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Extension of the soil monitoring network via tea bag initiatives: A 3000 km latitudinal gradient in European Russia

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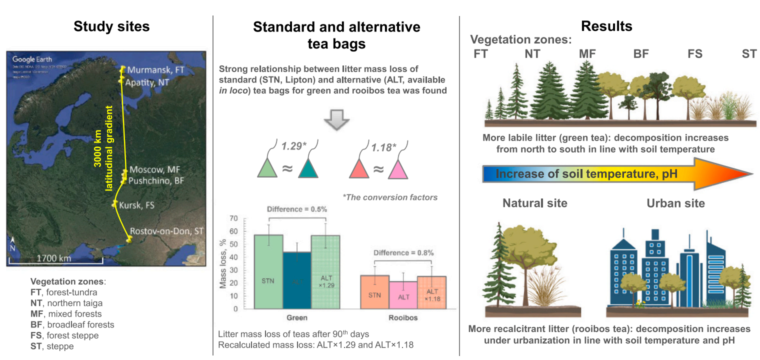
HIGHLIGHTS

- Local tea bags closely match standard ones in decomposition rate.
- Soil temperature is the main driver of green tea decomposition rate.
- Rooibos tea decomposes faster in urban compared to natural soils.
- Soil temperature and pH primary drive Rooibos tea decomposition.

GRAPHICAL ABSTRACT



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ABSTRACT

The increasing popularity and recognition of citizen science approaches to monitor soil health have promoted the idea to assess soil microbial decomposition based on a standard litter sample - tea bags. Although tea bag initiatives are expanding across the world, the global datasets remain biased in regard to investigating regions and

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biomes. This study aimed to expand the tea bag initiative to European Russia, which remains a "white spot" on the tea bag index map. We also added urban soils into the analysis, which were underestimated previously. We compared the standard and local tea brands to explore possible adaptations of the standard approach to regions with limited access to standard tea brands. The established monitoring network included natural and urban sites in six vegetation zones along a 3000 km latitudinal gradient. There was a very close linear relationship (R^2 = 0.94–0.98) in the mass loss of alternative and standard tea litter. The mass loss of green tea in soil along the latitudinal gradient showed an increasing trend from north to south. Variations in the microbial decomposition of green tea were mainly explained by the latitudinal gradient, with low soil temperature identified as key factors hampering decomposition. Mass loss of the more recalcitrant rooibos tea was mainly determined via land use, with decomposition rates on average 1.3 times higher in urban soils. This pattern was in line with higher soil temperatures and pH in urban sites compared to natural counterparts. The findings of our study could prove valuable in extending the tea bag network of soil decomposition assessment into broader territories, including urban areas. Additionally, they could facilitate the involvement of citizen science and complete the database for C cycle modeling depending on climatic conditions.

1. Introduction

Capacity of soil for carbon (C) sequestration is determined by the balance between the C inputs to soil through litter and rhizodeposition and the C losses through decomposition of these inputs and of soil organic matter (SOM). Hence, the decomposition rate of litter is one of the key factors determining the C-climate feedback of terrestrial ecosystems (Bond-Lamberty et al., 2018). Litter decomposition experiments have been frequently used to obtain insights into these factors, controlling for the decomposition processes in soil (Bradford et al., 2016). Climatic conditions (temperature and soil moisture) (Chen et al., 2018), litter traits (Vivanco and Austin, 2019) and the interplay of both factors (Bradford et al., 2016) are considered among the main drivers of the litter decomposition rate. The search for decomposition drivers across biomes has resulted in the necessity to standardize litter decomposition surveys. Use of tea bags has been proposed to overcome the problems of site-specific litter and methodological differences (Keuskamp et al., 2013). The very first tea bags were buried in 2010, and, since that time, the method has diffused worldwide, involving independent research studies and citizen scientists. The approach grew into Teatime 4 Science (http://www.teatime4science.org/) and the TeaComposition Initiative (https://www.teacomposition.org/teacomposition-initiative/), which applied a common protocol and standard litter samples (tea bags of Lipton rooibos and green tea) (Keuskamp et al., 2013; Djukic et al., 2018). Although the TeaComposition Initiative has achieved impressive spatial coverage across Europe, providing valuable insights into soil decomposition dynamics in this region, its representation across the rest of the globe is less comprehensive. Countries with vast territories and diverse climate belts, such as Russia and Canada, and regions like Africa, remain underrepresented in the existing TeaComposition database. For example, Russian sites constitute only 8 out of the 336, with the majority located in south Siberia and only 1 in the European part of the country (Khibiny Station Russia, Murmansk region) (Djukic et al., 2018). This highlights how European Russia is a notable white spot on the tea bag map.

Djukic et al. (2018), in a global study, alongside climate effects, examined the role of land-use type in distribution patterns of tea litter loss. However, urban soils—important hotspots of anthropogenic activities—were not considered. Consequently, this makes it impossible to estimate the impact of urbanization on the litter decomposition patterns globally and disentangle climate and urbanization effects.

