Soil C/N ratios cause opposing effects in forests compared to grasslands on decomposition rates and stabilization factors in southern European ecosystems

Juan A. Blanco [a,*], María Durán [b], Josu Luquin [a], Leticia San Emeterio [b], Antonio Yeste [a], Rosa M. Canals [b]

[a] Institute for Multidisciplinary Applied Biology (IMAB), Departamento de Ciencias, Universidad Pública de Navarra (UPNA), 31006 Pamplona, Spain
[b] Institute of Innovation and Sustainable Food Chain (IS-FOOD), Departamento de Agronomía, Biotecnología y Alimentación, Universidad Pública de Navarra (UPNA), 31006 Pamplona, Spain

HIGHLIGHTS

- Forests and grassland soils are fundamental in understanding carbon cycling.
- Decomposition rates were assessed in 16 European ecosystems with the Tea Bag Index.
- Precipitation was positively related to litter decomposition and stabilization.
- Plant cover type strongly interacted with soil C/N ratios affecting decomposition.
- Forest increase will strongly modify soil organic matter stabilization and turnover.

GRAPHICAL ABSTRACT
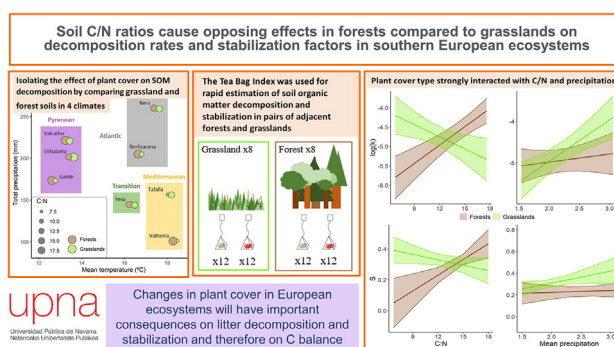
ARTICLE INFO

ABSTRACT

Soils store an important amount of carbon (C), mostly in the form of organic matter in different decomposing stages. Hence, understanding the factors that rule the rates at which decomposed organic matter is incorporated into the soil is paramount to better understand how C stocks will vary under changing atmospheric and land use conditions. We studied the interactions between vegetation cover, climate and soil factors using the Tea Bag Index in 16 different ecosystems (eight forests and eight grasslands) along two contrasting gradients in the Spanish province of Navarre (SW Europe). Such arrangement encompassed a range of four climate types, elevations from 80 to 1420 m.a.s.l., and precipitation (P) from 427 to 1881 mm year^{-1}. After incubating tea bags during the spring of 2017, we identified strong interactions between vegetation cover type, soil C/N and precipitation affecting decomposition rates and stabilization factors. In both forests and grasslands, increasing precipitation increased decomposition rates (k) but also the litter stabilization factor (S). In forests, however, increasing the soil C/N ratio raised decomposition rates and the litter stabilization factor, while in grasslands higher C/N ratios caused the opposite effects. In addition, soil pH and N also affected decomposition rates positively, but for these factors no differences between ecosystem types were found. Our results demonstrate that soil C flows are altered by complex site-dependent and site-independent environmental factors, and that increased ecosystem lignification will significantly change C flows, likely increasing decomposition rates in the short term but also increasing the inhibiting factors that stabilize labile litter compounds.

1. Introduction

Forest area in Europe is expanding, causing changes in carbon stocks and flows that need to be better understood to develop effective forest

* Corresponding author at: Institute for Multidisciplinary Applied Biology (IMAB), Departamento de Ciencias, Universidad Pública de Navarra (UPNA), 31006 Pamplona, Spain.
E-mail address: juan.blanco@unavarra.es (J.A. Blanco).

policies for climate change mitigation and adaptation (Wieser et al., 2014; Palmero-Iniesta et al., 2021). Demographic and socioeconomic changes in the last century have increased forest cover in Europe over 25–30 % since the 1950s (EEA, 2017; Kauppi et al., 2018). In addition, climate change is promoting the expansion of some particular tree species, such as those more adapted to drought and intensified fire regimes (Bussotti et al., 2015). As modelization approaches have already suggested (Seely et al., 2010; Lo et al., 2015), both forest expansion and species substitution are expected to cause profound changes in the terrestrial carbon (C) budgets.

