton 2016) to test for direct and indirect relationships between the factors ecosystem type, incubation time (in days) and microbial community on remaining litter mass. The data used in this analysis were the tea-associated prokaryotic microbiomes samples (8 sites from the 3- and 12-month sampling; Figure 1, Table S1) and the associated/paired mass loss data. The microbial communities were represented by the first two axes of the principal coordinates analysis (PCO). The factor 'ecosystem type' encompasses inherent environmental differences and occur across a range

of climates zone that may affect the structure of microbial communities and litter decomposition rates (Ochoa-Hueso et al. 2019). A random effect identifying each plot and site combination was included to account for spatial and temporal dependencies in the data. We fitted separate models for each tea type to examine differences in pathways between the two tea types and removed non-significant paths from our final models, and Fisher´s tests of directed separation were used to evaluate model fit (Lefcheck and Freckleton 2016). To estimate the influence of the categorical variable ecosystem type, we used χ^2 likelihood ratio tests for each individual piecewise model, and compared among ecosystem types using post-hoc means adjusted Tukey tests at $\alpha = 0.05$ with the emmeans package (Lenth et al. 2018).

Results and discussion

Ecosystem-driven decomposition. During the first year of decomposition, the proportion of remaining tea mass was consistently lower for green tea than for rooibos tea litter. This was mainly due to the initial mass loss during the first three months of incubation, amounting to 70-90% and 29-45% for green and rooibos tea, respectively (all raw data presented in Figure S1; see Table S1 for site-level data). A significant ecosystem effect was driven by the greater amount of mass loss for both tea types in mangrove and seagrass ecosystems relative to tidal marsh and freshwater wetlands (Figure 2a, Table S2). A significant ecosystem*tea interaction appears to be related to small differences among tidal marsh and freshwater wetlands: rooibos tea mass loss was lowest in tidal marshes, but green tea mass loss was lowest in freshwater wetlands (Figure 2a; Table S2). Single exponential decay rates for green tea were more than doubled those in mangrove and seagrass ecosystems compared to freshwater wetlands and tidal marshes. Ecosystem differences in decay rates for rooibos tea followed a similar but more constrained pattern, likely due to the relatively reduced leaching (Figure 2b). Further,

316 compared to studies using a similar modelling approach, the decay rates of the tea litter were
317 in the range of belowground litter and anaerobic decomposition for seagrass (0.0018 - 0.01 d^{-1};
318 Trevathan-Tackett et al. 2020b), and mangrove and tidal marsh plants (means of 0.0015
319 and 0.0012 d^{-1}, respectively; Ouyang et al. 2017). In contrast, green decay rates were closer to
320 those of non-woody freshwater plant families, primarily belonging to submergent-, floating-
321 or herb-type groups (0.01 - 0.05 d^{-1}), while rooibos tea decay rates were closest to woody
322 plants and emergent or grassy non-woody plant families (0.001 - 0.005 d^{-1}; Webster and
323 Benfield 1986). By tracking tea decomposition over a full year, we showed that an
324 exponential decay rate modelling approach can be applied to tea litter data, and that the decay
325 rates of both tea substrates are within the realm of natural litters, and may be capturing
326 approximate endmember decay rates (Trevathan-Tackett et al. 2020a).
327
328 We hypothesise that the high decomposition rates in the mangrove and seagrass ecosystems
329 are related to the greater inundation at these sites facilitating leaching-induced mass loss. In
330 this case, instead of water-logged conditions creating an anoxic environment conducive to
331 slower decay, porewater flushing led to high leaching, particularly for the green tea litter, and
332 thereby an abiotic-driven reduction in mass. Similarly, water column incubations enhanced
333 leaching compared to tea litter at the soil surface or buried (Seelen et al. 2019), while
334 positions lower in the tidal frame have been found to have significantly higher tea litter
335 stability compared to positions higher in the tidal frame (Mueller et al. 2018). Although, we
336 do not have tidal or inundation data for all the sites in this study, all the seagrass sites were
337 classified as subtidal, while mangroves in mainland Australia typically inhabit the intertidal
338 zone from approximately mean sea level to upper intertidal elevations where they may be
339 replaced by tidal marsh.

340 Climate effect within ecosystem type. Climate had a significant effect on decomposition
341 dynamics for the subset of freshwater wetland and tidal marsh ecosystems analysed. While
342 deployments across a broader range of sites within each climate are ideal for robust
343 interpretation, our analyses highlight some preliminary trends. First, tea litter in the temperate
344 climates generally decomposed more slowly than the warmer climates, shown by 7-16%
345 higher remaining mass across both ecosystems and tea types (Figure 2c,d, Table S3). For the
346 freshwater wetland sites, temperate climates exhibited the least amount of decomposition,
347 compared with the higher decomposition in the Mediterranean (green tea) and warm
348 temperate (rooibos tea) climates after the year of decomposition *in situ* (Figure 2c, Table S3).
349 This resulted in 1.7- to 2-fold slower decay rate for the green tea and 1.6- to 1.8-fold slower
350 decay rate for the rooibos in the temperate climates. For the tidal marshes, climate effects on
351 green tea litter were significant at both 3- and 12-month samplings (Figure 2d; $k = 0.00871$
352 and $0.0124\ d^{-1}$ for temperate and warm-temperate, respectively), while the enhanced
353 decomposition of the rooibos tea in the warm temperate climate was significant only after 12-
354 months of burial (Figure 2d; $k = 0.00153$ and $0.00214\ d^{-1}$ for temperate and warm-temperate,
355 respectively).

356 By accounting for climatic differences with ecosystem types over a continental scale, we
357 showed that warmer climates may enhance belowground litter decomposition. Enhanced tea
358 decomposition rates due to elevated temperatures has been shown in the laboratory
359 (Keuskamp et al. 2013, Trevathan-Tackett et al. 2020a) and in the field (Djukic et al. 2018,
360 Mueller et al. 2018, Petraglia et al. 2019, Seelen et al. 2019). Similar to this study, (air)
361 temperature only affected tea decay in temperate tidal marsh ecosystems within the same
362 regional biome (Mueller et al. 2018) or globally when aggregated at the biome level (Djukic
363 et al. 2018). Further, our use of a categorical climate approach integrates temperature and
364 precipitation conditions that may be particularly important for freshwater wetlands that

depend on the annual wet season for water. Petraglia et al. (2019) found that variable moisture conditions affected the impact of, or ability to detect, the effects of elevated temperatures on tea litter decomposition, likely due to changing oxygen availability. In contrast, under stable inundated conditions, e.g. lakes and subtidal environments, elevated temperature can enhance tea decomposition (Seelen et al. 2019, Trevathan-Tackett et al. 2020a). Further research aimed at teasing apart this potential temperature-inundation relationship would be useful for parameterising climate change effects across multiple ecosystems, particularly for ecosystems like seagrass meadows whose hydrology would be influenced by currents, tides and storms, but potentially less influenced by daily fluctuations in temperature in subtidal environments.

Ecosystem and decomposition effects on the prokaryotic microbiomes

Alpha diversity metrics, ASV richness and evenness, were 1.1-1.8-fold higher in the bulk soil compared to the two tea types ($p < 0.001$ for both; Figure S2a,b), suggesting a degree of specialisation of the prokaryotic taxa colonising the tea litter (Pioli et al. 2020). Rooibos tea had higher richness and evenness than the green tea prokaryotic microbiome ($p < 0.001$; Figure S2). Similarly, richness and evenness also increased from the 3- to 12-month incubation ($p < 0.001$ and $p = 0.003$, respectively; Figure S2c,d).

Ecosystem effects. While we were limited in the number of sites from each ecosystem sampled for the prokaryotic microbiome community analysis, and have likely missed a level of within-ecosystem heterogeneity across sites (Behera et al. 2019, Palacios et al. 2021), we found that ecosystem type drove the differences in community composition (beta diversity; significant ecosystem*substrate and ecosystem*time interactions, Table S4). The ecosystem

389 effect also explained 33.4% of the variation explained in the principle coordinates analysis,
390 driven by the distinction between the freshwater wetlands and coastal wetlands and between
391 tidal marsh and mangrove+seagrass ecosystems (PCO1; Figure 3). Soil communities were
392 stable over time (Table S4), whereas the tea-associated composition shifted and became more
393 similar to the soil communities over time (PCO2; Figure 3). In the pairwise analyses, the soil
394 and tea litter communities differed in all ecosystems, except between the two tea litters within
395 tidal marshes. Indicator analysis resulted in 650 highly significant ASVs ($p < 0.001$) from
396 132 families (Figure S4; see detailed substrate results in Supplementary Materials). The
397 Rhizobiales order, well-known for its nitrogen-fixing and rhizosphere members, dominated
398 freshwater wetland microbiome communities, but were substrate-specific (Figure S4). The
399 prokaryotic microbiomes from tidal marsh and mangrove ecosystems consisted of putative
400 sulphate-reducers and methane cyclers, though comprising of distinct families within each
401 ecosystem. Syntrophobacteraceae ASVs, potentially involved in methane and sulphide
402 production (Kuever et al. 2014), were indicative of both mangrove and seagrass soils. The
403 tea-associated ASVs in the seagrass ecosystems consisted of a diversity of putative anaerobic
404 functional groups from green tea, including methanogenic *Methanogenium* archaea, and
405 rooibos tea litters.

