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An affordable and reliable assessment of aquatic decomposition: Tailoring the Tea Bag Index to surface waters

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ABSTRACT

Litter decomposition is a vital part of the global carbon cycle as it determines not only the amount of carbon to be sequestered, but also how fast carbon re-enters the cycle. Freshwater systems play an active role in the carbon cycle as it receives, and decomposes, terrestrial litter material alongside decomposing aquatic plant litter. Decomposition of organic matter in the aquatic environment is directly controlled by water temperature and nutrient availability, which are continuously affected by global change.

We adapted the Tea Bag Index (TBI), a highly standardized methodology for determining soil decomposition, for lakes by incorporating a leaching factor. By placing Lipton pyramid tea bags in the aquatic environment for 3 h, we quantified the period of intense leaching which usually takes place prior to litter (tea) decomposition. Standard TBI methodology was followed after this step to determine how fast decomposition takes place (decomposition rate, k_1) and how much of the material cannot be broken down and is thus sequestered (stabilization factor, S). A Citizen Science project was organized to test the aquatic TBI in 40 European lakes located in four climate zones, ranging from oligotrophic to hyper-eutrophic systems. We expected that warmer and/or eutrophic lakes would have a higher decomposition rate and a more efficient microbial community resulting in less tea material to be sequestered.

The overall high decomposition rates (k_1) found confirm the active role lakes play in the global carbon cycle. Across climate regions the lakes in the warmer temperate zone displayed a higher decomposition rate (k_1) compared to the colder lakes in the continental and polar zones. Across trophic states, decomposition rates were higher in eutrophic lakes compared to oligotrophic lakes. Additionally, the eutrophic lakes showed a higher stabilization (S), thus a less efficient microbial community, compared to the oligotrophic lakes, although the variation within this group was high. Our results clearly show that the TBI can be used to adequately assess the decomposition process in aquatic systems. Using “alien standard litter” such as tea provides a powerful way to compare decomposition across climates, trophic states and ecosystems.

By providing standardized protocols, a website, as well as face to face meetings, we also showed that collecting scientifically relevant data can go hand in hand with increasing scientific and environmental

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literacy in participants. Gathering process-based information about lake ecosystems gives managers the best tools to anticipate and react to future global change. Furthermore, combining this process-based information with citizen science, thus outreach, is in complete agreement with the Water Framework Directive goals as set in 2010.

1. Introduction

The global carbon cycle traces the flow of carbon as it is being recycled and reused throughout the biosphere. The cycle entails long term processes such as carbon sequestration to, and release from, carbon sinks. This biogeochemical process exchanges carbon among various compartments including land, ocean and atmosphere (Falkowski et al., 2000). The relationships and exchanges between these three compartments have been researched in depth (i.e. Hayes and Waldbauer, 2006; Bristow and Kennedy, 2008; Knauth and Kennedy, 2009; Macbean and Peylin, 2014). While historically, carbon fluxes in inland waters have received little attention, recently freshwaters have been recognized as an important part in the global carbon cycle (Kortelainen et al., 2004; Cole et al., 2007; Biddanda, 2017). Freshwater systems are both an export pipeline from land to ocean and an active compartment in which up to 1,0 Pg C y^{-1}, or 48% of terrestrial C, is sequestrated and stored in its sediments (Cole et al., 2007; Butman et al., 2016). Organic carbon burial in inland water sediments can even exceed the organic carbon sequestration on the ocean floor (Tranvik et al., 2009). Inland waters not only include rivers and streams of various sizes but also standing waters, ranging from ponds to large lakes and reservoirs. They play a distinct role in the global carbon cycle as they are very active sites of carbon cycling, especially considering the small area lakes occupy in the landscape; i.e. only 3% of the earth's total land surface area (Tranvik et al., 2009; Williamson et al., 2009).

1.1. Drivers of the decomposition process in lakes

A core ecosystem process in the carbon cycle is decomposition. Only a small fraction of the organic carbon produced by terrestrial plants is incorporated into biomass by herbivores or is sequestrated and stored long term in soils (Cebrian and Duarte, 1995; Battle et al., 2000). The remaining 90% of organic carbon is broken down by microbes and invertebrates and quickly reenters the carbon cycle. This makes decomposition of plant litter one of the most important ecosystem processes in the biosphere (Cebrian, 1999).

Factors controlling terrestrial and aquatic decomposition include temperature, moisture, concentration of acid-unhydrolyzable residue (AUR -formerly referred to as lignin), phosphorus, polyphenol and AUR:N ratio (Zhang et al., 2008). In terrestrial systems moisture is the first factor that can influence the rate and completeness of decomposition, followed by temperature (Prescott, 2010; Bradford et al. 2017). If these factors surpass their optimal range, 60–75% moisture and 30–40 °C, decomposition is influenced or even inhibited (Prescott, 2010). For aquatic systems, optimal ranges of factors controlling decomposition are not yet clear. The sustained contact with water inherent to aquatic environments is thought to substantially increase leaching, which in turn importantly affects weight loss and decomposition rate (Gessner et al., 2010).

1.1.1. Decomposition and spatial variability in lakes

Lakes are heterogeneous in space and time. Spatial variability in lakes, i.e. a littoral and pelagic zone, has direct consequences for decomposition, as these zones are directly related to substrate availability (Wetzel, 2001). In the littoral zone, both allochthonous and autochthonous leaf litter material accumulates, supplying benthic detritivores and microorganisms with substrate to break down. The released nutrients in the littoral can be taken up by either macrophytes or microorganisms including (cyano-) bacteria, algae (green algae and diatoms) and periphyton (Wetzel, 2001). In general, this is the most ideal place for decomposition because with respect to the pelagic, the littoral has more substrate, higher water temperatures and contact with the sediment, from which detritivores benefit. Decomposition in the pelagic is slower and less complete due to temperature and substrate limitations (Nishri et al., 2011).

1.1.2. Decomposition and environmental change

As is the case in terrestrial habitats, the decomposition process in water is carried out by microbial decomposers and invertebrate detritivores (Gessner et al., 1999) and is sensitive to changes in environmental conditions, such as increases in nutrient availability (Woodward et al., 2012). An increase in nutrient availability in aquatic environments has been shown to accelerate the decomposition process as microbes grow faster, thus stimulating microbial and invertebrate consumption (Gulis and Suberkropp, 2003; Woodward et al., 2012). Eutrophication may, however, also decrease decomposition efficiency through suppression of the diversity of the decomposing community (Gessner et al., 2010). Land use change and climate change have altered nutrient dynamics throughout the world (Vitousek et al., 1997; Rockström et al., 2009; Ferreira et al., 2015), thereby intensifying eutrophication effects on decomposition (Murdoch et al., 2000).

The process rate of (exo-) enzymes, generally the rate-limiting factor in microbial decomposition, is strongly determined by the temperature of the environment (Peterson et al., 2007; Cunha et al., 2010; Blagodatskaya et al., 2016). Global warming of surface waters (O'Reilly et al., 2015) has a direct accelerating effect on the decomposition process, with up to 80% of decomposition being driven by microbial activity (Pascoal and Cássio, 2004; Griffiths and Tiegs, 2016). Moreover, eutrophication may intensify the effect of global warming on litter decomposition in lakes (Ferreira and Chauvet, 2011).