Plant litter decomposition makes up approximately 70 % of the global carbon flux from soils to the atmosphere (Raich and Potter, 1995). How the substitution of vegetation cover caused by expansion of forest area is influencing the processes of soil organic matter (SOM) transformation (decomposition and stabilization) and their consequences on regional C balance compared to less lignified natural plant communities (grasslands and open shrublands) is therefore a matter of interest. Afforestation implies changes in plant biomass production and its composition (increased lignification and tissues recalcitrance to decomposition), as well as in the environmental conditions (microclimate). Such changes are expected to strongly determine the type and activity of soil detritivore and decomposer communities (Wardle et al., 2004; de Vries et al., 2012; Kaiser et al., 2016). Hence, understanding changes in carbon flows in such landscape transitions is becoming increasingly important to better comprehend how carbon pools may change in the near future.

Climate is a crucial environmental driver of soil organic matter decomposition through two main controlling factors: temperature and water availability (Aerts, 1997). A wet and mild environment will allow plants to produce more biomass and hence higher litterfall rates. Such environment also produces higher SOM decomposition rates than arid or extreme environments (Althuizen et al., 2018; Becker and Kuzyakov, 2018). Both temperature and precipitation affect decomposition rates directly (through freeze-thaw and wetting and drying cycles), but also indirectly by influencing the type, growth and activity of decomposer communities. Such communities are constituted by detritivore invertebrates and by decomposer microorganisms very sensitive to environmental conditions (Schimel and Hättenschwiler, 2007; Saura-Más et al., 2012; Sylvain et al., 2014; Herrera-Alvarez et al., 2020). To that respect, aridity indexes defined as a balance between annual precipitation and potential evapotranspiration have been shown to be strongly correlated to biotic decomposition rates (Alessandro and Nyman, 2017). However, unlike decomposition rates, the effects of temperature and soil moisture on SOM stabilization have been less studied, even though they are expected to drive the process in the opposite way: that is, harsher environmental conditions reduce SOM decomposition and enhance SOM stabilization (Keuskamp et al., 2013).

At the landscape level, climate is highly modulated by altitude, topography and plant cover. Decomposition rates decrease at increasing altitudes coinciding with the expected temperature drop (Wang et al., 2017). Regarding topography, it strongly determines the level of exposure to solar radiation, which highly affects the energy arriving to the soil surface and evapotranspiration rates. Topography also has an effect on water retention capacity by soils and moisture levels. Topographic effects affecting SOM transformation rates have been reported to be particularly strong in water-limited systems (Gabriel and Kellman, 2014; Alessandro and Nyman, 2017). Plant cover also has a strong effect on climate modulation and on solar radiation arriving to the soil surface. The occurrence of a tree cover decreases light penetration and increases transpiration rates with respect to bare soil evaporation (Huxman et al., 2005). Although the effect of forest cover on microclimate and organic matter decomposition is expected to be as variable as forest structure itself can be (i.e., tree cover and diversity, among other factors) (Rahman et al., 2018), differences in SOM transformation rates are expected to occur when comparing forests with shrublands and grasslands ecosystems (Prescott, 2002). This hypothesis has not always been easy to prove. For example, Wang et al. (2017) did not find differences in soil enzyme activities and decomposition rates between conifer canopies and *Rhododendron* sp. shrublands in the Alps.

Hence, more understanding is needed to estimate how the multiple, usually linked, environmental factors are already modifying C flows in changing European landscapes. The most suitable methods to obtain estimations of relative importance of environmental factors on SOM decomposition and stabilization are experiments of organic materials in situ (Åkesson, 2017). Traditionally, decomposition studies have used litterbags to understand decomposition rates and chemical changes in decomposing material (Blanco et al., 2011), but such approach produces results that are dependent on the plant material. On the other hand, resins have been used to explore soil processes, but in this case, the limitation relies on the artificial nature of the resin used (Reynolds and Hunter, 2001). Hence, both approaches are not suitable to test simultaneous changes in vegetation and environmental factors.