406 Together these results indicate that ecosystem largely influenced the prokaryotic microbiome
407 community. This was reflected in the overall community composition, as well as the putative
408 metabolic pathways utilised by the indicator taxa, e.g. nitrogen-cycling and methylotrophy in
409 freshwater wetlands and tidal marshes, and methanogenesis, sulphur cycling and fermentation
410 in mangroves and seagrasses. These differences reflect general salinity conditions (e.g.
411 marine taxa and sulphate reducers), and potentially the fluctuating conditions in the tidal
412 range allowing for different biogeochemical niches to be formed and utilised (Yin and Yan
413 2020). However, within the putative functional guilds in each ecosystem, the taxa that

414 colonised the tea litter from the soils were diverse, indicating association with the two tea
415 types and suggesting a degree of functional redundancy in the capacity to degrade different
416 OM compositions under predominantly anaerobic conditions (Pioli et al. 2020).

417

418 Shifts in the prokaryotic tea microbiome during the decomposition process. Focussing only
419 on tea-associated samples to further investigate successional shifts in prokaryotic microbiome
420 communities involved in tea litter decay, ecosystem type remained the key driver in the tea
421 prokaryotic microbiomes with significant ecosystem*time and ecosystem*substrate
422 interactions (Table S4). Indicator analysis of the ecosystem*time interaction resulted in 290
423 highly significant ASVs ($p \leq 0.001$; Figure 4). Several taxonomic groups were identified as
424 common to early or later stages of decay. For example, Anaerolineae family A4b and
425 *Spirochaeta* were common early on in the freshwater wetland and tidal marsh ecosystems,
426 while Deltaproteobacteria groups (*Desulfovibrio*, *Desulfuromonas*, Myxococcales) and
427 Bacteroidetes family BD2-2 were indicative of early decomposers in the three tidal
428 ecosystems. Tea decomposition at the later stages within the freshwater wetlands and tidal
429 marshes were indicated by methane-oxidiser *Methyloceanibacter* and cellulolytic fermenter
430 SBR1031 (Xia et al. 2016). Bathyarchaeota, known to consume a diversity of recalcitrant OM
431 types (fatty acids, aromatics, carbohydrate polymers) using a variety of anaerobic metabolic
432 pathways (Zhou et al. 2018), were shared by all the ecosystems except mangroves.
433 Furthermore, the tidal ecosystems were characterised by sulphur-cycling taxa at the 12-month
434 sampling, but from different genera compared to the 3-month sampling, including
435 *Desulfosarcina*, *Desulfatitalea*, *Desulfococcus* and *Sulfurovum*. This could suggest a potential
436 difference in r- and k-strategist lifestyles within the Deltaproteobacteria.

Despite these similarities across multiple ecosystem types, the colonisation and successional shifts in indicator taxa were mostly distinct within each ecosystem (Figure 4). For the freshwater wetlands, the indicator taxa shifted from aerobic Sphingomonadaceae and Bacteroidia (*Pedobacter* and *Sporocytophaga*) at three months to Xanthobacteraceae (*Bradyrhizobium* and purple sulphur bacteria Rhodopseudomonas) and Bacilli groups at 12 months. There were also ammonium-oxidising *Ellin6067* and *Nitrocosmicus* genera, as well as *Methanobacterium* and *Methyloceanibacter* ASVs in conjunction with *Syntrophobacter* in the later stages of decay in the freshwater wetlands, suggesting the use of multiple nitrogen- and methane-cycling pathways utilised one year into decomposition (Yarwood 2018). Later decay in the tidal marsh was also represented by ammonium- and sulphur- oxidising groups Nitrosococcaceae, *Nitrosopumilus*, Thiotrichaceae and *Thioalkalispira*. Mangrove tea communities shifted from anaerobic Bacteriodia indicator taxa to a community more indicative of sulphur cycling (*Sulfurimonas*, *Sulfurovum*, Desulfobacteraceae), as well as Woesearchaeia, recently implicated in assisting with methanogensis in seagrass soils (Burkholz 2018). In contrast, seagrass tea samples shifted from sulphate reducer-dominant Deltaproteobacteria to increased Gammaproteobacteria as decay progressed, including *Thiodiazotropha*, recently found to be a seagrass root endosymbiont involved in detoxifying sulphides within the rhizosphere (Martin et al. 2020b).

These results indicated shifts in key taxa contributing to early and later stages of microbial breakdown of the tea litter in wetland soils. We found that some of these shifts in indicator taxa were within similar putative functional groups, and potentially linked to changing availability and OM quality as decomposition progressed. Most notably, the sulphate reducers within the Deltaproteobactera shifted from *Desulfovibrio* and *Desulfuromonas* indicating earlier tea decay, to *Desulfatitalea* and *Desulfosarcina* from Desulfobacteraceae indicating later decay. Additionally, we found a greater diversity in the metabolic pathways

462 associated with the taxa during the 12-month sampling, including sulphur-oxidising,
463 ammonium-oxidising and methane cycling. These results emphasise the key importance of
464 multiple biogeochemical pathways for carbon degradation as decomposition progresses. For
465 example, recent reports have highlighted the magnitude of methanogenesis and methane
466 cycling in blue carbon ecosystems and the potential of these emissions to reduce net carbon
467 preservation (Garcias-Bonet and Duarte 2017, Rosentreter et al. 2018, Al-Haj and Fulweiler
468 2020). Further, while the taxa colonising the tea litter were primarily anaerobic (Figures 4,
469 S4), the presence of taxa that utilised oxidative pathways for OM breakdown may be linked
470 to root in-growth. The increased intrusion of roots into the litter bag over time may reflect the
471 rhizosphere microbes and plants utilising the rich source of tea OM or contributing
472 prokaryotic taxa to the tea microbiome itself, thereby highlighting the importance of the
473 rhizosphere in nutrient cycling and detoxification for plant and soil health (Zheng et al. 2016,
474 Martin et al. 2020a).
475
476 *Determining direct and indirect effects of ecosystem type*
477 Our model implies that ecosystem type affects remaining mass directly and indirectly through
478 changes in microbial taxonomic composition. The prokaryotic microbiome PCO shows
479 differences in species composition among ecosystem types along the first axis (PCO1),
480 whereas the second axis (PCO2) reflects changes in species composition between the 3rd and
481 12th month of incubation (as seen in Figure 3). The bulk soil microbial communities did not
482 change in a similar magnitude as the tea-associated communities over the incubation (Figure
483 3), confirming that the PCO2 changes were likely related to the decomposition process rather
484 than local or seasonal changes. Thus, the path between incubation time and remaining mass
485 was included as a correlation, as there is no expected directional causality between them.

Mass remaining was affected by incubation time indirectly via the microbial community for both tea types, and by ecosystem directly but only for green tea (Figure 5, Table S5-S6). For the green tea, we postulate that the direct ecosystem effect was likely a reflection of the influence of ecosystem-specific leaching prior to the first mass loss sampling at 3-month (Figure 5a), an ecosystem-driven pattern that was maintained throughout the decomposition period (Figure 5a). In contrast, rooibos tea mass loss was not dependent on ecosystem type in the SEM ($\chi^2 = 1.62$, DF = 3, $p = 0.65$; Table S5). Rather, the shift in microbial community composition as incubation period progressed was strongly related to the remaining mass of the tea, particularly of the rooibos tea (Figure 5b). These results reflect that the mass loss between 3 and 12 months was greater for the rooibos tea (11-19% versus 4-6% for green tea), and that the associated prokaryotic microbial communities also shifted as decay progressed. We suspect that the green tea was more homogeneous after the leaching process, containing more recalcitrant carbohydrate and lipid/wax residues (Duddigan et al. 2020). Rooibos tea, conversely, may be representing greater diversity in (usable) OM over the year of decomposition, likely as polysaccharides, carbohydrates and cell-wall compounds (Duddigan et al. 2020). These potential shifts in OM quality over time for both tea litters match the indicator taxa at 12 months that have the putative functions for cellulose and recalcitrant OM degradation. Further, the greater amount and diversity of accessible OM for the rooibos substrate may be linked to the higher community alpha diversity compared to the green tea litter (Figure S2). This, in addition to the magnitude of change of the rooibos tea community during decomposition, likely underlies the tighter links to the microbial communities utilising this substrate.