Of course these drivers do not act in isolation, but results from published studies are ambiguous on the combined effect of eutrophication and temperature on decomposition (Marcarelli and Wurtsbaugh, 2006; Durance and Ormerod, 2007; Ferreira and Chauvet, 2011). Other environmental factors, such as detritivore density or litter recalcitrance, may also change along a climatic gradient thus confounding results of large-scale manipulations (Ferreira et al., 2015). To disentangle the effect of temperature and litter quality on the decomposition process among climate regions, a standardized litter is needed (Keuskamp et al. 2013; Tiegs et al. 2013).

1.2. Measuring decomposition

Traditionally, both in terrestrial and aquatic environments the decomposition process is studied by using leaf litter packs (Petersen and Cummins, 1974; Wieder and Lang, 1982; Gessner et al., 1999; Gulis and Suberkropp, 2003; Prescott, 2010; Ferreira and Chauvet, 2011; Keuskamp et al., 2013; Ferreira et al., 2015; García-Palacios et al., 2016; Griffiths and Tiegs, 2016; Bradford et al., 2017). When leaf litter enters the environment, a leaching event will take place (Gessner et al., 1999). Water soluble substances leach from the material, while microbes colonize and start to break down the litter. Macroinvertebrates shred the material by eating both the microbial and leaf biomass after which only the most indigestible part of the leaf material remains and is eventually stored in the sediment or sequestered (Gessner et al., 1999). Litter packs capture the integrated result of these processes by their weight loss in time. The rate at which mass loss takes place is often approximated by the decomposition constant (k_1 value) (Prescott, 2010). K-estimators have been frequently used to capture differences in decomposition at a temporal or spatial scale, and among different sources of litter material (Gessner et al., 1999; Hayes and Waldbauer, 2006; García-Palacios et al., 2016). Additionally, the amount of material which is not broken down by the decomposing community (stabilization factor *S*) gives information about the effectiveness of the decomposing community under the current environmental conditions. This component of the decomposition process has only been added to decomposition analyses fairly recently (Harmon et al., 2009). The resulting two-phase decomposition process provides insight in the carbon burial potential of ecosystems in which decomposition is measured. In light of climate change, mapping, or even exploiting the carbon burying potential of ecosystems might provide an opportunity to minimize the effects of anthropogenic CO_2 emissions (Keuskamp et al., 2013).

1.2.1. The Tea Bag Index

To see the effects of environmental factors on the decomposition process, a standard uniform litter should be subjected to different levels of the desired factor. To be able to accurately assess the impact of these factors, the decomposition process has to be mapped across scales, thus ecosystems and climate regions, and at high spatial resolution.

The Tea Bag Index (TBI) is a method, designed by Keuskamp et al. (2013), where Lipton tea bags are used as standard leaf litter to determine a decomposition constant (k_1) and stabilization factor (*S*). By determining the amount of recalcitrant material, this stabilization factor is a measure for the effectiveness of the decomposing community under current environmental conditions. The TBI was originally designed to provide process-driven information on soil functions at local, regional and global scales. In this study, we tested its usefulness in the aquatic environment. Using a fixed incubation period and two types of tea, i.e. green tea to determine the stabilization factor and rooibos tea to determine the decomposition constant, the TBI allowed for modelling of both decomposition rate (k_1), as stabilization factor *S*. As it is an affordable, easy and fast standardized methodology, the TBI allows for decomposition experiments at a much larger scale and, offers opportunities for citizen science projects.

1.3. Opportunities of citizen science

While Citizen Science can refer to the remediation of environmental issues or problems by any action, individual or group (Sivek and Hungerford, 1990; Bonney et al., 2009), this definition has been broadened as seen in the 2014 edition of the Oxford Dictionary: “scientific work undertaken by members of the general public, often in collaboration with or under the direction of professional scientists and scientific institutions.” In recent years, Citizen Science has received increasing attention from the scientific community as a way to combine outreach, to address environmental literacy and as a means of doing scientific research at larger scales (e.g. Arslan, 2012; Henderson et al., 2012, Frontiers in Ecology and the Environment Special Issue “Citizen Science”; Forawi, 2016). Additionally, a call for participatory science has been set out at the governmental level. The European Water Framework Directive (WFD) (The European Parliament and the Council of the European Union, 2000) underlines the importance of public involvement for the successful integration of sustainable water use. To successfully protect the ecological quality of all Europe's water, the WFD promotes citizens' engagement in water quality assessment and solutions, encourages water managers to invest in outreach initiatives that deal with water awareness and further collaborations with non-government organizations (NGOs; Dickinson et al., 2012). A standardized, affordable method such as the TBI index offers a unique opportunity to collect scientifically robust data while contemporarily addressing environmental issues and increasing scientific literacy of Citizen Science participants.

2. Objectives

The objectives of our study were twofold, (1) to assess the potential of the Tea Bag Index (TBI) as an standard litter decomposition method for the aquatic environment, and (2) to test to what extent large scale (trophic state, climate zone) and small scale patterns (lake zone) drive decomposition as assessed by TBI methodology. To this end, a multi-lake survey (40 lakes) was initiated by a consortium of scientists and citizen scientists working together in EU-COST program Networking Lake Observatories in Europe (EU Cost action 1201; NETLAKE).

3. Material and methods

This research consisted of a development phase in which we adapted the terrestrial TBI method to the aquatic environment. In the second phase we applied the adapted TBI method to assess decomposition rates in multiple lakes across climate zones and a gradient of trophic states.

3.1. Developing the Tea Bag Index method in the aquatic environment

3.1.1. Placement of teabags in lakes

The Tea Bag Index was carried out following Keuskamp et al. (2013). In short, 2–5 tetrahedron-shaped synthetic tea bags containing green tea (Lipton pyramid bags EAN 87 22700 05552 5) and 2–5 tetrahedron-shaped synthetic tea bags containing Rooibos tea (Lipton pyramid bags EAN 87 22700 18843 8) were placed together in the littoral, i.e. on top of the sediment (1), in the pelagic (2) and buried 8 cm in the sediment following standard TBI protocol (3). By placing the tea bags at different position in the lake, we aimed to follow the spatial variability in decomposition. Placing the tea bags 8 cm in the lake sediment coincides with the traditional TBI as it prevents tea bag loss due to predation or scouring but still allows the littoral decomposing community to reach the tea bag. The standard retrieval time of the tea bags is approximately 90 days after deployment. This time scale was used in upscaling part of this research (paragraph 2.3). After the tea bags were retrieved, they were gently washed with tap water to remove adhering sediment and macroinvertebrates. The tea bags were then air dried for 5 days at room temperature, after which the remaining dry weight was determined using the same balances to determine initial weight, and weighted to the nearest 0.01 g (Keuskamp et al. 2013).