Anthropogenic perturbations in urban environments (e.g., contamination, sealing, mechanical disturbance) make urban soils substantially different from their natural analogues (Vasenev and Kuzyakov, 2018). The important role of urban soils for ecosystem services and in the sustainable development of the urban environment is recognized worldwide, especially in studies focused on C stocks and fluxes in urban soils (Pouyat et al., 2017; Ivashchenko et al., 2019; Guo et al., 2024). Tea bags have been previously used in several urban studies to explore the effect of land cover (Pino et al., 2021; Sandén et al., 2021; Kotze and Setälä, 2022) and management (Tresch et al., 2018; Somerville et al., 2020) on microbial decomposition patterns. However, climate–urbanization interactions remain largely overlooked because climatic variability was not considered in the methodological setup of these studies. Thus, the primary aim of our study was to assess the early stage (90 days) of tea litter decomposition patterns across a latitudinal gradient spanning six vegetation zones in European Russia and to disentangle climate and urbanization effects.

However, this study had a methodological constraint due to logistical limitations because the standard Lipton rooibos (EAN 87 22700 18843) and Lipton green tea (EAN 87 22700 05552 5) are not commercially available products in Russia. Russia is not the only country where international tea delivery is limited by customs regulations, but even if the standard brand was available, deviations in chemical properties cannot be excluded due to, for instance, variations in tea growing conditions. In the course of time, tea-producers may change tea composition or even give up on using nondegradable bags to become more ecofriendly. Consequently, to extend the monitoring network, alternative, precalibrated commercial tea bags brands for tea litter decomposition studies, according to standard Lipton tea, are needed (Middelanis et al., 2023). Moreover, a more flexible protocol allowing local alternative tea brands could increase the attractiveness of the approach for citizen science projects and facilitate further expansion of the monitoring network. Therefore, our second aim was to test the locally available alternative tea bag brands to calculate the conversion factors between them and thereby expand the network for litter decomposition assessment for broader areas, facilitating the engagement of citizen scientists and Russian researchers in data collection. The possibility of extensive monitoring of C stabilization and decomposition in Eurasian soils will facilitate the acquisition of insights into continental climate, boreal forests—primary terrestrial C sink—and permafrost regions exposed to accelerated global warming and considered as an important climate tipping point (Strauss et al., 2017).

2. Materials and methods

2.1. Study sites and setup of the tea bag experiment

This study represents a 3000 km latitudinal gradient of European Russia (Fig. 1A, Table 1). The gradient crosses six vegetation zones (forest-tundra, northern taiga, mixed forests, broadleaf forests, forest steppe, and steppe), which correspond to boreal forest, temperate forest, and woodland/shrubland Whittaker's biomes (Fig. 1B). In each vegetation zone, we selected the urban and natural sites. We chose the urban sites within residential areas characterized by the presence of lawns with trees. Specifically, the urban sites were located within Murmansk (population 0.3 million), Apatity (0.05 million), Moscow (12 million), Pushchino (0.02 million), Kursk (0.4 million), and Rostov-on-Don (1.1 million), representing forest-tundra, northern taiga, mixed forests, broadleaf forests, forest steppe, and steppe vegetation zones, respectively. We selected the corresponding natural counterparts at the outskirts of each city. At each urban and natural site, we then chose a representative area of approximately 900 m^2, and we randomly distributed 10 observation plots in the area for soil sampling and tea bag burying (Fig. 2A). We buried the two replicates per each plot of standard tea bags set (Lipton, according to Keuskamp et al., 2013) and alternative tea bags available in Russia (Vkusvill rooibos and Berton green) at a soil depth of 8 cm at the beginning of the growing season (end of May to beginning of June 2021). All the tea bags were weighed prior to the experiment. In total, 960 tea bags (80 per each site × 2 land use types × 6 vegetation zones) were buried.

When choosing the alternative tea bags, we considered the following important criteria: (1) nylon pouch fabric with average mesh d = 0.20 mm detected via a Tescan Vega 3 SBH scanning electron microscope (Czech Republic (Fig. S1A)) and (2) having no additives and flavorings. The Standard (Lipton) tea bags were produced in 2020 using nonwoven fabric (polypropylene). The hole size between the threads of the fabric ranged from 0.03 to 0.10 mm (Fig. S1B). The majority of soil microorganisms are <0.10 mm, and only the small forms of microfauna (e.g., tardigrades, nematodes, rotifers) can penetrate 0.10–0.20 mm. However, it has been demonstrated the decomposition of litter in tea bags over a 90-day period is independent of the mesh size (Mori et al., 2021).

The tea bags were kept in the soil for 90 days. Then we sampled the tea bags, placed them in individual craft bags, and delivered them to the laboratory, where soil particles and roots were removed. We dried the remaining litter at 70 °C over 48 h and weighed it. Fig. 2B shows the burial and removal of tea bag procedures.

Keuskamp et al. (2013) suggested calculating the tea bag indexes (TBI): decomposition rate (k) and litter stabilization factor (S). However, recent studies highlight the potential for under- and overestimations of k and S factors depending on soil properties (Mori et al., 2022). New approaches to TBI calculations are still in the developing stage (Mori, 2022b; Middelanis et al., 2023). Therefore, the TBI was not calculated in our study. Instead we focused on the green and rooibos tea mass losses to evaluate the early-stage microbial decomposition of litter of different quality (labile and recalcitrant, respectively).

During tea bag incubation in the soil, we monitored topsoil temperature (Checktemp sensor, resolution 0.1 °C, accuracy ±0.3 °C, Hanna Instruments, Germany) and moisture (Delta-T Devices, accuracy ±3 %, UK) every 2 weeks near each plot. At the same day when the tea bags were buried, we collected topsoil samples (0–8 cm) from each plot to analyze the chemical and microbial properties. We measured soil bulk density based on the dry weight of the ring samples (V = 100 cm^3).