Perspectives on microbial-driven decomposition of tea litter in wetlands

The key drivers in both tea litter decay and tea-associated prokaryotic communities were the differences between freshwater/tidal marsh wetlands versus mangrove/seagrass ecosystems. We hypothesise that the ecosystem differences in decay rates were in part from abiotic tidal flushing and inundation, whereas salinity and potentially inundation-linked anoxic conditions defined the prokaryotic microbiome structure and putative functions. The division due to marine influence is not surprising as salinity is shown to be a strong influence in the formation of the prokaryotic microbiome communities and diversity (Lozupone and Knight 2007, Balmonte et al. 2020). When considering the decomposition process over a time series, salinity alone has been shown to be a smaller influence on the rate of aerobic organic matter breakdown than the quality and amount of organic matter available (Balmonte et al. 2020). In contrast, this study focussed on belowground organic matter turnover where the selective forces of both salinity and anoxic conditions (e.g. mangrove and seagrass soils) on the microbiome structure and functional groups, coupled with the differing quality of the tea litters, were important for the differential litter breakdown in wetlands. This combined effect has been shown to enhance litter decomposition in tidal marsh (Zhai et al. 2021) and mangrove (Kristensen et al. 2008, Ouyang et al. 2017) ecosystems, including belowground cellulose decomposition (Stagg et al. 2018). At the same time, the mangrove and seagrass ecosystems likely experiencing different inundation patterns also drove abiotic leaching early-on in the decomposition process, which is independent of the redox effects of water-logged soils (Godshalk and Wetzel 1978). While we cannot tease apart the roles of salinity, oxygen and inundation separately, it is likely we are quantifying enhanced decomposition in the mangrove and seagrass ecosystems via multiple biotic and abiotic pathways in response to flooding (Stagg et al. 2017). Such combinations of flooding and salinity intrusion have been predicted to impact decomposition in tidal wetlands differently according to the position

along the tidal frame (e.g. marine to freshwater tidal), and dependent on litter quality (Stagg et al. 2017, Stagg et al. 2018). Future studies using the standardised tea litters may be promising for teasing apart these impacts on decomposition along a salinity and tidal gradient.

We were able to identify cross-ecosystem commonalities (e.g. potential functional redundancies) and differences (e.g. community structure linked to salinity and oxygen) of microbial turnover of tea litter. The shift in the communities over time highlights the taxa that may be employing *K*-strategist lifestyles during the decomposition process, as well as the increasing presence of oxidising groups found in all ecosystem types. Both strategies may be important to the decomposition of more structurally complex forms of carbon, such as lignocellulose and lipids/waxes (Cragg et al. 2020). Further, the decay rates and putative metabolic functions of the prokaryotic microbiomes observed in this study could be reflecting the enzymatic activity of the microbiota across freshwater and coastal soils, which are influenced by functional and enzymatic diversity, temperature, pH and substrate availability (Arnosti 2011, Arnosti et al. 2014). Linking decay rates with enzyme diversity and activity has been shown to be a key facet to explaining organic matter turnover and preservation in terrestrial ecosystems (Wieder et al. 2013, Bradford et al. 2017). Inclusion of these variables could help parameterise the carbon cycling and preservation models both for within- and cross-ecosystems comparisons.

In conclusion, this cross-ecosystem study on standardised tea litter decomposition and associated prokaryotic microbiomes showed that wetland type was a dominant indicator of microbial-driven tea litter decay. Tea litter decomposition was driven directly by wetland type, likely via abiotic factors like inundation, whereas the tea-associated microbial

communities and their putative functions, which were likely influenced by salinity, oxygen availability and shifts in OM quality, indirectly drove decay. We found preliminary evidence of a climatic effect across a continental scale, whereby the warmer climates exhibited enhanced mass loss over 12 months of decomposition for both tea types. Together, the results presented here provides new insight into the factors influencing tea litter decomposition by prokaryotic microbiota, and advances our understanding of belowground processes in wetlands over a range of spatiotemporal scales and ecosystems.

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605 Literature Cited

606 Al-Haj, A. N., and R. W. Fulweiler. 2020. A synthesis of methane emissions from shallow
607 vegetated coastal ecosystems. Global Change Biology **26**:2988-3005.
608 Anderson, M. J., R. N. Gorley, and K. R. Clarke. 2008. PERMANOV + for PRIMER: Guide
609 to Software and Statistical Methods. PRIMER-E Ltd, Devon, UK.
610 Arnosti, C. 2011. Microbial extracellular enzymes and the marine carbon cycle. Annual
611 Review of Marine Science **3**:401-425.

Arnosti, C., C. Bell, D. Moorhead, R. Sinsabaugh, A. Steen, M. Stromberger, M. Wallenstein, and M. Weintraub. 2014. Extracellular enzymes in terrestrial, freshwater, and marine environments: perspectives on system variability and common research needs. Biogeochemistry **117**:5-21.

Baird, M. E., K. A. Wild-Allen, J. Parslow, M. Mongin, B. Robson, J. Skerratt, F. Rizwi, M. Soja-Woznaik, E. Jones, M. Herzfeld, N. Margvelashvili, J. Andrewartha, C. Langlais, M. P. Adams, N. Cherukuru, M. Gustafsson, S. Hadley, P. J. Ralph, U. Rosebrock, T. Schroeder, L. Laiolo, D. Harrison, and A. D. Steven. 2020. CSIRO Environmental Modelling Suite (EMS): scientific description of the optical and biogeochemical models (vB3p0). Geoscientific Model Development **13**:4503-4553.

Balmonte, J. P., H. Hasler-Sheetal, R. N. Glud, T. J. Andersen, M. K. Sejr, M. Middelboe, A. Teske, and C. Arnosti. 2020. Sharp contrasts between freshwater and marine microbial enzymatic capabilities, community composition, and DOM pools in a NE Greenland fjord. Limnology and oceanography **65**:77-95.

Bates, D., M. Maechler, B. Bolker, S. Walker, and R. Haubo Bojesen Christensen. 2015. lme4: Linear mixed-effects models using Eigen and S4. R package version 1.1–7. 2014.

Behera, P., M. Mohapatra, J. Y. Kim, T. K. Adhya, A. K. Pattnaik, and G. Rastogi. 2019. Spatial and temporal heterogeneity in the structure and function of sediment bacterial communities of a tropical mangrove forest. Environmental Science and Pollution Research **26**:3893-3908.

Berg, B., and C. McClaugherty. 2008. Decomposition as a Process. Pages 11-33 *in* B. Berg and C. McClaugherty, editors. Plant Litter. Springer-Verlag, Berlin Heidelberg.

Bolyen, E., J. R. Rideout, M. R. Dillon, N. A. Bokulich, C. Abnet, G. A. Al-Ghalith, H. Alexander, E. J. Alm, M. Arumugam, and F. Asnicar. 2018. QIIME 2: Reproducible, interactive, scalable, and extensible microbiome data science. 2167-9843, PeerJ Preprints.

Bonan, G. B., M. D. Hartman, W. J. Parton, and W. R. Wieder. 2013. Evaluating litter decomposition in earth system models with long-term litterbag experiments: an example using the Community Land Model version 4 (CLM 4). Global Change Biology **19**:957-974.

Bradford, M. A., B. Berg, D. S. Maynard, W. R. Wieder, and S. A. Wood. 2016. Understanding the dominant controls on litter decomposition. Journal of Ecology **104**:229-238.

Bradford, M. A., G. M. Tordoff, T. Eggers, T. H. Jones, and J. E. Newington. 2002. Microbiota, fauna, and mesh size interactions in litter decomposition. Oikos **99**:317-323.

Bradford, M. A., G. C. Veen, A. Bonis, E. M. Bradford, A. T. Classen, J. H. C. Cornelissen, T. W. Crowther, R. Jonathan, G. T. Freschet, and P. Kardol. 2017. A test of the hierarchical model of litter decomposition. Nature Ecology & Evolution **1**:1836-1845.