3.1.2. Adapting Tea Bag Index method to the aquatic environment

Because decomposition in aquatic environments entails a much larger leaching effect compared to the terrestrial domain, we aimed to add this additional leaching effect to the TBI analyses. Henceforth, we experimentally determined a 'leaching factor' to adapt the TBI method to aquatic environments. To this end, on 29 April 2016, 288 Lipton green and rooibos tea bags were placed in the pelagic, littoral and buried 8 cm in the sediment of a small pond in Wageningen, the Netherlands ('Wadi', 0.03 ha; z_{max} 1.1 m; 51.987561, 5.670941). We placed the pelagic tea bags on 20 locations in the Wadi, in which every location differed in water depth, thickness of the organic layer and amount of macrophyte growth. We placed the tea bags between 10 and 100 cm water depth, the littoral ones at a water depth of 30–100 cm and the tea bags in the sediment at 8 cm but with different thickness of the organic matter layer (0–9 cm). Macrophyte growth ranges from 0 to 60% cover, the water temperature ranges from 9.5 °C to 14 °C during the leaching period. A bamboo stick was used to facilitate placement and retrieval of the tea bags.

The variability of the amount and time frame of the leaching phase in aquatic environments is high (France et al. 1997; Gessner et al., 1999). However, the TBI uses commercial tea which has been dried for several days before packaging. This kills the cells resulting in a loss of structural integrity which facilitates the rapid leaching of soluble components (Gessner et al., 1999). To compensate for initial intense leaching we have estimated leaching over the first 3 h of submersion. This period is regarded as a pre-conditioning phase, to reflect leaching that takes place prior to colonization by microbes, and can start relatively fast after entering the system (Krevš et al. 2017).

The fraction of weight loss relative to the initial weight of the green tea and rooibos tea bags after 3 h submersion in water was calculated for each zone (littoral, pelagic and sediment). In this time frame, microbial decomposition is unlikely to break down detectable amounts of material, but leaves sufficient time to capture the period of intense leaching from the tea material. Differences in intense leaching among tea type and location in the water column (zone) was tested using a pairwise *t*-test with Bonferroni correction. This leaching factor was used as a correction factor for the initial tea weight, as leaching is not an active part of the decomposition process, in further use of the TBI methodology in this paper (see below for model calculations).

3.1.3. Two phase decomposition model with leaching factor

Decomposition rates and the amount of recalcitrant material (i.e. remaining fraction) are estimated using a model in which a two phase decomposition process is assumed (as described by Wieder and Lang, 1982; Keuskamp et al., 2013)

$$W_t = ae^{-k_1 t} + (1 - a) \qquad \text{eq. 1}$$

In which W_t is the remaining mass of leaf litter (in grams) after the incubation period (t in days), k_1 is the decomposition rate (in day^{-1}), and a is the decomposable fraction.

Function (eq. (1)) was parameterized by fitting the weight losses of both tea types over 4 incubation periods: 3 h and 27, 89, 138 days in the same Wadi pond located in Wageningen, the Netherlands from 29 April 2016 until 14 September 2016.

In the TBI methodology, green and rooibos tea bags from the same location are coupled to recreate this two phase decomposition model using only one incubation period in which rooibos tea allows for estimation of k, and green tea for a (as established by Keuskamp et al. 2013). As the decomposition process progresses, part of the labile, decomposable fraction stabilizes and becomes recalcitrant (Prescott, 2010). This stabilization is conditional on the environment and will thus result in deviations of the actual decomposed fraction a. This inhibiting effect of the environment on the decomposition process is captured in the stabilization factor S, which is calculated using equation (2).

$$S = 1 - \frac{a_g}{H_g} \qquad \text{eq. 2}$$

In which S is the stabilization factor, a_g is the decomposable fraction and H_g is the hydrolysable fraction of green tea. a_g is determined by dividing the final weight by its initial weight of green tea. H_g is the sum of the nonpolar extractives, water solubles and acid solubles of green tea as determined by Keuskamp et al. (2013) using a sequential carbon fraction extraction technique by Ryan et al. (1990).

Subsequently, equation (1) was rewritten to calculate the decomposition constant k_1 using the one time point approach of the TBI methodology. For the decomposable fraction (a), the decomposable fraction of rooibos tea was used (a_r), calculated by multiplying the hydrolysable fraction of rooibos H_r by (1-S).

The leaching factor was used as a correction factor for the initial tea weight, by subtracting the multiplication of the initial tea mass times the leaching factor, specific for the position of the tea bag in the water column.

As leaching reduces the initial tea weight, it specifically reduces the relative size of the hydrolysable fraction for both green tea and rooibos tea (H_g and H_r). Therefore, we subtracted the specific leaching factor for green tea or rooibos tea from respectively H_g and H_r and divided this by 1-leaching factor. The resulting corrected hydrolysable fraction was thereafter used to calculate the stabilization factor S and decomposition rate k_1.

To assess the effect of the inclusion of a leaching factor on our results we compared the results obtained using the leaching factor, to the results obtained when using the traditional TBI calculation, i.e. not applying the leaching factor. The variance within groups was larger compared to when the leaching factor was used, leading to non-significant results between groups (results not shown, available upon request). Moreover, leaching in the aquatic environment plays a larger role compared to the terrestrial ecosystem. We therefore recommend not to disregard the leaching factor when deploying the TBI method in the aquatic environment.

3.2. Scaling up: testing large and small scales patterns using citizen science

Citizen Science relies on willing individuals with an interest in their environment (lakes) and curious about science. Therefore, the lakes were selected based on the following criteria: the presence of a citizen scientist teamed up with science partner to ensure quality assurance when collecting data and timely feedback during the sampling period. Prior limnological knowledge of the lake was considered to be an important factor when choosing among different lakes in a single area. Additional quality assurance check points in the sampling procedure included standardization of scales used and photographic checks of tea bag placement.

To ensure standardization of the TBI methodology we trained scientists and citizen scientists during a NETLAKE EU COST Action (1201) workshop in the Czech Republic in 2016 (visit http://ekolbrno.ibot.cas.cz/en/2016/06/20/zprava-z-training-school-cost-netlake/to see the report including photos from this workshop). During this workshop the protocols were further fine-tuned and a website and Facebook group was created, after which 25 groups signed up to study a lake for this European wide citizen scientist project. Six lakes repeated the TBI measurement in 2017, and additional 8 lakes joined in 2017. The results from TBI placement in

7 lakes in 2015 were included, bringing the total number of lakes to 40 (Fig. 1; Appendix A). For the TBI methodology, tea bags were weighted to the nearest 0.01 g according to the provided protocol (visit www.nioo.knaw.nl/netlake-citizen-science to access the protocols).

3.2.1. Study sites for citizen science

The TBI adapted to the aquatic environment was deployed in 40 lakes across Europe in 2015–2017 (Fig. 1). Using the Köppen – Geiger climate classification, lakes were assigned to their specific climate region (Rubel and Kottek, 2010). For the Italian lakes the more detailed classification by Rubel et al. (2017) was used. Volunteers placed more than 500 tea bag pairs in the littoral, pelagic and sediment zones across four Köppen- Geiger climate zones: 27 lakes in temperate regions without a dry season (Köppen- Geiger climate region Cf), 3 lakes with a dry summer (Cs), 5 lakes in cold continental regions without a dry season (Df) and 5 lakes in a polar region with a tundra climate (ET). In addition, we placed (IButton®) temperature loggers in the littoral zone in 34 out of the 40 European lakes.