An alternative approach is the Tea Bag Index (TBI) method, based on a standardized data collection of SOM transformation that is based on the comparison of the mass loss of a labile (green tea) and a recalcitrant (rooibos tea) plant material (Keuskamp et al., 2013). This standardized method generates two main outputs (the SOM decomposition rate k, and the stabilization factor S), which allow to estimate the C fluxes and the long-term C sequestration potential for a particular habitat. The method has been widely used in experimental studies on SOM transformation during the last decade, in order to determine the role of different environmental drivers (Althuizen et al., 2018; Rahman et al., 2018; Blume-Werry, 2021). Although some criticism has lately been raised related to the absolute values of estimated decomposition rates (Mori, 2022), the method remains valid for comparative studies in which relative differences among sites (rather than the absolute quantitative values) are the factor of interest. In fact, if such method were used simultaneously in a broad range of vegetation, climate and soil sites, a detailed picture of the relative influences of such factors and their interactions on C flows in European terrestrial ecosystems could be drawn.

For the reasons presented above, our main hypothesis is that the existence of a tree cover modulates the rates of organic matter decomposition through the change in the environmental conditions and their interactions. Based on this hypothesis, our main objective was to test whether the presence of a tree canopy causes changes on decomposition processes always in the same direction or, contrarily, whether such direction depends on the specific prevailing environmental conditions (related to the specific particular vegetation type, climate, topography or soil of a given site). We established a unique experimental set-up to test our hypothesis, taking advantage of the location of the Navarre province, a hinge region of 10,391 km^2 in southwestern Europe between the temperate and the Mediterranean climates. Hence, our experimental set-up included the high elevation of the Pyrenees as well as the humid lowlands near the Atlantic Ocean and the arid region in the Ebro Valley. We characterized decomposition rates and stabilization factors using standardized litter material (tea bags) in paired biomes of adjacent native forests and grasslands along a strong gradient of altitude (50–1420 m.a.s.l.), aridity (0–30 Thornthwaite's aridity index), continentality and distance to the Atlantic Ocean (10–122 km), and soil attributes (i.e. pH 3.7–8.3). By using standardized plant material, the differences in litter quality among biomes (the most direct regulator of litter decomposition in large-scale studies; Zhang et al., 2008) were excluded, allowing us to focus the research on the effects of the environmental changes induced by the forest expansion of different species of native dominant trees colonizing open ecosystems.

2. Material and methods

2.1. Study sites

Eight sites were selected in Navarre province (northern Spain). Each site was composed by two adjacent ecosystems: a native forest and a native grassland. Sites were selected based on the availability of natural vegetation cover, accessibility for setting and recovering the tea bags, and closeness to weather stations. To maximize the range of environmental variables, the sites were organized into two transects, with four sites per transect.

Table 1

Main features of experimental sites. Tm: average daily temperatures during the incubation period. P: total rainfall during the incubation period. MAT: Mean annual temperature. MAP: Mean annual precipitation. Additional detailed information for each site is provided in the Supplementary Information (Tables S2–S9).

Municipality name	Altitude	Climate during Incubation period		Historical climate annual data		Forest type	Grassland type
	m.a.s.l.	Tm	P	MAT	MAP		
		°C	mm	°C	mm year^{-1}		
Bera	80–100	15.7	247.1	14.3	1871.3	Chestnut closed stand	Temperate meadow
Bertizarana	172–250	17.1	194.8	13.7	1559.8	European beech closed stand	Temperate meadow
Valcarlos	1075–1100	12.9	221.7	9.3	1637.9	European beech closed stand	Mountain grassland
Orbaizeta	1075–1094	13.3	200.2	9.4	1881.2	European beech closed stand	Mountain grassland
Garde	1405–1420	12.5	176.5	8.3	1362.5	Scots pine closed stand	Mountain grassland
Yesa	745–750	16.3	145.7	12.2	856.1	Portuguese oak open stand	Mediterranean grassland
Tafalla	469–470	17.5	175.7	13.4	546.9	Evergreen oak open stand	Mediterranean grassland
Valtierra	392–397	17.7	122.2	13.3	427.6	Aleppo pine open stand	Mediterranean grassland

being the decomposed fraction in the field and H_g the hydrolysable fraction of green tea as chemically estimated in the lab (Keuskamp et al., 2013), S can be estimated with Eq. (2).