2.2. Chemical composition of alternative tea bags

We determined the nonpolar extractives (NPE, e.g., fats and waxes), water solubles (WS, e.g., carbohydrates and phenolics), acid solubles (AS, e.g., cellulose), and acid insolubles (AIS) fractions of the alternative green tea and rooibos tea by a sequential extraction technique (Ryan et al., 1990). We extracted NPE with dichloromethane in a water bath for 24 h and determined WS fraction through hot water extraction. We divided the AS and AIS fractions using lignin content determination. We isolated lignin by acid hydrolysis in 72 % sulfuric acid (TAPPI, 2011). Then we combusted lignin fraction at 600 °C to determine the ash content. The remaining material was a mineral fraction. We determined the content of every fraction gravimetrically (Sartorius CP124S, Germany) and expressed it as a percentage of dry weight using extraction coefficients for calculation. The hydrolysable fraction (easily mineralized by soil microorganisms) was calculated as the sum of the NPE, WS, and AS fractions.

2.3. Soil chemical and microbial properties

We determined total C and N content in the soil samples via the dry combustion method using an automated HCNS analyzer (Vario Cube, Germany). We measured the pH in a soil:water suspension (1:2.5 ratio) using a Starter Bench pH Meter (USA), and we determined microbial biomass carbon (MBC) by the substrate-induced respiration (SIR) method based on the maximal initial response of microorganisms to the addition of glucose (Anderson and Domsch, 1978; Ananyeva et al., 2008). We assessed the SOM decomposition rate in standard conditions (22 °C, 55–65 % water holding capacity) using basal respiration (BR) through determination of CO_2 (ISO 16072), and we measured SIR and BR by a Krystall 2000 M Meta-Chrom gas chromatograph (Russia), equipped with a thermal conductivity detector for measuring CO_2 concentration. The fresh MBC and BR sample measurements were taken from soils pre-incubated at 25 °C for 72 h (Creamer et al., 2014). We performed all analyses in triplicate.

2.4. Statistics

To compare the soil variables and mass losses of tea litters between vegetation zones, we used a one-factor analysis of variance (ANOVA). We performed a comparison of soil properties between urban and natural soils via an independent t-test for each vegetation zone.

We used principal component analysis (PCA) to show the variations and relationships between the studied soil properties or predictors (quantitative factors) across vegetation zones and land use types. We

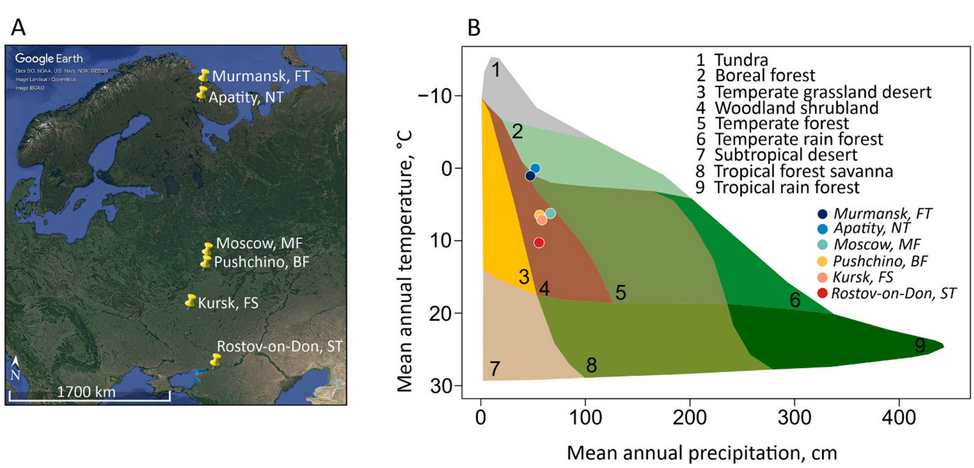

Fig. 1. Study sites along 3000 km latitudinal gradient: cities and vegetation zones are indicated (A); location of study sites on Whittaker Biome Diagram basing on mean annual temperature and mean annual precipitation data (B). Hereinafter: FT, forest-tundra; NT, northern taiga; MF, mixed forests; BF, broadleaf forests; FS, forest steppe; ST, steppe.

used redundancy analysis (RDA) to (i) show the total variance of mass loss of tea litters across the climatic gradient for both natural and urban ecosystems; and (ii) test the relationships between this variance and soil properties. We checked the variation inflation factor (VIF) for each predictor, and it was excluded in RDA if VIF > 4. To evaluate the quantitative factors in combination to the qualitative factors (climatic gradient and land use types) the linear mixed effects models were applied (lmer function from package "lme4"). The significance of quantitative factors (fixed factors) was checked by *t*-tests using Satterthwaite's method. In prior statistical analysis, both dependent and predictor variables were log-transformed (i.e., mass loss of rooibos tea, C, N, C:N, MBC, BR) or squared (bulk density) according to normal distribution adjustment. We performed statistical analysis and visualization of experimental data in R. A total of 109 out of 120 plots were included in the data analysis, because, for 11 plots, we could not detect the buried alternative teabags (for standard teabags it was 16). The possible reason for this is human and wildlife activity in urban and natural sites, respectively.