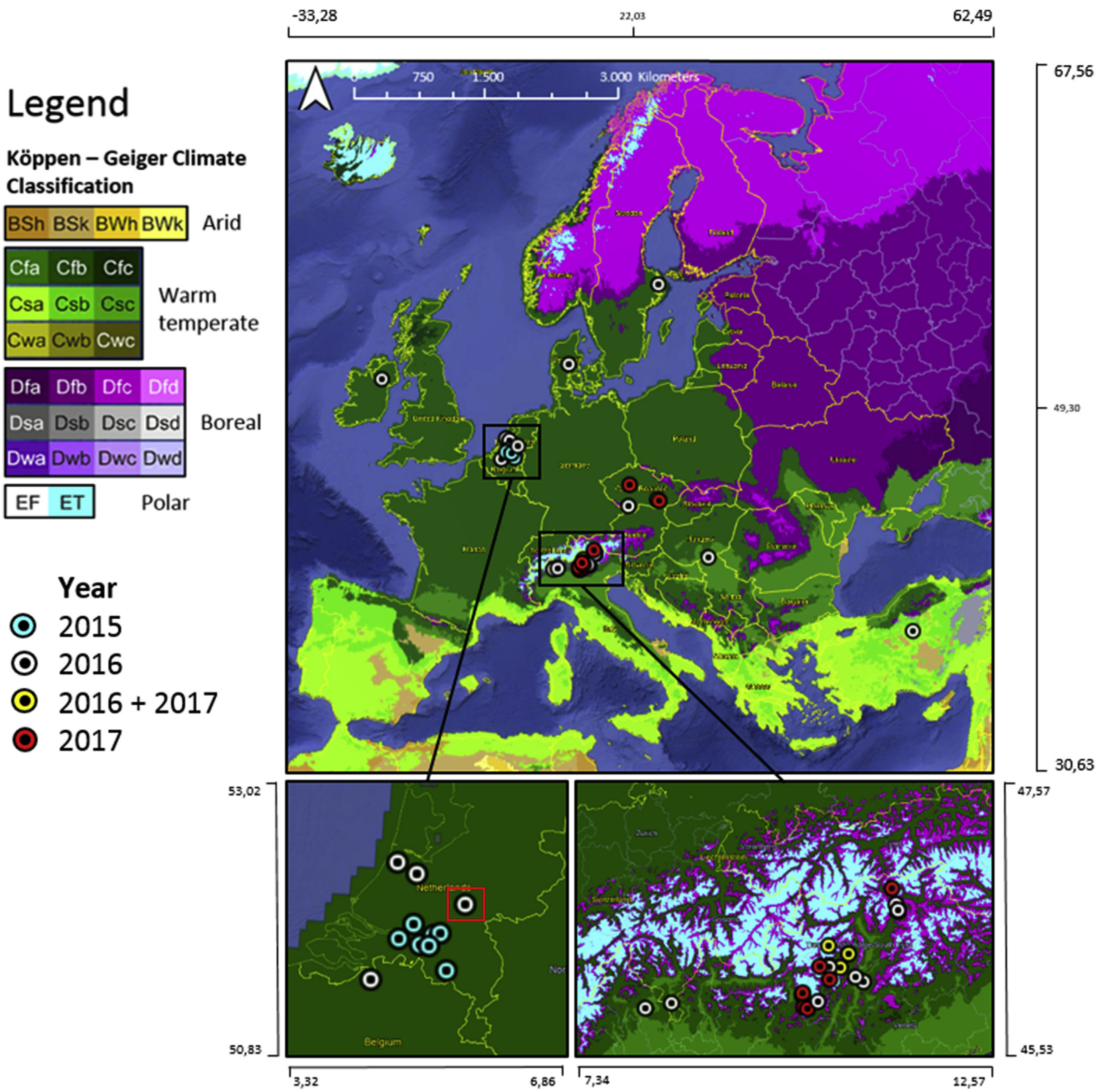

Fig. 1. Overview of participating lakes in the NETLAKE citizen science COST Action 1201 in 2015 (blue), 2016 (white), 2017 (red) and both in 2016 and 2017 (yellow). Red square indicates location of validation of TBI methodology in 2016. The background map displays Köppen-Geiger climate regions (Rubel and Kottek, 2010; Rubel et al., 2017), longitude and latitude geographic coordinates (decimal degrees) are indicated around each panel.

Table 1
Weight loss (grams and fraction of start weight) of green tea and rooibos tea during a 3 h leaching event. Tea bags were placed at 3 locations in the lake; pelagic zone ± 5 cm under de water surface, in the littoral between submerged plants, and buried 8 cm in the sediment according to the standard TBI protocol (mean ± SD).

	Green tea		Rooibos tea	
	grams	fraction	grams	fraction
Pelagic	0.581 (±0.042)	0.280 (±0.014)	0.252 (±0.044)	0.113 (±0.019)
Littoral	0.454 (±0.170)	0.218 (±0.081)	0.199 (±0.083)	0.090 (±0.037)
Sediment	0.480 (±0.080)	0.230 (±0.037)	0.201 (±0.057)	0.090 (±0.025)
Lake average	0.505 (±0.124)	0.243 (±0.059)	0.217 (±0.068)	0.098 (±0.030)

To be able to assign trophic state to each of the participating lakes, volunteers were asked to measure Secchi depth. Using the trophic state index (TSI) for lakes (Carlson, 1977), Secchi depths were converted to a trophic state index using equation (3).

$$TSI = 10(6 - \frac{lnSD}{ln2}) \quad \text{eq. 3}$$

Where TSI = Trophic State Index and SD = Secchi disk depth (m). A TSI score of above 70 indicates a hypereutrophic lake, a score between 50 and 70 is classified as eutrophic, between 40 and 50 as mesotrophic and below 40 as oligotrophic.

In total across our lakes, 105 sites were chosen to determine the decomposition rate and stabilization constant. Of these 105 sites, 60 sites were located in the littoral zone, 34 sites in the pelagic and 11 sites in the sediment (Appendix A). Each site was composed of 2–5 tea bag pairs.

Of the deployed teabags, 313 tea bag pairs where used for further analysis. Causes of exclusion included loss of tea bags due to vandalism or weather conditions or inaccurate final tea weight determination due to trapped macrofauna, sediment particles or biofilm formation.

3.2.2. Data analyses

Differences in fraction mass loss for green and rooibos tea among climate zones and TSI was tested using an ANOVA, modified to use permutation tests instead of normal theory tests (aovp). This same test was used to determine differences among the decomposition constant (k_1) and stabilization factor (S) between climate regions, TSI and position in the water column. To avoid pseudoreplication (Hurlbert, 1984), we used "lake position in the water column" as our experimental unit, allowing us to test for differences in decomposition at lake zones across a climatic and trophic gradient. As we expected that our unbalanced design could have an effect on the outcome of our analyses, we ran a separate analyses where we randomly selected 5 lakes in the Cf climatic region (temperate climate without a dry season; overrepresented in our dataset) and all other climate regions were retained in the original sample size.

All analyses were carried out using R version 3.2.3 with base package stats and the dplyr v0.4.3, lmPerm v2.1.0, ggplot2 v2.2.1 and minpack.lm v1.2-0 package (Wickham, 2009; Elzhov et al. 2015; R core Team, 2015; Wickham and Francois, 2015; Wheeler and Torchiano, 2016).