$$S = 1 - \left(a_g / H_g \right) \qquad (2)$$

Assuming that due to the short incubation time the mass loss of recalcitrant compounds is negligible and that the chemically hydrosoluble fraction of the rooibos (Hr) is the value provided by Keuskamp et al. (2013) due to

the standardized fabrication process by Lipton™, and that the value of S is the same for both teas, the labile fraction of the rooibos tea (a_r) could be estimated following Eq. (3).

$$a_r = H_r \left(1 - S \right) \qquad (3)$$

With $W_r(t)$ and a_r known, k can be estimated using Eq. (1) particularized for *rooibos* values. For details on these equations, see Keuskamp et al. (2013).

Fig. 2. Experimental set up used to place two sets of tea bags (green tea and rooibos) in each pair of ecosystem types found at each experimental site.

2.3. Climate and soil data

Meteorological and climate data were collected from the network of weather stations managed by the regional Government of Navarre (Meteonavarra, 2022). Most sites were nearby to permanent weather stations and hence weather records were directly used, except for those in the highest eleva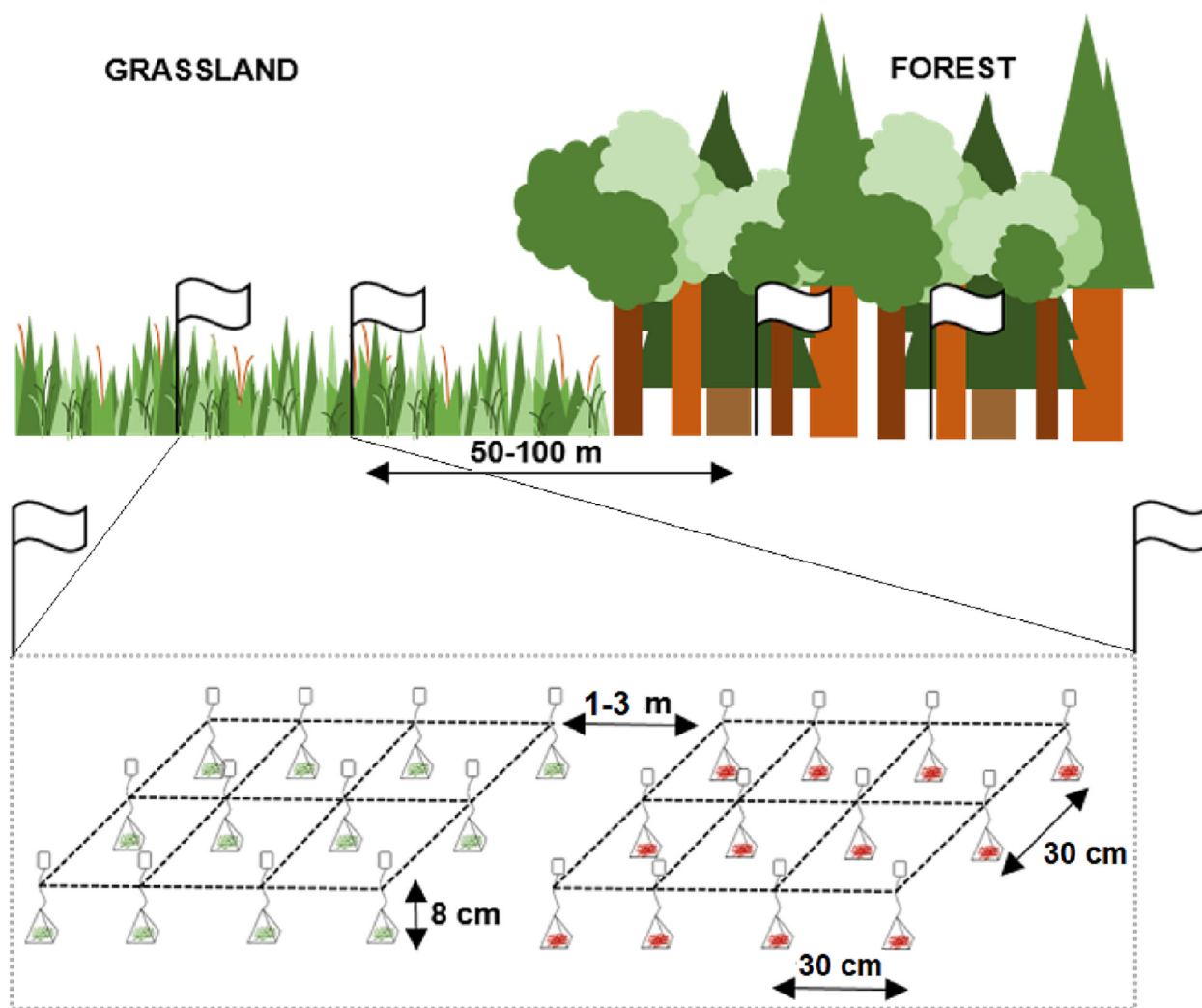tion areas (Garde, Orbaizeta) for which temperature and precipitation were corrected using the MTCLIM model (Running et al., 1987), following guidelines by Lo et al. (2011). Temperature and precipitation were registered for the field incubation period.