Brinson, M. M., A. E. Lugo, and S. Brown. 1981. Primary productivity, decomposition and consumer activity in freshwater wetlands. Annual review of ecology and systematics **12**:123-161.

Burkholz, C. 2018. The Effect of Increasing Temperature on Greenhouse Gas Emissions by *Halophila stipulacea* in the Red Sea. King Abdullah University of Science and Technology.

Cáceres, M. D., and P. Legendre. 2009. Associations between species and groups of sites: indices and statistical inference. Ecology **90**:3566-3574.

660 Callahan, B. J., P. J. McMurdie, M. J. Rosen, A. W. Han, A. J. A. Johnson, and S. P. Holmes.
661 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Nature
662 methods **13**:581-583.
663 Chambers, L. G., T. Z. Osborne, and K. R. Reddy. 2013. Effect of salinity-altering pulsing
664 events on soil organic carbon loss along an intertidal wetland gradient: a laboratory
665 experiment. Biogeochemistry **115**:363-383.
666 Chimner, R. A., and K. C. Ewel. 2005. A tropical freshwater wetland: II. Production,
667 decomposition, and peat formation. Wetlands Ecology and Management **13**:671-684.
668 Connolly, C. T., W. V. Sobczak, and S. E. Findlay. 2014. Salinity effects on Phragmites
669 decomposition dynamics among the Hudson River's freshwater tidal wetlands.
670 Wetlands **34**:575-582.
671 Cragg, S. M., D. A. Friess, L. G. Gillis, S. M. Trevathan-Tackett, O. M. Terrett, J. E. Watts,
672 D. L. Distel, and P. Dupree. 2020. Vascular plants are globally significant
673 contributors to marine carbon fluxes and sinks. Annual Review of Marine Science
674 **12**:469-497.
675 Didion, M., A. Repo, J. Liski, M. Forsius, M. Bierbaumer, and I. Djukic. 2016. Towards
676 harmonizing leaf litter decomposition studies using standard tea bags—a field study
677 and model application. Forests **7**:167.
678 Djukic, I., S. Kepfer-Rojas, I. K. Schmidt, K. S. Larsen, C. Beier, B. Berg, K. Verheyen, A.
679 Caliman, A. Paquette, and A. Gutiérrez-Girón. 2018. Early stage litter decomposition
680 across biomes. Science of The Total Environment **628**:1369-1394.
681 Duddigan, S., L. J. Shaw, P. D. Alexander, and C. D. Collins. 2020. Chemical Underpinning
682 of the Tea Bag Index: An Examination of the Decomposition of Tea Leaves. Applied
683 and Environmental Soil Science **2020**:6085180.
684 Dufrêne, M., and P. Legendre. 1997. Species assemblages and indicator species: the need for
685 a flexible asymmetrical approach. Ecological Monographs **67**:345-366.
686 Ewers Lewis, C. J., M. A. Young, D. Ierodiaconou, J. A. Baldock, B. Hawke, J. Sanderman,
687 P. E. Carnell, and P. I. Macreadie. 2020. Drivers and modelling of blue carbon stock
688 variability in sediments of southeastern Australia. Biogeosciences **17**:2041-2059.
689 Fanin, N., S. Bezaud, J. M. Sarneel, S. Cecchini, M. Nicolas, and L. Augusto. 2020. Relative
690 Importance of Climate, Soil and Plant Functional Traits During the Early
691 Decomposition Stage of Standardized Litter. Ecosystems **23**:1004-1018.
692 Garcias-Bonet, N., and C. M. Duarte. 2017. Methane production by seagrass ecosystems in
693 the Red Sea. Frontiers in Marine Science **4**:340.
694 Godshalk, G. L., and R. G. Wetzel. 1978. Decomposition of aquatic angiosperms. III. *Zostera*
695 *marina* L. and a conceptual model of decomposition. Aquatic Botany **5**:329-354.
696 Janousek, C. N., K. J. Buffington, G. R. Guntenspergen, K. M. Thorne, B. D. Dugger, and J.
697 Y. Takekawa. 2017. Inundation, vegetation, and sediment effects on litter
698 decomposition in Pacific Coast tidal marshes. Ecosystems **20**:1296-1310.
699 Kelleway, J. J., O. Serrano, J. A. Baldock, R. Burgess, T. Cannard, P. S. Lavery, C. E.
700 Lovelock, P. I. Macreadie, P. Masqué, M. Newnham, N. Saintilan, and A. D. L.
701 Steven. 2020. A national approach to greenhouse gas abatement through blue carbon
702 management. Global Environmental Change **63**:102083.
703 Keuskamp, J. A., B. J. Dingemans, T. Lehtinen, J. M. Sarneel, and M. M. Hefting. 2013. Tea
704 Bag Index: a novel approach to collect uniform decomposition data across
705 ecosystems. Methods in Ecology and Evolution **4**:1070-1075.
706 Kirwan, M. L., and J. P. Megonigal. 2013. Tidal wetland stability in the face of human
707 impacts and sea-level rise. Nature **504**:53-60.
708 Kristensen, E., S. Bouillon, T. Dittmar, and C. Marchand. 2008. Organic carbon dynamics in
709 mangrove ecosystems: a review. Aquatic Botany **89**:201-219.

Kuever, J., F. Rainey, and F. Widdel. 2014. The family syntrophobacteraceae. The Prokaryotes: Deltaproteobacteria and Epsilonproteobacteria:289-299.
Lamont, K., N. Saintilan, J. J. Kelleway, D. Mazumder, and A. Zawadzki. 2020. Thirty-year repeat measures of mangrove above-and below-ground biomass reveals unexpectedly high carbon sequestration. Ecosystems **23**:370-382.
Lefcheck, J., and R. Freckleton. 2016. Piecewise SEM: piecewise structural equation modelling in R for ecology, evolution and systematics. Methods Ecol Evol 7 (5): 573–579.
Lenth, R., H. Singmann, J. Love, P. Buerkner, and M. Herve. 2018. Emmeans: Estimated marginal means, aka least-squares means. R package version **1**:3.
Lozupone, C. A., and R. Knight. 2007. Global patterns in bacterial diversity. Proceedings of the National Academy of Sciences **104**:11436-11440.
Macreadie, P. I., D. A. Nielsen, J. J. Kelleway, T. B. Atwood, J. R. Seymour, K. Petrou, R. M. Connolly, A. C. Thomson, S. M. Trevathan-Tackett, and P. J. Ralph. 2017. Can we manage coastal ecosystems to sequester more blue carbon? Frontiers in Ecology and the Environment **15**:206-213.
Marley, A. R., C. Smeaton, and W. E. Austin. 2019. An assessment of the tea bag index method as a proxy for organic matter decomposition in intertidal environments. Journal of Geophysical Research: Biogeosciences **124**:2991-3004.
Martin, B. C., M. S. Alarcon, D. Gleeson, J. A. Middleton, M. W. Fraser, M. H. Ryan, M. Holmer, G. A. Kendrick, and K. Kilminster. 2020a. Root microbiomes as indicators of seagrass health. FEMS microbiology ecology **96**:fiz201.
Martin, B. C., J. A. Middleton, M. W. Fraser, I. P. Marshall, V. V. Scholz, and H. Schmidt. 2020b. Cutting out the middle clam: lucinid endosymbiotic bacteria are also associated with seagrass roots worldwide. bioRxiv.
Mitsch, W. J., B. Bernal, A. M. Nahlik, Ü. Mander, L. Zhang, C. J. Anderson, S. E. Jørgensen, and H. Brix. 2013. Wetlands, carbon, and climate change. Landscape Ecology **28**:583-597.
Moomaw, W. R., G. Chmura, G. T. Davies, C. Finlayson, B. A. Middleton, S. M. Natali, J. Perry, N. Roulet, and A. E. Sutton-Grier. 2018. Wetlands in a changing climate: science, policy and management. Wetlands **38**:183-205.
Morrissey, E. M., J. L. Gillespie, J. C. Morina, and R. B. Franklin. 2014. Salinity affects microbial activity and soil organic matter content in tidal wetlands. Global Change Biology **20**:1351-1362.
Mozdzer, T. J., S. E. Drew, J. S. Caplan, P. E. Weber, and L. A. Deegan. 2020. Rapid recovery of carbon cycle processes after the cessation of chronic nutrient enrichment. Science of The Total Environment **750**:140927.
Mueller, P., L. M. Schile-Beers, T. J. Mozdzer, G. L. Chmura, T. Dinter, Y. Kuzyakov, A. V. d. Groot, P. Esselink, C. Smit, A. D'Alpaos, C. Ibanez, M. Lazarus, U. Neumeier, B. J. Johnson, A. H. Baldwin, S. A. Yarwood, D. I. Montemayor, Z. Yang, J. Wu, K. Jensen, and S. Nolte. 2018. Global-change effects on early-stage decomposition processes in tidal wetlands–implications from a global survey using standardized litter. Biogeosciences **15**:3189-3202.
Nahlik, A. M., and M. S. Fennessy. 2016. Carbon storage in US wetlands. Nature communications **7**:1-9.
Nicastro, A., Y. Onoda, and M. J. Bishop. 2012. Direct and indirect effects of tidal elevation on eelgrass decomposition. Marine Ecology Progress Series **456**:53-62.
Ochoa-Hueso, R., M. Delgado-Baquerizo, P. T. A. King, M. Benham, V. Arca, and S. A. Power. 2019. Ecosystem type and resource quality are more important than global