4. Results

4.1. Developing the Tea Bag Index method for the aquatic environment

The average weight loss of tea bags placed in a shallow eutrophic lake for 3 h differed based on the location. On average, 0.243 mg (±0.059 SD) of green tea and 0.098 mg (±0.030 SD) of rooibos tea was leached from the tea bags over the 3 h (Table 1). Additionally, the leaching fraction differed significantly between the tea bags placed in the pelagic zone and tea bags placed in the littoral zone or buried 8 cm in the sediment (Fig. 2, $p = 0.003$).

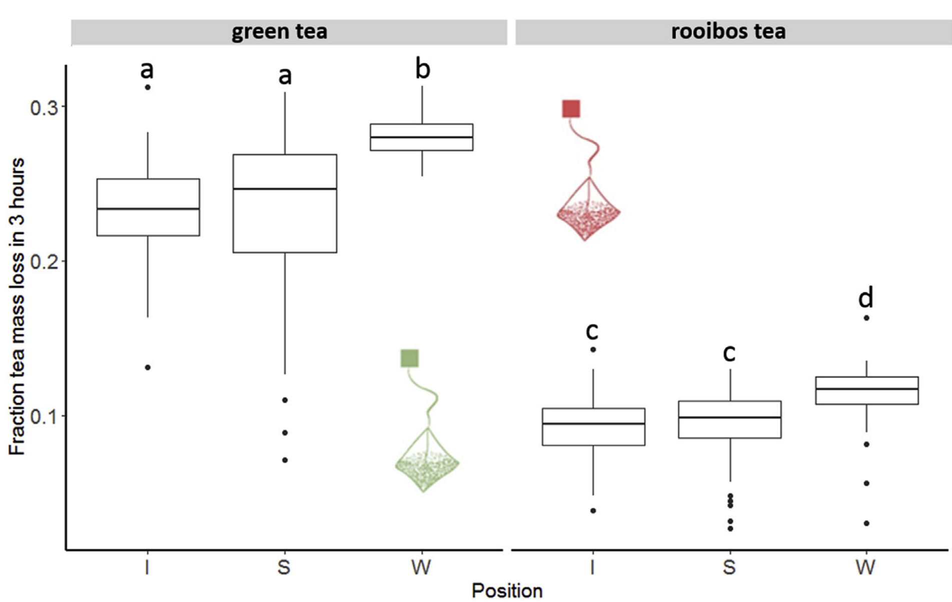

Fig. 2. Fraction mass loss of green tea and rooibos tea relative to start weight after 3 h submersion to capture the period of intense leaching in pond Wadi, Wageningen, the Netherlands. Position indicates the difference between placing the tea bags buried 8 cm in the sediment (S), laying on top of the sediment - littoral zone (L) or hanging in the water column – pelagic zone (P). Letters indicate significant pairwise differences between the different positions and tea types ($p < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

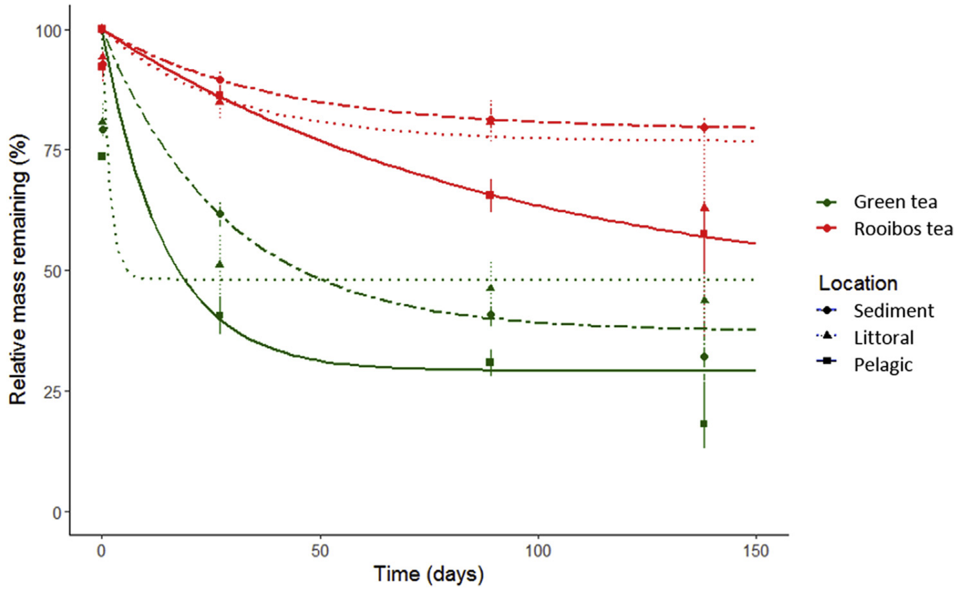

Fig. 3. Relative mass remaining of rooibos and green tea as measured in shallow lake in different positions in the water column (Pelagic, hanging in the water column; littoral, laying on top of the sediment; sediment, buried 8 cm in the sediment). The tea bags were retrieved after 3 h, and 27, 89 and 136 days of incubation (n = 45). Lines show fitting to exponential decay function (eq (1)).Vertical bars represent standard errors. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Water depth, macrophyte cover or thickness of the organic matter layer on top of the sediment did not significantly influence the leaching factor. To determine the decomposition constant and stabilization factor using the TBI in an aquatic environment, this leaching fraction, specific for the position of the tea bags in the water column (resulting in pelagic, littoral and sediment fraction), was used to correct the initial tea weight.

Recreating the decomposition dynamics of rooibos and green tea in an aquatic environment required a multi-harvest approach as previously described by Keuskamp et al. (2013) (Fig. 3). Green tea decomposed relatively fast and began to level off after 30–60 days depending on the location in the water column. Rooibos tea decomposed much slower and only started to level off at the end of the experiment (±130 days). As green tea reached its limit value relatively fast, it allowed for estimation of S, while rooibos was still being actively decomposed allowing the estimation of k_1. Based on these results the TBI incubations for the across site assessment where kept at 90 days, similar to the standard TBI protocol for soils.

4.2. Rolling out to larger scale using citizen science

4.2.1. Decomposition constant (k_1) and stabilization factor (S)

The calculated decomposition constant (k_1) and stabilization factor (S) per lake was compared to the range of k_1 and S as found by Keuskamp et al. (2013). The values for S found in our experiment ranged from −0.39 to 0.8 (average 0.106 ± 0.191 SD). The decomposition constant (k_1) values calculated in our experiment ranged from 0.00001 to 0.06 day^{-1} (average 0.0078 day^{-1} ± 0.0067 SD). These values are somewhat higher than the ones obtained by Keuskamp et al. (2013) (Fig. 4).

4.2.2. Decomposition and spatial variability in lakes

We placed tea bags at different positions in the lake i.e. in the pelagic, in the littoral between aquatic plants, and buried in the lake sediment, to follow different decomposing communities. A significant difference was found for relative mass loss of rooibos tea ($p = 0.002$) and for the relative mass loss of green tea ($p = 0.001$) between the pelagic, littoral and sediment in lake zones. Likewise, a significant difference between these positions in the water column was found for the stabilization factor S ($p = 0.042$) and for the decomposition constant k_1 ($p = 0.045$).