3. Results

3.1. Chemical composition of the standard and alternative tea bags

The C:N ratio characterizing the rooibos tea litter was higher by 2.4–3.5 times compared to green tea (Table 2). The highest C:N ratio in rooibos tea is responsible for its slowest long-term microbial decomposition in soil compared to green tea litter. The portions of hydrolysable fractions of standard and alternative tea bags were similar—though the contribution of acid-soluble and mineral fractions in the alternative tea bags was 1.5–1.8 and 2.0–7.0 times higher, respectively, as compared to standard tea. Such differences can provide slightly different rates for standard and alternative tea litter decomposition in the soil.

3.2. Topsoil properties across latitudinal gradient and land use types

Mean annual soil temperature and soil pH increased from northern to southern sites (Fig. S2A, Table S1). The soil temperature, pH, and bulk density for urban soils were higher than natural sites (Fig. S2B). Soil BR, MBC, and C and N content in urban sites were significantly lower compared to the natural sites.

PCA summarized the information of the spatial distribution of the soil properties (Fig. 3). The highest contribution to the first PCA axis was common for soil bulk density ($r = -0.87$), pH ($r = -0.67$), BR ($r = 0.88$) and C ($r = 0.91$), whereas the C:N ratio ($r = -0.71$) and soil temperature ($r = 0.71$) contributed mainly to the second axis (Fig. 3A). More northern sites of forest tundra and northern taiga were grouped separately from southern sites of steppes and forest steppes, whereas the sites of the mixed and broadleaf forests took the middle position (Fig. 3B). Consequently, the considered latitudinal zones could be clustered mainly by temperature to the three conventional groups: "cold", "medium", and "warm". Variance of soil properties along the latitudinal gradient for natural sites was expressed stronger than for urban ones (Fig. 3C). The differentiation of natural and urban sites along the first axis was mainly related to changes in soil bulk density, pH, organic matter content, and microbial activity, expressed as BR. Thus, selected sites had different soil chemical, physical, and microbial properties, as determined by the vegetation zone and land use type. Such broad variability of soil properties and bioclimatic conditions provides opportunities for the calibration of the alternative tea bag index approach in alignment with the standard tea bags.

3.3. Mass losses of alternative and standard litter from tea bags

As expected, the green tea decomposed 2.2 and 2.1 times faster than rooibos tea for standard and alternative approaches, respectively (Fig. 4). Both types of litter in alternative tea bags decomposed slowly compared to the standard one. The differences in mass loss averaged 1.3 and 1.2 times for green and rooibos tea, respectively. This discrepancy can be attributed to a higher amount of recalcitrant litter fraction in alternative tea litter compared to standard Lipton tea (Table 2).

We found a strong linear relationship ($R^2 = 0.98$ and 0.94 for green and rooibos, respectively) between the mass losses from the standard and alternative tea bags for all data set, despite the difference in bag fabric and chemical composition of the tea litter (Table 2, Fig. S3). To adjust the differences in the tea weight losses, we calculated the conversion factors from alternative to standard approaches. For green tea, it amounted to 1.29 and, for rooibos tea, to 1.18. There were no substantial differences in the calculated conversion factors between natural and urban sites (Fig. S4). The differences between the standard and recalculated mass loss values using conversion factors amounted on average 0.5–0.8 % (Fig. 4). Given the robust relationship established between alternative and standard mass losses, subsequent analyses was further focused on decomposition patterns of only alternative tea bags, including the impact of climate and land use type.

3.4. Mass loss of tea under consideration of qualitative factors

The mass loss of green tea litter in soils increased along the latitudinal gradient (Fig. 5A). The lowest mass loss of green tea litter was common at latitudes with average soil temperature for the growing season below +12 °C ("cold" climate cluster: forest-tundra and northern taiga zones). The loss of green tea litter mass in urban soils (on average 45 %) was similar to that in natural soils (43 %) (Fig. 5B). For rooibos tea, the highest mass loss was observed for sites with an average soil temperature for the growing season higher than +19 °C ("warm" climate cluster: forest-steppe and steppe zones). (Fig. 5C). The mass loss of rooibos tea in urban soils was 1.3 times larger than in natural soils (Fig. 5D). Important to note that the variation in rooibos mass loss between natural and urban soils was site-specific (Fig. S5). The highest difference between land use types was observed in the forest steppe belt, while the least variation was found in the northern taiga. We can thus summarize the decomposition rate of the more available substrate (green tea) was more sensitive to the latitudinal (climate) gradient, whereas the decomposition of the more recalcitrant rooibos tea was primary determined by the land use type.

Table 1
Location, soil types and mean annual temperature (MAT) and mean annual precipitation (MAP) for the study sites.

Vegetation zone	City	Natural soils (IUSS Working Group WRB, 2022)	Land use types (NE coordinates)		MAT, °C	MAP, mm
			Natural	Urban	1991–2020	1991–2020
Forest tundra	Murmansk	Albic Podzols	68.983623, 33.017952	68.972977, 33.088903	1.08	521
Northern taiga	Apatity	Albic Podzols	67.578723, 33.297863	67.569801, 33.400842	0.04	568
Mixed forests	Moscow	Albic Retisols	55.818633, 37.553158	55.737377, 37.620192	6.26	712
Broadleaf forests	Pushchino	Luvic Phaeozems	54.829566, 37.650065	54.832479, 37.625452	6.51	610
Forest-steppe	Kursk	Haplic Chernozems	51.571898, 36.091595	51.661231, 36.128212	7.13	631
Steppe	Rostov-on-Don	Calcic Chernozems	47.504316, 40.155043	47.254819, 39.711088	10.30	604