For soil data, a sample was collected at each ecosystem type from the top 10 cm soil layer for further analyses of texture, pH, conductivity, organic matter (OM), organic carbon (OC) and total nitrogen (N). Texture class (percentage of coarse and fine sand, silt and clay; USDA) was assessed by the gravimetric method. Soil pH and electrical conductivity of the <2 mm fraction were measured in 1 M KCl in a soil-solution ratio of 1:2.5 with a pH/conductivity meter (Crinson Micro pH-2001/Micro-CM-2202, Barcelona, Spain). The determination of soil organic matter and organic carbon was based on the Walkley-Black chromic acid wet oxidation method. Total nitrogen was estimated by the Kjeldahl method. Standard procedures for previous analyses are described by Pansu and Gautheyrou (2007). Details on soil features can be found in the Supplementary Information, whereas the accumulated precipitation, average temperature and C/N ratios are represented in Fig. 3.

2.4. Data analysis

Linear mixed models (LMMs, *nlme* R package; Pinheiro et al., 2020) were used to assess the effects of ecosystem type, climate, soil chemical properties and their interactions on the variables S, k and S/k ratio.

Sampling site was included as a random effect, while ecosystem type, pH, conductivity, soil organic matter, total nitrogen, C/N ratio, mean daily precipitation during the incubation period, and averages of daily mean (T_{ave}), maximum (T_{max}) and minimum (T_{min}) temperatures during the incubation period were included as fixed effects. Alternative models with the different combinations of fixed effects and interactions were constructed and compared using the Akaike information criterion in order to choose the best fixed effects structure (AIC, Burnham and Anderson, 2002). Once the best fitting model was obtained for each variable, the significance of the fixed effects was evaluated by using restricted maximum likelihood estimation procedures. Differences at $p < 0.05$ were regarded as statistically significant. The amount of variability explained by the best fitting models was calculated following Nakagawa et al. (2017) method, and using *MuMIn* R package (Barton, 2022). Data for k and S/k ratio were log transformed prior to the analysis in order to meet normality assumptions as a Gaussian error structure was used in the models (Quinn and Keough, 2002). We tested the influence of outliers following the same procedure of model selection for models including and excluding outliers. Outliers' exclusion did not change the results, as we obtained the same best-fitted models with and without outliers. Hence, we present results of models including outliers. All statistical analyses were performed using R 4.1.1 software (R Core Team, 2021).