4.2.3. Climate region

After correcting for the overrepresentation of the temperate region without a dry season (Cf), a significant difference among the relative weight loss per rooibos tea bag was found ($p < 0.0001$), which resulted in a significant difference for decomposition factor (k_1) among climate regions ($p = 0.0007$, Fig. 5A). No significant difference in the rate at which green tea decomposed across the climate regions was found ($p = 0.052$), consequently, no significant climate region effect in the stabilization factor S ($p = 0.078$) was found. The temperate regions (Cf and Cs) had significantly higher decomposition rates than the cold continental region (Df) and polar region (ET) ($p < 0.05$).

4.2.4. Trophic gradient

The trophic state index could not be calculated for 8 of the 40 lakes as no Secchi depth was available during the tea bag incubation period. Twelve oligotrophic and 17 eutrophic lakes were subsampled and tested against 7 mesotrophic lakes per tested parameter. Hypereutrophic lakes (n = 2) and lakes where trophic state could not be determined were excluded from the analysis. Significant differences among trophic state were found in mass loss of rooibos tea ($p = 0.026$) and for the decomposition constant k_1 ($p = 0.012$). On average, a higher decomposition rate was found in eutrophic lakes, however the highest decomposition rates were found in an oligotrophic lake ($p < 0.05$). Additionally, a significance difference of mass loss of green tea were found ($p = 0.007$), leading to a significant difference for stabilization factor S ($p = 0.004$) over trophic states (Fig. 5B). Eutrophic sites had significantly higher stabilization constants that oligotrophic and mesotrophic sites ($p < 0.05$).

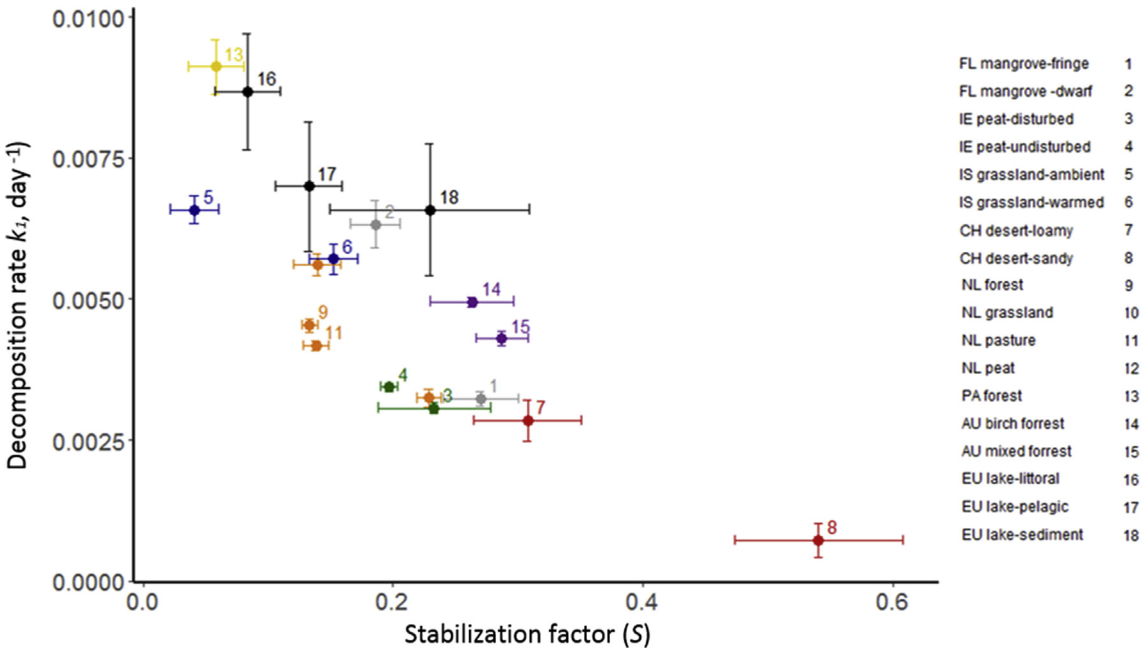

Fig. 4. Decomposition constant (k_1, day^{-1}) and stabilization factor (S) across terrestrial ecosystems ([1] – [15], in color; data from Keuskamp et al. [2013]) and European lakes per decomposition zone (littoral [16], pelagic [17] and in the sediment [18]; in black).

5. Discussion

Litter decomposition is widely used as an indicator of ecosystem functioning in streams and rivers (Gessner et al., 1997). The weight loss of two tea types, a highly standardized litter material, was followed in order to determine the suitability for the use of this tea bag methodology (TBI) in an aquatic environment. We introduced the leaching factor to correct for the initial leaching event which plays a much larger role in aquatic decomposition compared to terrestrial decomposition. We recommend that this leaching factor should be added to standard TBI methodology to calculate the decomposition constant and stabilization factor in aquatic environments. By placing tea bags in 40 lakes across four climate zones and trophic states we show that the TBI, with leaching factor adjustment, is able to pick up a temperature and eutrophication signal. Here, we offer an extension of the TBI methodology in an effort to create a validated global decomposition map across ecosystems as proposed by Keuskamp et al. (2013) and García-Palacios et al. (2016).

5.1. Tea Bag Index

The Tea Bag Index (TBI) methodology is a standardized litter bag experiment to determine terrestrial decomposition, similar to those performed since the 1970s (Winterboum, 1978). However the simultaneous use of two tea types (used in the current study as well) allows for only one time point to calculate decomposition rates. This greatly reduces the amount of samples needed and effort required by (citizen) scientists. Additionally, using the TBI provides not only the traditional decomposition constant k_1 (reflecting the decomposition rate) but also a stabilization factor S (reflecting the amount of material that cannot be broken down). This gives additional information about the decomposition curve but also about the amount of the tea material that can be broken down by the microbial community *in situ* under the present environmental conditions. Adapting this methodology to aquatic environments creates opportunities to include these ecosystems in global carbon maps and for non-experts to contribute to decomposition databases. The TBI excludes detrivorous macrofauna that play a role in natural decomposition as they feed not only on the litter itself but also on the fungal mycelium colonizing the litter (Gessner et al., 1999; García-Palacios et al., 2016). As with the traditional fine mesh litter bag method, by excluding these detritivores their contribution to the decomposition process and rate is not taken into account (Benfield, 2006). As macroinvertebrates are especially sensitive to changes, they are likely to be impacted by degradation of their ecosystem quite early on, thus effecting the decomposition process early on (Gessner et al., 2010; Hodkinson and Jackson, 2005). If the TBI methodology would be used for the early detection of ecosystem degradation, the exclusion of detritivores might lead to an underestimation of k_1 and S. However, the microbial community can also respond quickly to environmental change as shown by Suberkropp and Chauvet (1995), where significant changes in microbial decomposition of leaves were seen after 5 days of transferring the leaf material to a stream with contrasting water chemistry.