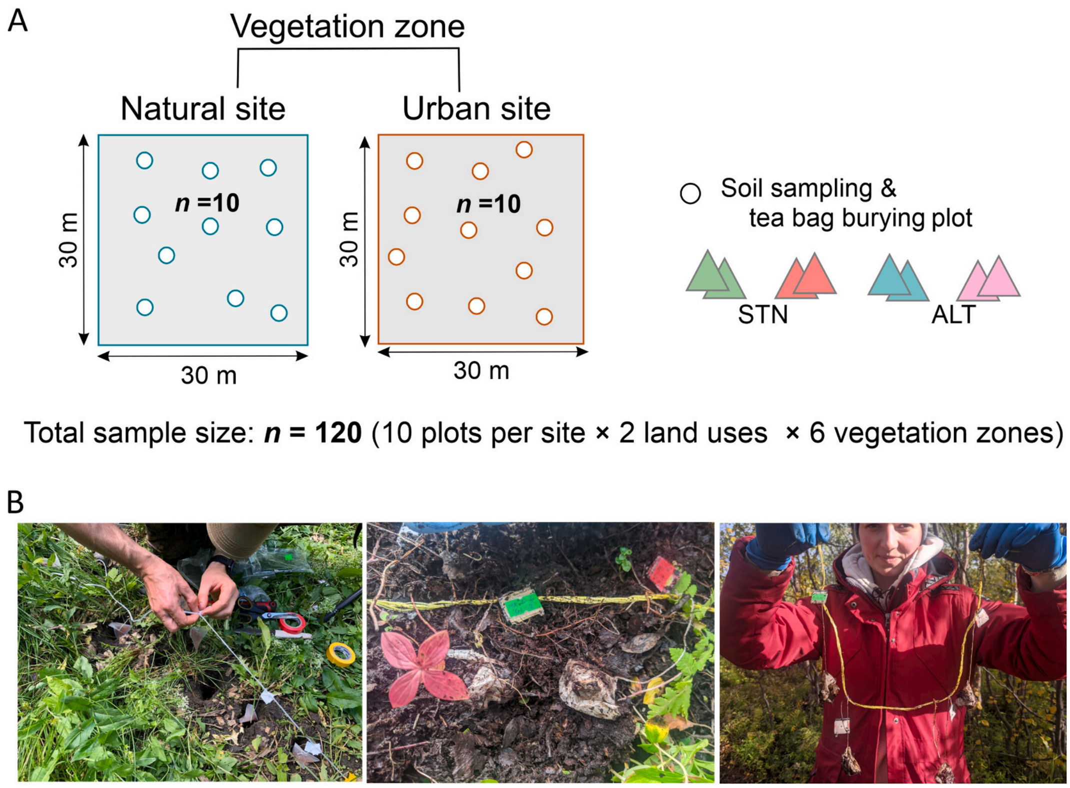

Fig. 2. Soil sampling and tea bag burying scheme of standard (STN) and alternative (ALT) tea bags (A). Burial and removal of tea bag procedures (B).

Table 2
Chemical properties of standard (Keuskamp et al., 2013) and alternative tea bags. The values present the mean ± standard deviation.

Properties	Green tea		Rooibos tea	
	Standard	Alternative	Standard	Alternative
C, %	49.0 ± 0.1	46.0 ± 0.9	50.5 ± 0.3	43.7 ± 0.6
N, %	4.0 ± 0.0	3.2 ± 0.0	1.2 ± 0.0	1.3 ± 0.1
C:N	12.2 ± 0.1	14.3 ± 0.1	42.9 ± 1.8	34.7 ± 2.6
Nonpolar extractives, %	6.6 ± 0.3	3.2 ± 0.1	4.9 ± 1.3	1.2 ± 0.2
Water soluble, %	49.3 ± 2.1	35.7 ± 0.6	21.5 ± 0.9	15.9 ± 0.3
Acid soluble, %	28.3 ± 1.7	42.6 ± 0.1	28.9 ± 4.0	51.3 ± 0.3
Acid insoluble, %	15.6 ± 0.9	19.3 ± 0.1	44.4 ± 4.0	31.6 ± 0.3
Mineral fraction, %	0.2 ± 0.0	1.4 ± 0.1	0.4 ± 0.1	0.8 ± 0.1
Hydrolysable fraction, %	84.2 ± 2.3	81.5 ± 0.7	55.2 ± 5.0	68.4 ± 0.1

3.5. Quantitative factors affecting tea litter mass loss

The first RDA axis described 28 % of the total variance (Fig. 6), whereas the contribution of the second axis was not significant (Table S2). Regarding the green tea decomposition mass loss, the variation between the study sites was attributed to temperature conditions, leading to distinct clusters that could be categorized as "cold" and "medium + warm." The studied sites were grouped by land use types according to the mass loss of rooibos tea, and this was clearly manifested in the "warm" cluster. Among the studied quantitative factors, soil temperature, pH, and C:N ratio had a high contribution to the first axis, with correlation coefficients of 0.97, 0.81, and − 0.73, respectively (Table S2). Hence, to understand their impacts on the mass loss of tea litter in the context of the climatic gradient and land use types change, these factors have been considered in mixed effects model. In the case of green tea, the significant factors were soil temperature ($p \leq 0.05$) and climatic gradient (26 % of the variance explained) (Table S3). This