3. Results

3.1. Decomposition rates and stabilization factors

There was a clear segregation between Mediterranean and temperate sites in decomposition rates, with Mediterranean sites reaching decomposition rates in the range 0.003–0.012 day^{-1}, whereas temperate sites reached higher decomposition rates in the range 0.009–0.024 day^{-1} (Fig. 4).
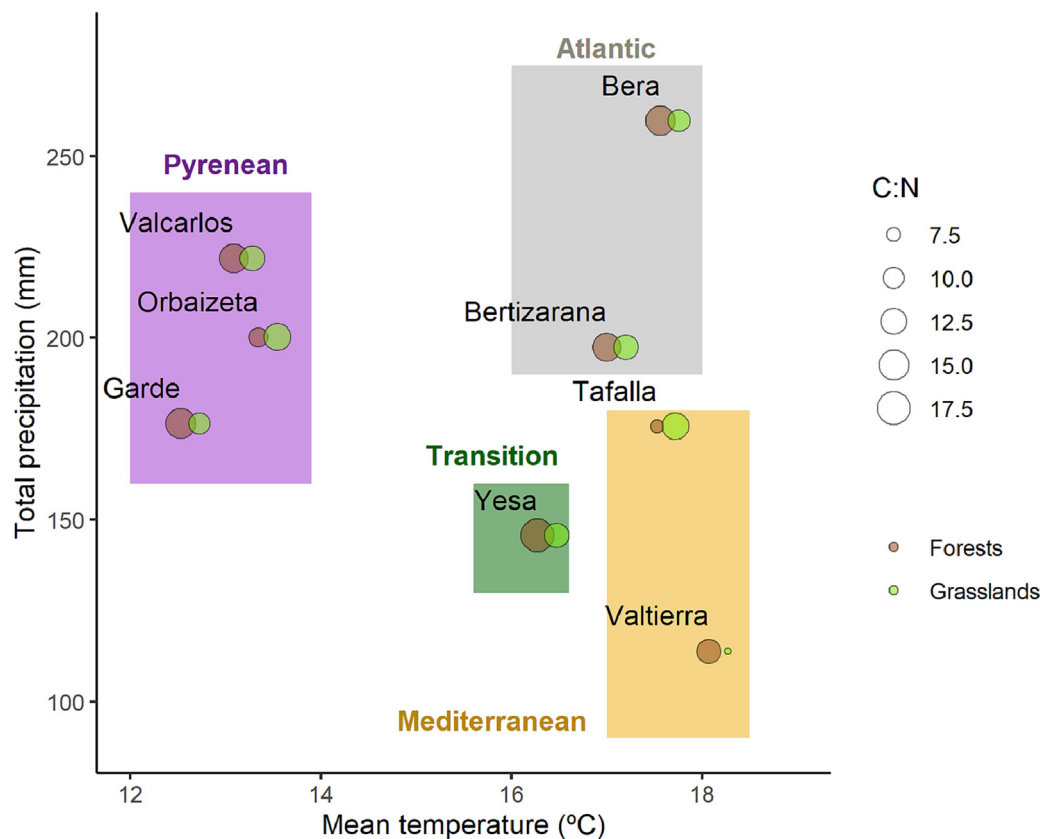

Fig. 3. Total precipitation (vertical axis) and mean temperature (horizontal axis) during incubation (spring 2017) at the eight sites (a pair of forest/grassland location in each site). Dot size is proportional to soil C/N ratio in the top 10 cm. Colored areas illustrate different climate regimes.
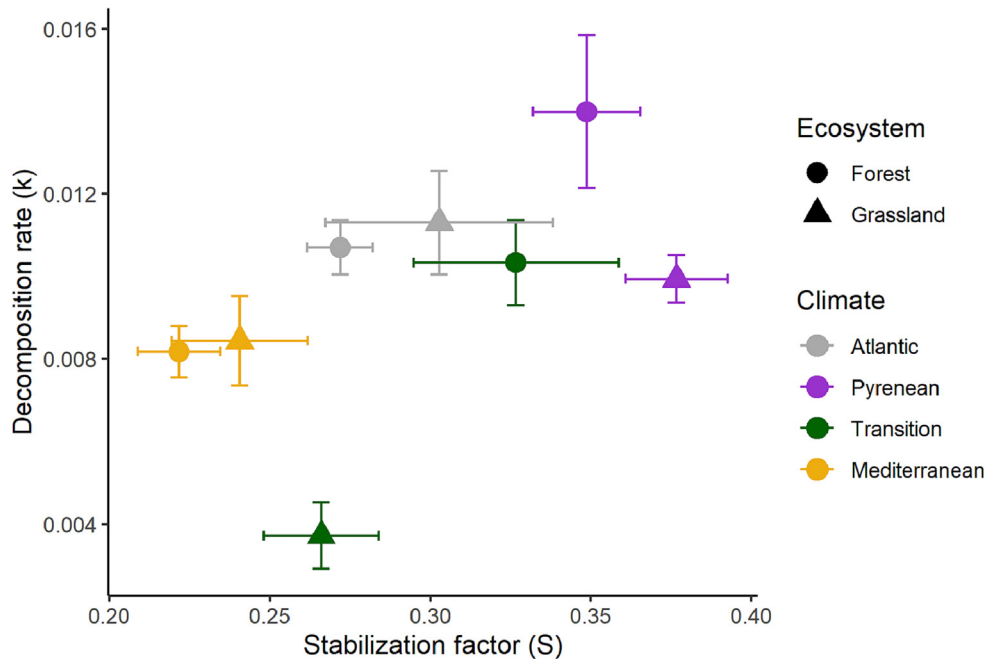
Fig. 4. Average decomposition rates (k, day^{-1}) and stabilization factors (S) for sites grouped by climate type (see Section 2.1 and Table 1). Atlantic sites: Bertizarana and Bera; Pyrenean sites: Orbaizeta, Valcarlos and Garde; Transition site: Yesa; Mediterranean sites: Tafalla and Valtierra. Error bars indicate standard deviation.