759 change drivers in regulating early stages of litter decomposition. Soil Biology and
760 Biochemistry **129**:144-152.
761 Ouyang, X., S. Y. Lee, and R. M. Connolly. 2017. The role of root decomposition in global
762 mangrove and saltmarsh carbon budgets. Earth-Science Reviews **166**:53-63.
763 Palacios, M. M., S. M. Trevathan-Tackett, M. E. Malerba, and P. I. Macreadie. 2021. Effects
764 of a nutrient enrichment pulse on blue carbon ecosystems. Marine Pollution Bulletin
765 **165**:112024.
766 Pester, M., N. Bittner, P. Deevong, M. Wagner, and A. Loy. 2010. A 'rare
767 biosphere'microorganism contributes to sulfate reduction in a peatland. The ISME
768 journal **4**:1591-1602.
769 Petraglia, A., C. Cacciatori, S. Chelli, G. Fenu, G. Calderisi, D. Gargano, T. Abeli, S.
770 Orsenigo, and M. Carbognani. 2019. Litter decomposition: effects of temperature
771 driven by soil moisture and vegetation type. Plant and Soil **435**:187-200.
772 Pioli, S., J. Sarneel, H. J. Thomas, X. Domene, P. Andrés, M. Hefting, T. Reitz, H. Laudon,
773 T. Sandén, and V. Piscová. 2020. Linking plant litter microbial diversity to
774 microhabitat conditions, environmental gradients and litter mass loss: Insights from a
775 European study using standard litter bags. Soil Biology and Biochemistry:107778.
776 Quast, C., E. Pruesse, P. Yilmaz, J. Gerken, T. Schweer, P. Yarza, J. Peplies, and F. O.
777 Glöckner. 2012. The SILVA ribosomal RNA gene database project: improved data
778 processing and web-based tools. Nucleic acids research **41**:D590-D596.
779 Rosentreter, J. A., D. T. Maher, D. V. Erler, R. H. Murray, and B. D. Eyre. 2018. Methane
780 emissions partially offset "blue carbon" burial in mangroves. Science advances
781 **4**:eaao4985.
782 Seelen, L. M., G. Flaim, J. Keuskamp, S. Teurlincx, R. A. Font, D. Tolunay, M. Fránková, K.
783 Šumberová, M. Temponeras, and M. Lenhardt. 2019. An affordable and reliable
784 assessment of aquatic decomposition: Tailoring the Tea Bag Index to surface waters.
785 Water Research **151**:31-43.
786 Serrano, O., C. E. Lovelock, T. B. Atwood, P. I. Macreadie, R. Canto, S. Phinn, A. Arias-
787 Ortiz, L. Bai, J. Baldock, C. Bedulli, P. Carnell, R. M. Connolly, P. Donaldson, A.
788 Esteban, C. J. Ewers Lewis, B. D. Eyre, M. A. Hayes, P. Horwitz, L. B. Hutley, C. R.
789 J. Kavazos, J. J. Kelleway, G. A. Kendrick, K. Kilminster, A. Lafratta, S. Lee, P. S.
790 Lavery, D. T. Maher, N. Marbà, P. Masque, M. A. Mateo, R. Mount, P. J. Ralph, C.
791 Roelfsema, M. Rozaimi, R. Ruhon, C. Salinas, J. Samper-Villarreal, J. Sanderman, C.
792 J. Sanders, I. Santos, C. Sharples, A. D. L. Steven, T. Cannard, S. M. Trevathan-
793 Tackett, and C. M. Duarte. 2019. Australian vegetated coastal ecosystems as global
794 hotspots for climate change mitigation. Nature communications **10**:1-10.
795 Spivak, A. C., J. Sanderman, J. L. Bowen, E. A. Canuel, and C. S. Hopkinson. 2019. Global-
796 change controls on soil-carbon accumulation and loss in coastal vegetated
797 ecosystems. Nature Geoscience **12**:685-692.
798 Stagg, C. L., M. M. Baustian, C. L. Perry, T. J. Carruthers, and C. T. Hall. 2018. Direct and
799 indirect controls on organic matter decomposition in four coastal wetland
800 communities along a landscape salinity gradient. Journal of Ecology **106**:655-670.
801 Stagg, C. L., D. R. Schoolmaster, K. W. Krauss, N. Cormier, and W. H. Conner. 2017.
802 Causal mechanisms of soil organic matter decomposition: deconstructing salinity and
803 flooding impacts in coastal wetlands. Ecology **98**:2003-2018.
804 Trevathan-Tackett, S. M., K. E. Brodersen, and P. I. Macreadie. 2020a. Effects of elevated
805 temperature on microbial breakdown of seagrass leaf and tea litter biomass.
806 Biogeochemistry.

807 Trevathan-Tackett, S. M., T. C. Jeffries, P. I. Macreadie, B. Manojlovic, and P. Ralph. 2020b.
808 Long-term decomposition captures key steps in microbial breakdown of seagrass
809 litter. Science of The Total Environment **705**:135806.
810 Trevathan-Tackett, S. M., P. I. Macreadie, J. Sanderman, J. Baldock, J. Howes, and P. Ralph.
811 2017. A global assessment of the chemical recalcitrance of seagrass tissues:
812 implications for long-term carbon sequestration. Frontiers in Plant Science **8**:925.
813 Walter, H., and S.-W. Breckle. 1999. Vegetation und Klimazonen. Ulmer, Stuttgart **544**.
814 Walters, W., E. R. Hyde, D. Berg-Lyons, G. Ackermann, G. Humphrey, A. Parada, J. A.
815 Gilbert, J. K. Jansson, J. G. Caporaso, and J. A. Fuhrman. 2016. Improved bacterial
816 16S rRNA gene (V4 and V4-5) and fungal internal transcribed spacer marker gene
817 primers for microbial community surveys. Msystems **1**:e00009-00015.
818 Webster, J., and E. Benfield. 1986. Vascular plant breakdown in freshwater ecosystems.
819 Annual review of ecology and systematics **17**:567-594.
820 Wieder, R. K., and G. E. Lang. 1982. A critique of the analytical methods used in examining
821 decomposition data obtained from litter bags. Ecology **63**:1636-1642.
822 Wieder, W. R., G. B. Bonan, and S. D. Allison. 2013. Global soil carbon projections are
823 improved by modelling microbial processes. Nature Climate Change **3**:909-912.
824 Xia, Y., Y. Wang, Y. Wang, F. Y. Chin, and T. Zhang. 2016. Cellular adhesiveness and
825 cellulolytic capacity in Anaerolineae revealed by omics-based genome interpretation.
826 Biotechnology for Biofuels **9**:111.
827 Yarwood, S. A. 2018. The role of wetland microorganisms in plant-litter decomposition and
828 soil organic matter formation: a critical review. FEMS microbiology ecology
829 **94**:fiy175.
830 Yin, Y., and Z. Yan. 2020. Variations of soil bacterial diversity and metabolic function with
831 tidal flat elevation gradient in an artificial mangrove wetland. Science of The Total
832 Environment **718**:137385.
833 Zhai, J., J. T. Anderson, G. Yan, L. Cong, Y. Wu, L. Dai, J. Liu, and Z. Zhang. 2021.
834 Decomposition and nutrient dynamics responses of plant litter to interactive effects of
835 flooding and salinity in Yellow River Delta wetland in northeastern China. Ecological
836 Indicators **120**:106943.
837 Zheng, Y., L. Hou, M. Liu, G. Yin, J. Gao, X. Jiang, X. Lin, X. Li, C. Yu, and R. Wang.
838 2016. Community composition and activity of anaerobic ammonium oxidation
839 bacteria in the rhizosphere of salt-marsh grass Spartina alterniflora. Applied
840 Microbiology and Biotechnology **100**:8203-8212.
841 Zhou, Z., J. Pan, F. Wang, J.-D. Gu, and M. Li. 2018. Bathyarchaeota: globally distributed
842 metabolic generalists in anoxic environments. FEMS microbiology reviews **42**:639-
843 655.