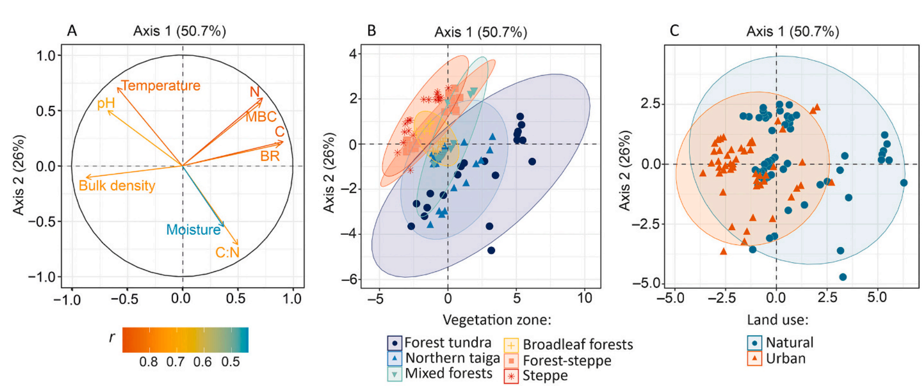

Fig. 3. Principal component analysis for topsoil properties (A): soil bulk density, pH, temperature and moisture, carbon (C), nitrogen (N), microbial biomass carbon (MBC) content and basal respiration (BR) grouped by vegetation zones (B) and land use types (C). Correlation coefficient between soil properties and axes is assigned as r ($n = 109$).

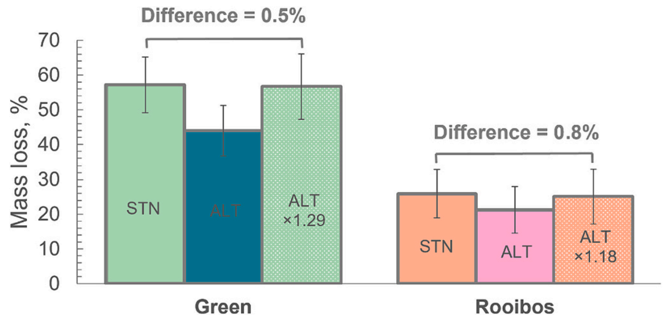

Fig. 4. Litter mass loss of standard (STN) and alternative (ALT) green and rooibos teas after 90th days. Recalculated mass loss of tea is presented as ALT×1.29 and ALT×1.18. The bar plot reflects the mean value with standard deviation.

indicates a slowing down of green tea decomposition from south to north is associated with a decrease in soil temperature. Instead, the soil C:N ratio and land use type (0.9 % of the variance explained) didn't affect the green tea mass loss in this model. For rooibos tea, interaction of soil pH and soil temperature contributed significantly ($p \leq 0.05$) to the variance in decomposition (Table S4). Land use type was a major contributor to rooibos mass loss (22 % of the variance explained), in contrast to the climate gradient (only 3 %). The rise of soil pH and soil temperature in urban areas elucidates the increased mass loss of recalcitrant rooibos litter compared to natural sites.

4. Discussion

4.1. Standard and alternative tea bags: limitations and perspectives for global soil surveys

The tea bag initiative has gained recognition among researchers and become an integral part of citizen science, involving students, teachers, and farmers (Pino et al., 2022). This innovative, well standardized, and cost-effective method, introduced by Keuskamp et al. (2013), relies on litter of two types of Lipton tea to assess microbial activity in regard to decomposing SOM and its stabilization. It follows a uniform standardized protocol for the global soil surveys. However, there is a challenge concerning efficient global distribution of tea bag initiative. Standard Lipton tea bags are distributed primarily through specific European shops and are not readily available in some countries. This has led to a geographical imbalance in tea bag initiative contributions, with a majority coming from European countries (Djukic et al., 2018). For instance, in Australia, efforts to carry out soil citizen science projects using the tea bags have required online orders and deliveries from Europe of Lipton tea bags (Pino et al., 2021). This procedure may not be accessible to people in rural areas, and it raises costs and complicates in general the tea bags applicability for certain countries and even continents. Indeed, researchers have highlighted the necessity for exploring tea bag alternatives.

Although alternative tea bags were tested in a study conducted in Scotland, there has been a lack of field comparisons between these alternative tea bags and standardized ones (Marley et al., 2019). Various alternative tea litter types have recently undergone laboratory and field testing in Germany (Middelanis et al., 2023). The authors extended the possibility of utilizing certain types of tea for decomposition studies. Various brands of black tea, for example, mirrored the decomposition patterns of Lipton green standardized tea even better than the alternative green tea brands. Instead, the decomposition of peppermint tea was characterized by excessively high variability between replicates and brands due to specific tea and packaging characteristics. We extended the geography of testing of alternative tea bags by including six vegetation zones within a 3000 km latitudinal gradient, also considering land-use represented by natural and urban sites. Despite the different fabric materials and chemical composition, there was a close relationship between standard and alternative tea mass loss. The close correspondence of the results obtained by standardized and alternative tea types means the method can be adapted by the application of other tea bag brands when the availability of standardized tea bags is limited. This enhances the approach applicability for diverse participants and countries and facilitates the expansion of the global database and application in citizen science. The approach adaptation to alternative tea bags