The TBI can thus provide a good estimation of the speed (k_1) and completeness (S) of microbial decomposition which is not confounded with detritivore effects. Other substantial benefits of the TBI are ease of deployment and low costs.

The use of “alien standard litter” is necessary to be able to compare decomposition rates (k_1) and stabilization factor (S) across climate zones and trophic states, but especially across ecosystems. This does not replace the need of decomposition experiments with allochthonous or local autochthonous litter in terrestrial and aquatic systems but rather adds additional information transcending ecosystem boundaries and allowing for global mapping of a key ecosystem process.

5.1.1. Tea Bag Index methodology standardization for the aquatic environment

The decomposition process in the aquatic environment can be

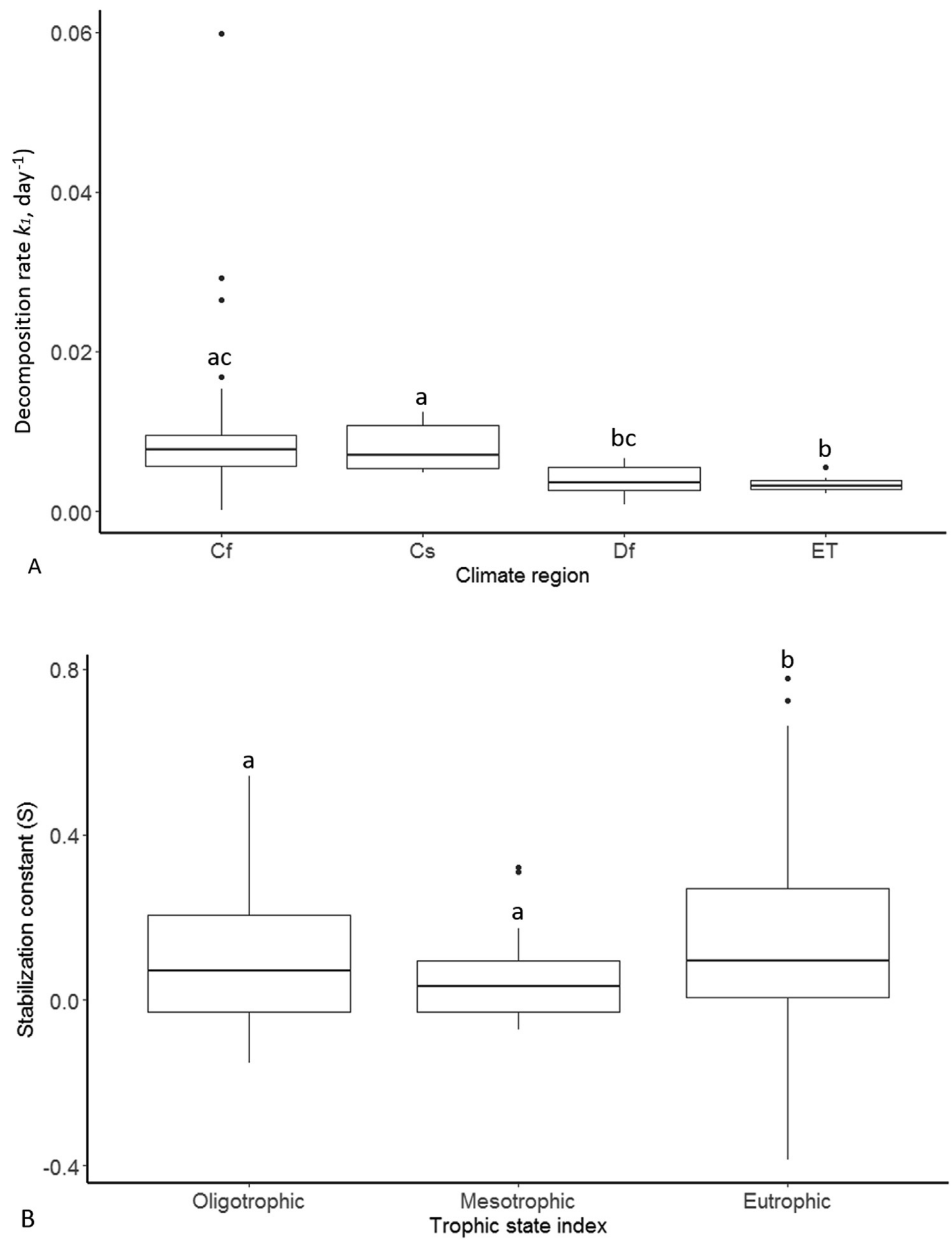

Fig. 5. A Measured variation in decomposition rate or constant (k_1, day^{-1}) among different climate zones in 40 lakes across Europe. Climate zones include temperate regions without a dry season (Köppen- Geiger climate region Cf, n = 27), with a dry summer (Cs, n = 3), cold continental regions without a dry season (Df, n = 5) and polar region with a tundra climate (ET, n = 5) (corrected for overrepresentation of Cf region). Letters indicate significant pairwise differences between climate regions ($p < 0.05$). B Measured variation among stabilization factor (S) among lakes with different trophic states based upon Secchi depth. Twelve oligotrophic and 17 eutrophic lakes were subsampled and tested against 7 mesotrophic lakes. Hypereutrophic lakes (n = 2) and lakes where no trophic state could be determined were excluded from the analysis. Letters indicate significant pairwise differences between trophic states ($p < 0.05$).

described in three stages; leaching, conditioning and fragmentation. In the decomposition process of tree leaves, leaching can take up to 24 h for autumn leaves, and up to several days or even weeks for fresh leaves (France et al. 1997; Gessner et al., 1999). We defined a leaching period of 3 h sufficient to capture the initial intense leaching of sun dried tea material (Whitworth et al., 2014). The contribution of intense leaching to mass loss in these 3 h differed between lake zones (Fig. 2). Stronger contact with water in the pelagic, resulted in more pronounced leaching from tea bags placed there compared to tea bags placed in the littoral or in sediment. These differences should be, and have been, taken into account in determining the k_1 and S in each lake zone. By measuring weight loss at five different time points, we were able to fit a two phase model for decomposition as an independent control of the quality of single-point TBI estimations of k_1 and S. (Fig. 3). Both green tea and rooibos tea decomposition in our lakes followed the same model as in the laboratory (dry) conditions (Fig. 2 in Keuskamp et al., 2013). This clearly shows that the TBI can be used to adequately assess the decomposition process in aquatic systems.

5.2. Factors influencing the decomposition process

The rate of terrestrial decomposition is determined by several factors, including temperature, moisture and pH, the quantity and quality of the decomposing material and the microbial community involved in decomposition (Chapin et al. 2002).

Temperature affects the decomposition process not only by determining the activity of decomposing community but also the leaching stage. Whitworth et al. (2014) looked at the effect of temperature on DOC leaching from natural litter. After 5 h of incubating sterile litter with Nanopure water, litter placed at 25 °C and 30 °C lost roughly double the amount of DOC compared to litter that incubated at 5 °C, 10 °C, 15 °C and 20 °C (Whitworth et al., 2014, Fig. 1). Additionally, the amount of DOC leaching at 5 °C, 10 °C, 15 °C and 20 °C is very similar. During the 3 h leaching event in this research, the water temperature ranged from 9.5 to 14 °C in lake Wadi. At the start of the European scale TBI experiment, the water temperatures ranged from 9.7 °C to 25.1 °C. Of our European lakes, 19 of the 34 lakes had a start temperature colder than 20 °C, while 15 have a temperature of above 20 °C at the leaching phase. For these 15 lakes, we might underestimate the weight loss by leaching when using the leaching factor determined in lake Wadi according to Whitworth et al. (2014). However, as we have used dried tea leaves as opposed to natural litter material, the difference between the amount of leaching at < 20 °C and >20 °C might be less pronounced.