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Figure Legends

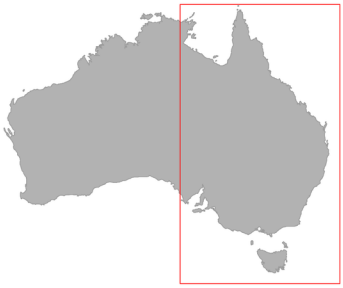
Figure 1: Tea litter decomposition site map.

Figure 2: Ecosystem and climate effects on tea decomposition in wetlands. Ecosystem type significantly ($p < 0.05$) affected the mass loss (a) and decay rates (b) of both the green and rooibos tea litter types. An analysis of a subset of sites that encompassed multiple climates revealed significant climatic effects on decomposition within freshwater wetlands (c; N = 10) and tidal marshes (d; N = 12). In all analyses, rooibos and green teas were significantly different from each other, so pairwise test letters indicate significant differences within each tea type. Capital letters represent pairwise differences for significant two-way interactions (tea*ecosystem/climate and time*ecosystem/climate), with horizontal and vertical brackets grouping the comparisons averaged across tea types and time, respectively; a, c). Lower-case letters represent pairwise differences for a significant three-way tea*climate*time interaction (d). Values are presented as the categorical time intervals and represent means ± standard error for proportion mass remaining and decay rate. Raw mass remaining data are presented in Figure S1.

Figure 3: Principal coordinates ordination of tea- and soil-associated prokaryotic microbiome communities. The soil-associated microbiome samples were collected at a similar depth as where the tea litter bags were decomposing. Samples from two sites were included per ecosystem, with replication of 2-3 samples for each site, substrate and time combination.

Figure 4: Sankey diagram of the relative abundance of the class-level taxa significant to the Ecosystem*Time interaction for tea samples. Only Amplicon Sequence Variants that were highly significant within this interaction ($p < 0.001$) are shown. The 290 ASVs were summarised into 35 classes that encompass 132 families. Thicker lines indicate higher relative abundance.

Figure 5: Structural equation model using prokaryotic microbial community compositions and tea biomass remaining for (a) green tea and (b) rooibos tea. Ecosystem (freshwater wetland, tidal marsh, mangrove, seagrass) and incubation time (in days) were the factors. Line colours indicate positive (black), negative (red) or categorical (grey) relationships, and numbers adjacent to lines are standardised path coefficients. For ecosystem type, unstandardized coefficients are given for each category, with letters indicating groupings based on posthoc Tukey tests. R2 are conditional values that include both random and fixed effects.



Legend

Ecosystem and sample type:

- Freshwater wetland
- Lotic
- Mangrove
- Seagrass
- Tidal marsh
- Mass loss only
- Mass loss and microbiome

Climate:

- Temperate climate
- Warm-temperate climate, humid climate
- Subtropical arid climate
- Mediterranean climate
- Equatorial humid climate
- Semi-arid tropical climate