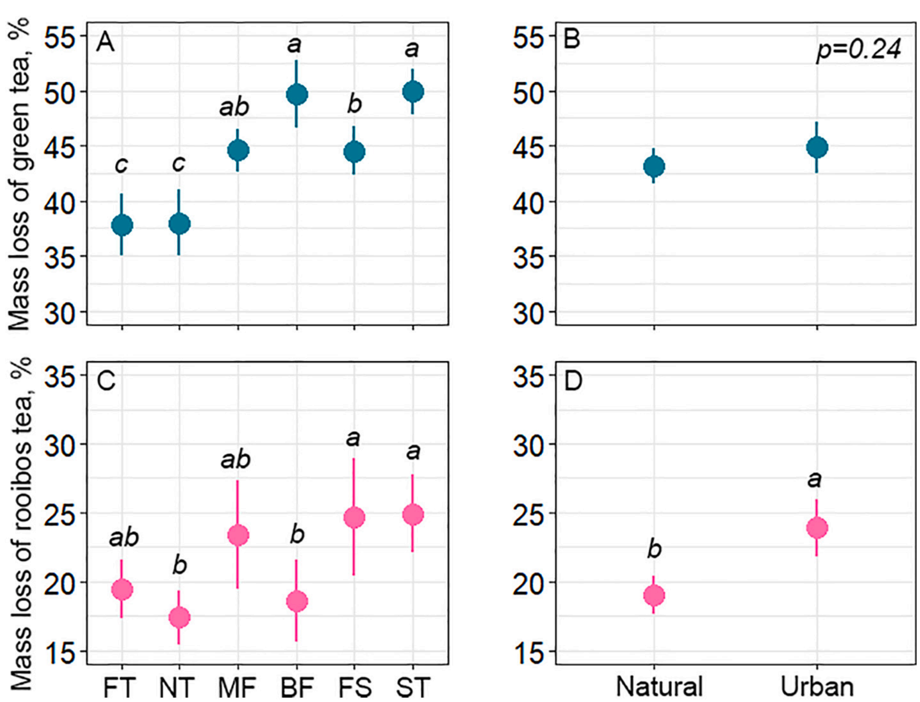

Fig. 5. Litter mass loss (means with 95 % confidence interval) of green and rooibos tea along the latitudinal gradient (A and C) and land use type (B and D, respectively).

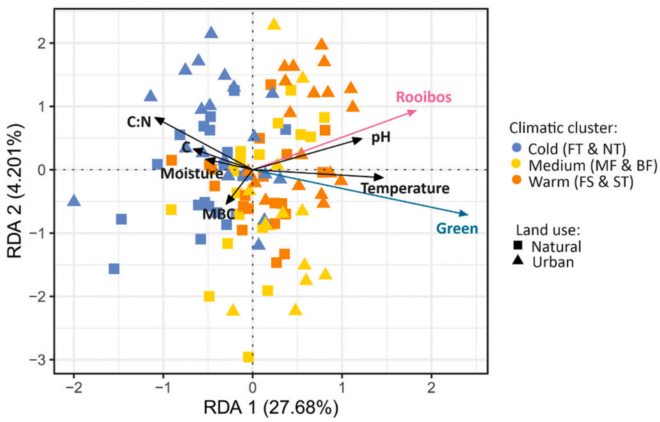

Fig. 6. RDA ordination showing the relationships between of mass loss of green and rooibos tea litter and soil properties (black arrows) across the land use types and latitudinal zones, grouped into "cold" (forest-tundra and northern taiga), "medium" (mixed and broadleaf forests) and "warm" (forest steppe and steppe) clusters ($n = 109$). Notes: MBC, microbial biomass carbon. Bulk density and basal respiration were excluded due to the VIF > 4 and high correlation of the variables with soil C (Fig. 3A).

involves the following steps: (1) controlled experiments on the natural composition of tea litter in the bags and (2) correct selection of the bag material with an appropriate mesh size and comparable size of tea particles (Mori, 2022a). In the case of an incomplete match between alternative and standard tea, as in our case, paired decomposition experiments under various environmental conditions and locations for the quantification of the conversion factors would be required. The next method development step would require creating a user-friendly database to catalog alternative tea bags and their validated conversion factors, regularly updated with new information. To enhance tea bag initiatives globally, we suggest appointing national coordinators for each country to facilitate collaboration, provide guidance, and serve as primary contacts. This comprehensive approach aims to enhance the accessibility, adaptability, and effectiveness of tea bag experiments across various geographic and social contexts.

4.2. Tea litter decomposition drivers

This study has recognized the sources of variation of early-stage microbial decomposition of different litter qualities (labile and recalcitrant, respectively) (Fig. 5). Decomposition (mass loss) of more labile litter (green tea) was sensitive to soil temperature along the 3000 km latitudinal gradient. Hydrothermal conditions have been generally identified as one of the drivers of tea litter decomposition (Djukic et al., 2018; Mueller et al., 2018; Sarneel et al., 2020; Górecki et al., 2021), but in this study, the soil temperature was the main factor of mass loss for green and rooibos tea litter (Fig. 6; Table S3;S4). The mean annual temperature strongly increases from north to south in European Russia, and precipitation remains comparatively stable (Fig. 1B), which was respectively reflected in soil temperature and moisture (Fig. S2).