In aquatic environments, as opposed to the terrestrial ecosystem, moisture is not a determining factor, while oxygen plays a much more important role (Pascoal and Cássio, 2004). As oxygen concentrations drop, microbial decomposition slows down substantially, albeit not coming to a full stop (Gomes et al. 2018). Aquatic microbial communities frequently encounter low oxic conditions due to anthropogenic factors such as intensified agriculture resulting in higher nutrient concentrations in waters. This stimulates microbial activity, depleting oxygen as it is used as the primary electron acceptor during respiration by aerobic organisms. In the aquatic environment, anaerobic organisms will then take over and will use other electron acceptors such as nitrate and sulfate, thus continuing the decomposition process (Krevš and Kučinskienė, 2012). These essential environmental differences between terrestrial and aquatic system contributed to the wider range of decomposition rates (k_1) and stabilization factors (S) as found in this study. The negative S values that were frequently obtained indicate the net loss of stable compounds, a phenomenon not frequently seen in terrestrial deployment of TBI (Keuskamp et al. 2013). The hypothetical un-decomposable fraction (resulting in S) includes not only untransformed material that will not decompose under the current conditions, but also stabilized primary and secondary compounds. Clearly, in our lakes, the microbial community was able to use (one of these) compounds for growth thus resulting in negative S values.

Additionally, higher decomposition rates (k_1) were found in this study compared to Keuskamp et al. (2013) (Fig. 4). Terrestrial decomposition rates can be limited by moisture (Prescott, 2010). For obvious reasons, moisture is not a limiting factor in lakes. As water is a more permeable environment than soil, the tea material could be easier accessible for the microbial community. The present lack of comparison between terrestrial versus aquatic decomposition hampers our understanding of this underlying mechanisms governing these differences. By disclosing the TBI methodology for the aquatic environment a whole new avenue of comparative research is opened up.

5.2.1. Climate regions

Temperature effects on decomposition have been researched extensively in various ecosystems (i.e. Zhang et al., 2008; Griffiths and Tiegs, 2016). Litter decomposition in terrestrial ecosystems (k) tends to decrease with latitude, and increase with temperature and nutrient conditions (Zhang et al. 2008; Prescott, 2010). The results of our multi-lake survey indicated that microbial decomposition rates in lakes in warmer climates are indeed higher than lakes in colder climates. This was also found in numerous studies with natural and artificial decomposable material and in other aquatic systems such as streams (Irons et al. 1994; Davindson and Janssens, 2006; Boyero et al. 2011).

Aquatic systems are very suitable to assess the impact of climate warming on decomposition as – contrary to terrestrial systems – moisture availability is not a confounding factor. Using lakes across latitudinal gradients as surrogates for future temperature changes (time-for-space substitution), the effects of climate change on decomposition can be predicted (Parmesan and Yohe, 2003). In this study we show that the highly standardized TBI methodology can pick up these effects of climate change using a latitudinal gradient.

5.2.2. Eutrophication gradient

By placing the TBI in lakes across Europe we show that the aquatic TBI is able to pick up an eutrophication signal on the in-lake decomposition process. This confirms our hypothesis that nutrient conditions can greatly influence the decomposition process. In a meta-analysis of the effect of nutrient addition to in-stream decomposition by Ferreira et al. (2015), nutrients were found to increase the decomposition rate on average by ± 50%. Likewise, in our study, the effect of eutrophication (indicated by the trophic state index) was found in the decomposition rate (k_1) and also in stabilization factor (S). The occasional high S values found for eutrophic lakes indicate a less efficient microbial community possibly caused by the fact that plenty of decomposable material is present. This reduces the need of microbes to decompose the harder, more recalcitrant, material for growth. Oligotrophic lakes could have less total litter material available for the decomposing community, making them more efficient when breaking down the limited amount of available material. Overall, the variation of S in eutrophic lakes is much higher than for mesotrophic and oligotrophic lakes probably due to a cofounding effect with lake zones and climate regions. As eutrophication is expected to increase in the future due to increased land use changes, population growth and climate change (Ferreira et al., 2015), a better understanding of its effect on ecosystem processes is vital to proper management of water bodies.

We encountered a high variability within certain trophic states and climate regions and an overrepresentation of certain bins, partly a consequence of our citizen science approach. Nevertheless, we were still able to pick up a climate and trophic signal using the TBI. For further research a targeted outreach to, for instance, lake associations and lakeshore inhabitants following a nested sampling design might result in a more evenly distributed dataset and allow deeper analysis of the effects of both climate change and eutrophication on aquatic decompositions. Additionally, the trophic state index based upon a limited number of Secchi readings is not ideal to determine the trophic state of a lake. For further research, the nutrient status of the lakes should be assessed using simple inexpensive kits and/or via remote sensing (e.g. Shi et al., 2018).

5.3. Opportunity for management

The European Water Framework Directive 2000/60/EU (WFD) has been implemented to restore Europe's waters and is the most substantial and ambitious piece of European environmental legislation to date. However, since its implementation 18 years ago, the main goal of good ecological status of all EU waters has not been achieved. Voulvoulis et al. (2017) assign this to the lack of integrated catchment management, i.e. considering the whole system, a pre-requisite for the effective implementation of the WFD. Methods such as the TBI can be applied in both terrestrial and aquatic systems to assess a key ecosystem process. Incorporation of decomposition rates and stabilization factors in standard monitoring regime can be of great use to achieve this shift to more system-based approach. Studying decomposition, a fundamental ecological process on the catchment scale, can advance integrative water management whilst at the same time contributing to scientific research on a global scale.

Besides achieving a good status for all Europe's water, the WFD aims to actively involve citizens in the catchment based approach. The TBI is highly suitable to combine these goals in one methodology. This standardized, affordable method offers a unique opportunity to combine the goals of the WFD while simultaneously addressing environmental and scientific literacy.

6. Conclusions

- The lack of a standardized litter decomposition method for aquatic vs. terrestrial ecosystems is an obstacle for the development of common decomposition models (García-Palacios et al., 2016).
- By adding a leaching factor to the TBI methodology, currently only validated for terrestrial systems, the TBI methodology can be deployed in aquatic systems.
- The TBI methodology can pick up patterns in climate and eutrophication across ecosystems. This is a first step in using the TBI in mapping global decomposition patterns in aquatic systems.
- Future studies should look at balanced design and more suitable predictor variables (temperature, oxygen and nutrient concentrations).
- Citizen science can contribute to gathering relevant data on ecosystem processes across ecosystems using standardized, easy and cheap methodologies.

Declaration of interests

- The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
- The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.watres.2018.11.081.

Data and R scripts available via https://doi.org/10.5061/dryad.h8j5648.

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