0 250 500 1,000 Kilometers

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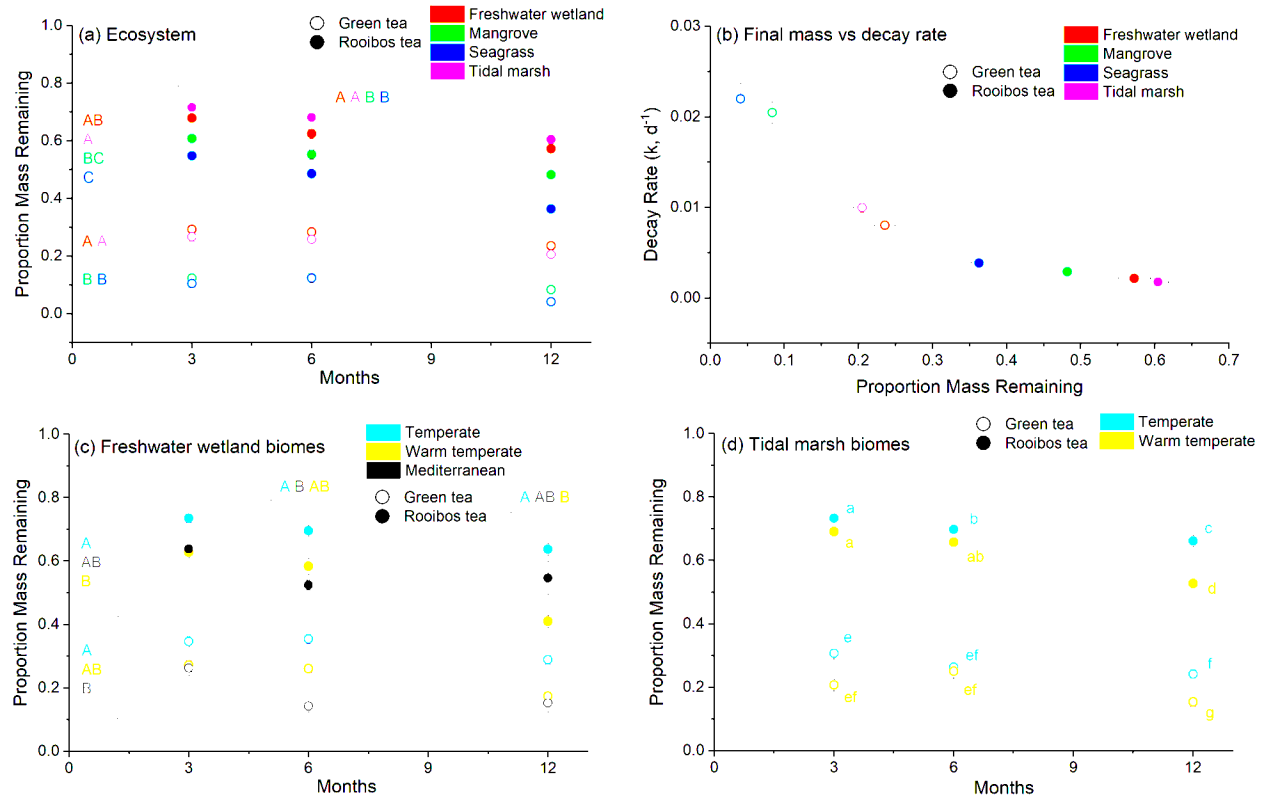
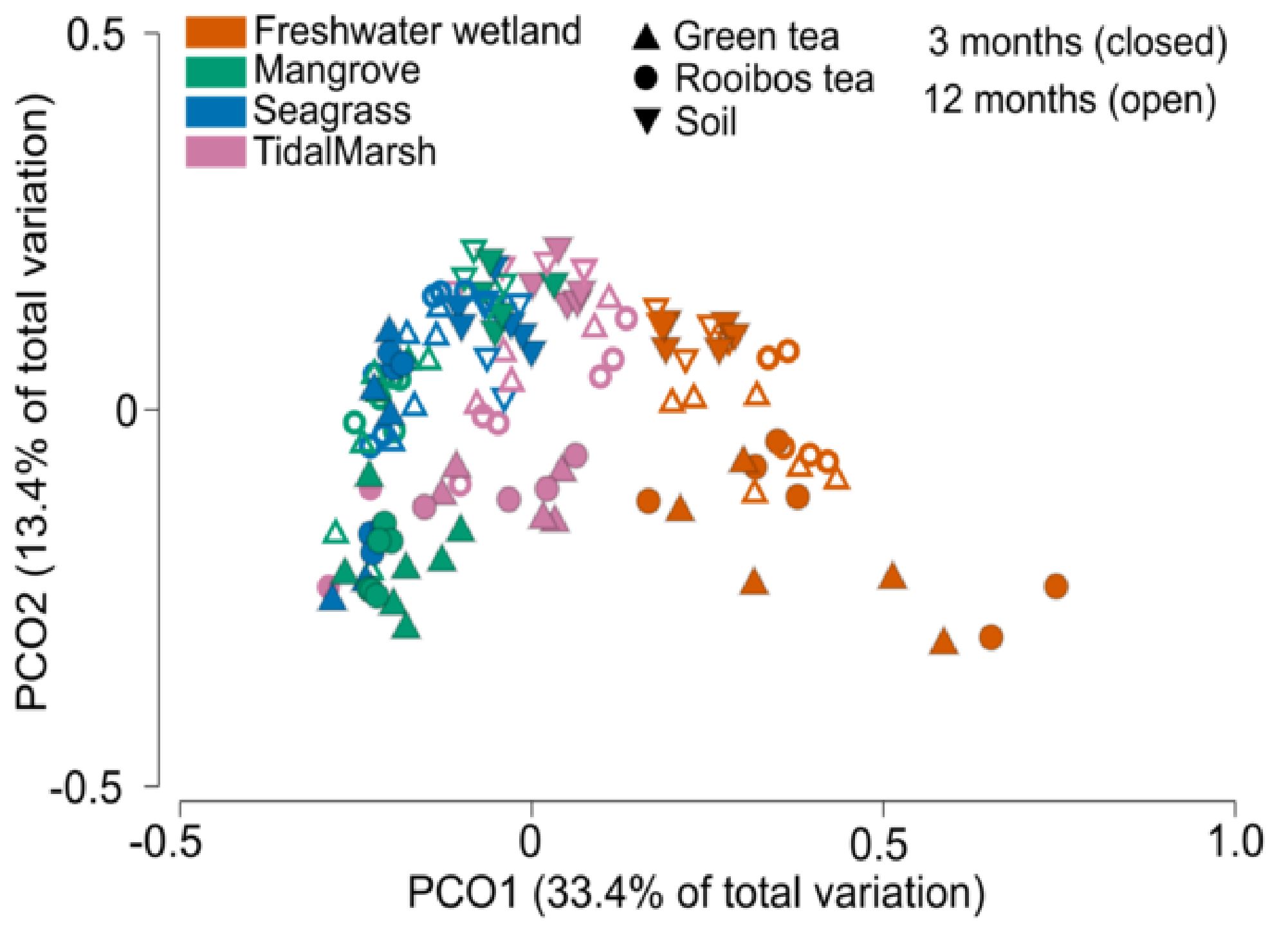
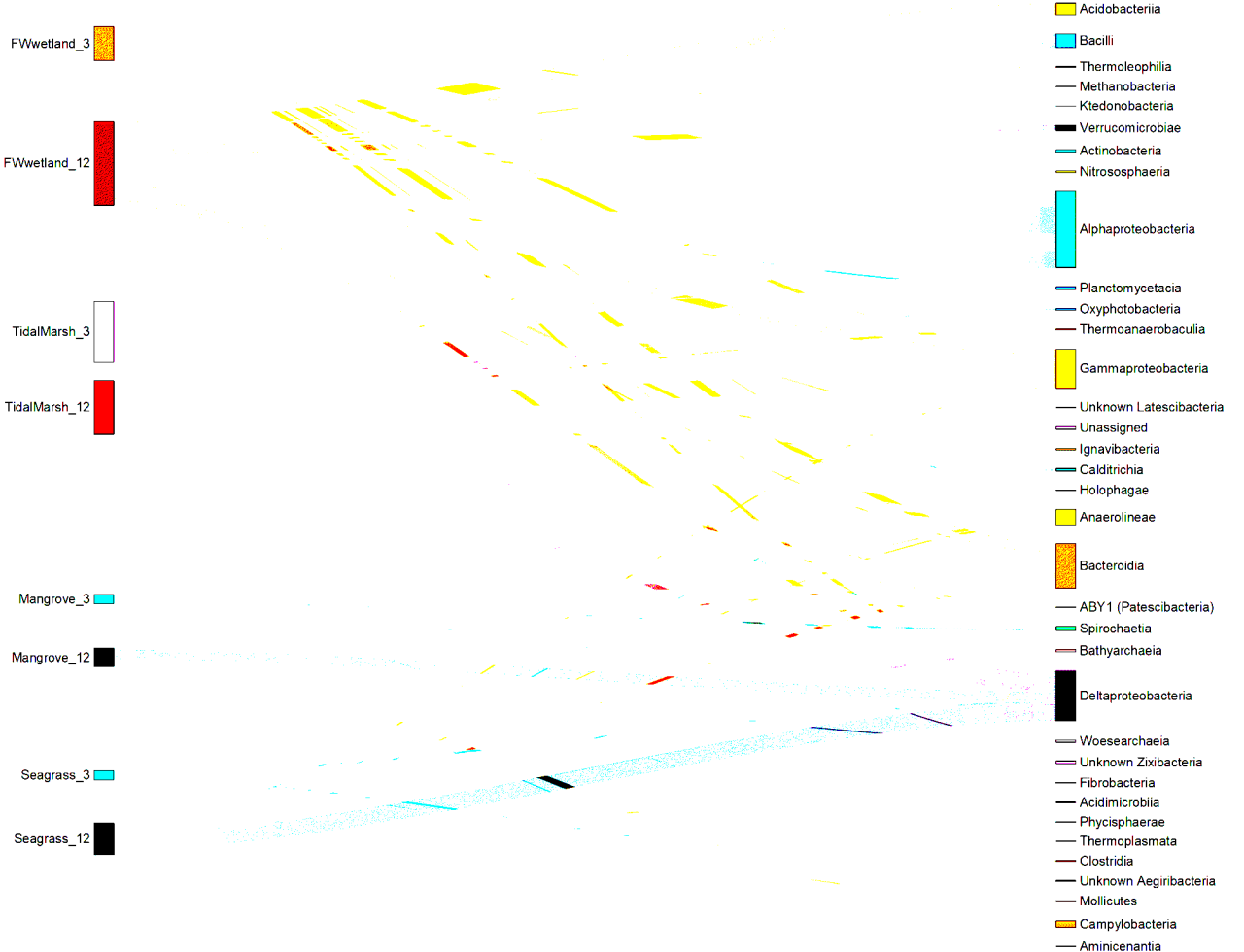
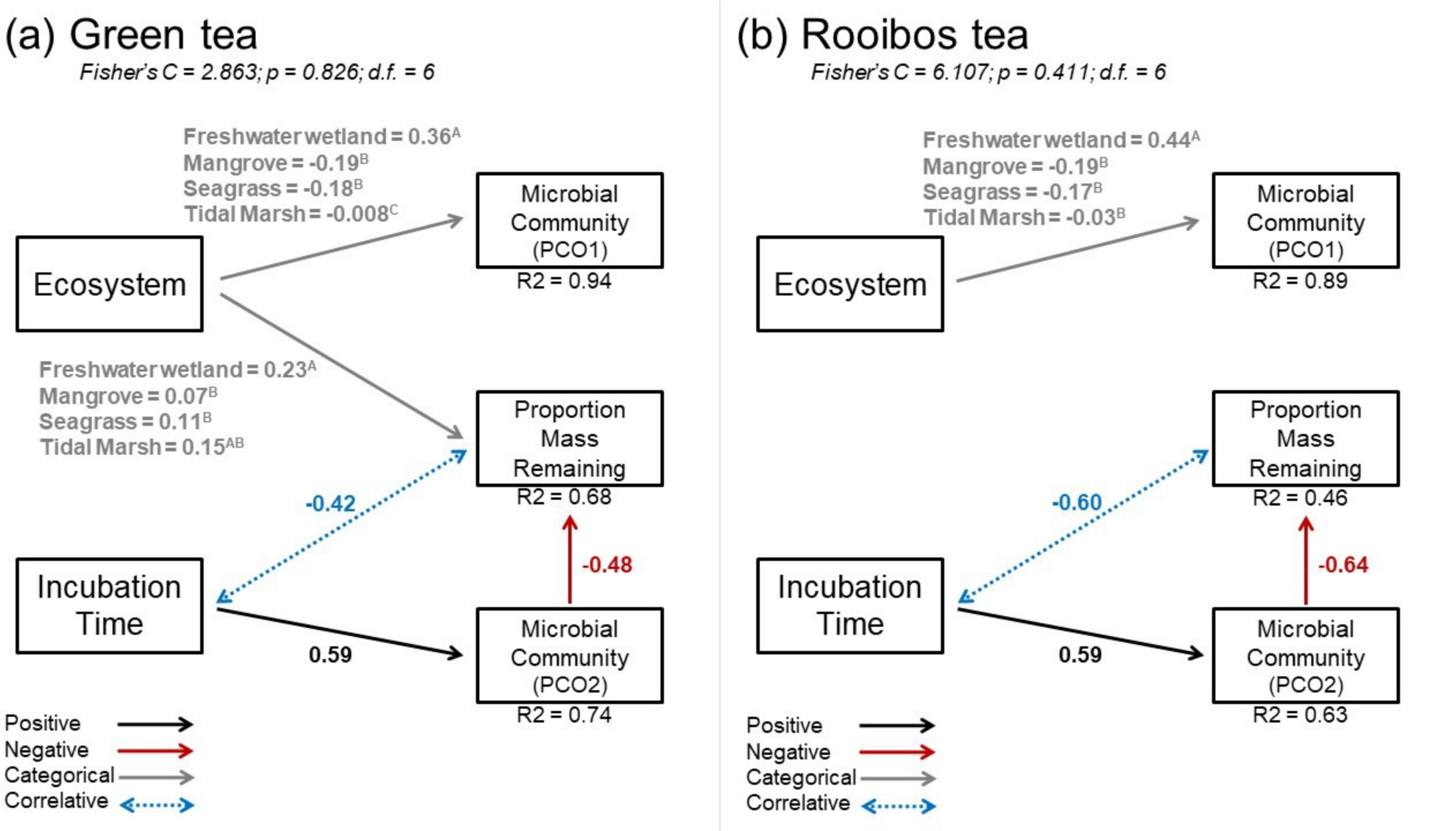
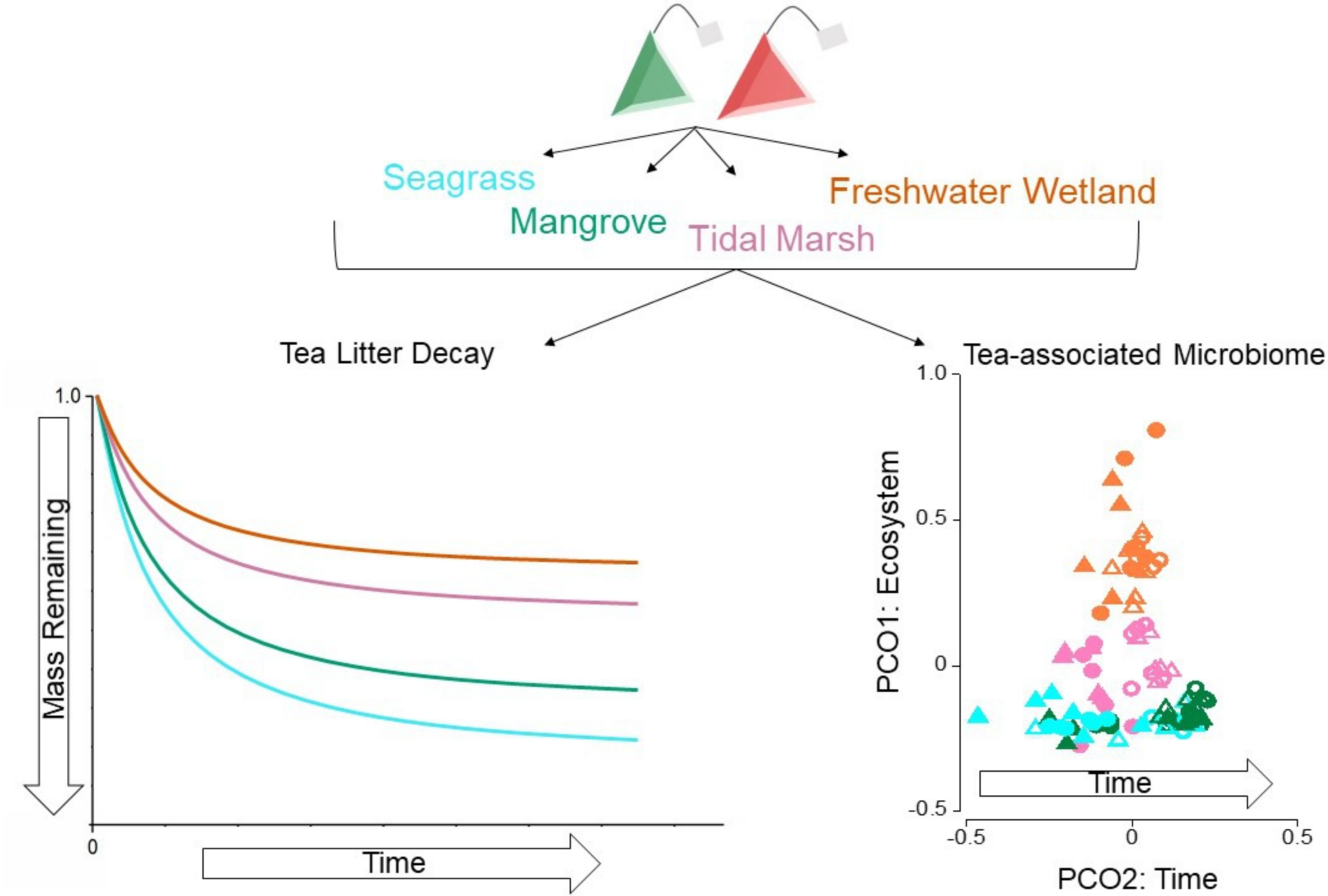