The decomposition of green tea increased with temperature following the latitudinal gradient, reducing the C stabilization. The temperature gradient indirectly determines the SOM composition. In the northern part of the transect (forest-tundra and northern taiga), SOM decomposes slowly, leading to accumulation of recalcitrant plant residuals. Consequently, the soil C:N ratio was the highest in northern sites compared to southern ones (Fig. S2). Based on mixed models we didn't find a significant effect of soil C:N ratio on the decomposition of green tea (Table S3). However, the interactive effects of precipitation and soil C:N ratio on tea litter mass loss were observed for forests and grasslands in southern Europe (Blanco et al., 2023). In our study, the green tea mass loss was primarily regulated by changes in soil temperature along a 3000 km latitudinal gradient.

Decomposition rates of recalcitrant rooibos tea were sensitive to land use type, with higher mass loss in urban soils compared to natural soils (Fig. 5D). Considering the effects of soil temperature and soil pH on the decomposition of rooibos tea, we surmise these factors largely contribute to the difference between natural and urban ecosystems that was confirmed by mixed models results (Table S4). In urban environments, factors such as litter exclusion and the introduction of organic amendments and pollutants may foster the formation of a higher fraction of recalcitrant SOM (Cachada et al., 2012; Ananyeva et al., 2023; Bekier et al., 2023). Consequently, the microbial community in urban settings should be ready to actively decompose such complex materials. Moreover, in urban environments, the rise in soil temperature (ranging from 0.2 °C to 3.0 °C, with an average value of 1.1 °C) (Fig. S2, Table S1) significantly stimulated tea litter decomposition compared to natural conditions. The urban heat island was found to boost microbial activity by 5–25 %, with soil temperature increases ranging between 1.7 and 2.0 °C during the vegetation period (Vasenev et al., 2021). This temperature increase resulted in the intensification of soil CO_2 emissions from urban soil (Vasenev et al., 2023). Temperature manipulation experiments conducted in situ demonstrated also that a small yet consistent increase in soil temperature accelerates decomposition and nutrient cycling (Gavrichkova et al., 2017). Temperature is the main factor for SOM decomposition when the microbial community faces high molecular weight recalcitrant compounds (Blagodatskaya et al., 2016). Besides the soil temperature effect, the pH increase in urban soils accelerates the decomposition of rooibos tea (see Fig. 6 and Table S4). The pH effect is executed through changes in microbial community structure and functioning and by impacting the nutrient availability (Lauber et al., 2009; Zhalnina et al., 2015; Anderson et al., 2018). The last was confirmed in a microcosm experiment where the rise of soil pH (from 4.7 to 6.7, 8.3, or 8.8) increased dissolved SOM concentration and microbial activity and shaped microbial community structure (Anderson et al., 2018). Thus, the pH together with soil temperature increase in urban soils accelerates the microbial decomposition of recalcitrant litter rather than the decomposition of labile green tea litter.

5. Conclusions

Based on a 3000 km latitudinal gradient along European Russia, encompassing natural and urban ecosystems, we propose using locally available alternative green and rooibos tea bags to study litter decomposition in the soil following the standard protocol (Keuskamp et al., 2013). Conversion factor was calculated to align the results of local tea litter with those obtained using standard Lipton tea bags.

Mass loss of green tea litter rose along the latitudinal gradient, whereas rooibos tea mass loss was larger in urban soils compared to natural ones. The decomposition of recalcitrant rooibos tea in urban soils was influenced by the temperature during the vegetation period (on average + 1.1 °C higher than in natural soils) and pH value (+1.1 units) compared to natural analogues. Consequently, microbial communities in urban soils decompose the recalcitrant organic matter faster than in natural soils. This study thus demonstrates the feasibility of participating in the Tea Bag Initiative project using locally available tea bags. It would facilitate the development of national databases, as well as the global tea bag initiative expansion.

CRediT authorship contribution statement

K. Ivashchenko: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **O. Gavrichkova:** Writing – review & editing, Writing – original draft, Methodology, Conceptualization. **M. Korneykova:** Writing – review & editing, Methodology, Conceptualization. **V. Vasenev:** Writing – review & editing, Project administration, Investigation, Funding acquisition. **N. Salnik:** Writing – review & editing, Methodology, Formal analysis. **N. Saltan:** Writing – review & editing, Methodology, Formal analysis. **D. Sarzhanov:** Writing – review & editing, Methodology, Formal analysis. **E. Babenko:** Writing – review & editing, Methodology, Formal analysis. **S. Urabova:** Writing – review & editing, Methodology, Formal analysis. **M. Slukovskaya:** Writing – review & editing, Methodology, Formal analysis. **M. Zavodskikh:** Writing – review & editing, Methodology, Formal analysis. **S. Gorbov:** Writing – review & editing, Methodology, Formal analysis. **D. Petrov:** Writing – review & editing, Methodology, Data curation. **A. Dolgikh:** Writing – review & editing, Methodology, Formal analysis. **Sotnikova Yu:** Writing – review & editing, Methodology, Data curation. **M. Vasileva:** Writing – review & editing, Methodology, Data curation. **P. Skripnikov:** Writing – review & editing, Methodology, Formal analysis. **O. Ryzhkov:** Writing – review & editing, Methodology, Formal analysis. **K. Nikerova:** Writing – review & editing, Methodology, Formal analysis. **S. Sushko:** Writing – review & editing, Methodology, Formal analysis. **N.D. Ananyeva:** Writing – review & editing, Methodology, Conceptualization. **T. Bochko:** Writing – review & editing, Methodology, Conceptualization. **Y. Kuzyakov:** Writing – review & editing, Writing – original draft, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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