(a) Ecosystem
Green tea
Rooibos tea
Freshwater wetland
Mangrove
Seagrass
Tidal marsh
Proportion Mass Remaining
Months
AB
A
BC
C
A A
B B
A A B B
(b) Final mass vs decay rate
Green tea
Rooibos tea
Freshwater wetland
Mangrove
Seagrass
Tidal marsh
Decay Rate (k, d-1)
Proportion Mass Remaining
(c) Freshwater wetland biomes
Temperate
Warm temperate
Mediterranean
Green tea
Rooibos tea
Proportion Mass Remaining
Months
A
AB
B
A
AB
B
A B AB
A AB B
(d) Tidal marsh biomes
Green tea
Rooibos tea
Temperate
Warm temperate
Proportion Mass Remaining
Months
a
a
b
ab
c
d
e
ef
ef
f
g


Freshwater wetland
Mangrove
Seagrass
TidalMarsh
Green tea
Rooibos tea
Soil
3 months (closed)
12 months (open)
0.5
0
-0.5
PCO2 (13.4% of total variation)
-0.5
0
0.5
1.0
PCO1 (33.4% of total variation)


FWwetland_3
FWwetland_12
TidalMarsh_3
TidalMarsh_12
Mangrove_3
Mangrove_12
Seagrass_3
Seagrass_12
Acidobacteriia
Bacilli
Thermoleophilia
Methanobacteria
Ktedonobacteria
Verrucomicrobiae
Actinobacteria
Nitrososphaeria
Alphaproteobacteria
Planctomycetacia
Oxyphotobacteria
Thermoanaerobaculia
Gammaproteobacteria
Unknown Latescibacteria
Unassigned
Ignavibacteria
Calditrichia
Holophagae
Anaerolineae
Bacteroidia
ABY1 (Patescibacteria)
Spirochaetia
Bathyarchaeia
Deltaproteobacteria
Woesearchaeia
Unknown Zixibacteria
Fibrobacteria
Acidimicrobiia
Phycisphaerae
Thermoplasmata
Clostridia
Unknown Aegiribacteria
Mollicutes
Campylobacteria
Aminicenantia


(a) Green tea
Fisher's C = 2.863; p = 0.826; d.f. = 6
Freshwater wetland = 0.36A
Mangrove = -0.19B
Seagrass = -0.18B
Tidal Marsh = -0.008C
Ecosystem
Microbial Community (PCO1)
R2 = 0.94
Freshwater wetland = 0.23A
Mangrove = 0.07B
Seagrass = 0.11B
Tidal Marsh = 0.15AB
Proportion Mass Remaining
R2 = 0.68
-0.42
-0.48
Incubation Time
0.59
Microbial Community (PCO2)
R2 = 0.74
Positive
Negative
Categorical
Correlative
(b) Rooibos tea
Fisher's C = 6.107; p = 0.411; d.f. = 6
Freshwater wetland = 0.44A
Mangrove = -0.19B
Seagrass = -0.17B
Tidal Marsh = -0.03B
Ecosystem
Microbial Community (PCO1)
R2 = 0.89
Proportion Mass Remaining
R2 = 0.46
-0.60
-0.64
Incubation Time
0.59
Microbial Community (PCO2)
R2 = 0.63
Positive
Negative
Categorical
Correlative


Seagrass
Mangrove
Tidal Marsh
Freshwater Wetland
Tea Litter Decay
Mass Remaining
1.0
0
Time
Tea-associated Microbiome
PCO1: Ecosystem
1.0
0.5
0
-0.5
-0.5
0
0.5
Time
PCO2: Time