However, sites were not so clearly segregated for the stabilization factor, although the highest values were reached at the sites with the highest altitude. As for ecosystem types, the lowest decomposition rates were recorded for the Mediterranean grasslands, whereas the highest rates were recorded in temperate mountain forests. However, no clear patterns for the stabilization factor in relation to ecosystem type was observed, being both forests and grasslands similarly spread along the observed range, although the lowest values averaged by climate were recorded in Mediterranean forests and the highest in Pyrenean grasslands (Fig. 4).

3.2. Model fitting and selected variables

The best fitting models explaining variation in S, k and S/k ratio (the models with the lowest AIC values) included the same random structure, with the sampling as a random effect. The complete structure of fixed effects after variable selection is shown in Table 2. The proportion of variability explained by the LMMs for S and k was $R^2 = 0.40$ and $R^2 = 0.55$, respectively. Ecosystem type, mean precipitation and their interaction were the only factors included in the three selected models, showing a marked effect of precipitation on grasslands that did not occur on forests. C/N ratio and total N had a significant effect on S and k: in the case of C/N ratio varying depending also on the ecosystem type. The rest of the soil variables were not as relevant, and the effect of

SOM was discarded in all three models after variable selection. Soil pH, conductivity and T_{min} were also included in the model constructed for explaining S variation (Table 2).

3.3. Stabilization factor (S) and decomposition rate (k) models

The models constructed for explaining S and k were similar, although the S model was more complex. Both included a similar precipitation effect, showing a positive correlation with both S and k on grasslands, while the effect on forests was only a small positive trend, almost null. The C/N ratio produced also different trends on S and k depending on the ecosystem type, with a positive correlation in forests but negative in grasslands (Figs. 5 and 6). In the case of total N, the effect was positive for both S and k, and for both ecosystems, grasslands and forests (Fig. 7). In addition, the stabilization factor S increased with pH for both ecosystems types (Fig. S1). Models were also produced for the S/k ratio, also showing significant interactions between cover type and climate, but without significant influence of soil features (Table S10, Fig. S2).

Table 2
LMM analysis results for the decomposition models, showing the significant terms and the best fitting model for each factor. LRT: Likelihood ratio test.

Best fitting model	Model term	LRT	p
log(k): Ecosystem	Ecosystem type:C/N	11.7	<0.001
type + N + C/N + P_{mean} + Ecosystem	Ecosystem type:P_{mean}	14.1	<0.001
type:C/N + Ecosystem type:P_{mean}	Total N	8.7	0.003
S: Ecosystem	Ecosystem type:C/N	11.6	<0.001
type + pH + Conductivity + N + C/N +	Ecosystem type:P_{mean}	8.0	0.005
P_{mean} + T_{min} + Ecosystem type:C/N +	pH	4.6	0.033
Ecosystem type:P_{mean}	Conductivity	6.3	0.012
	Total N	8.8	0.003
	T_{min}	19.8	<0.001

Fig. 5. Ecosystem-dependent partial effects of C/N ratios (left panel) and daily precipitation in spring 2017 (mm day^{-1}, right panel) on the decomposition rate k. Error bars indicate 95 % confidence interval.
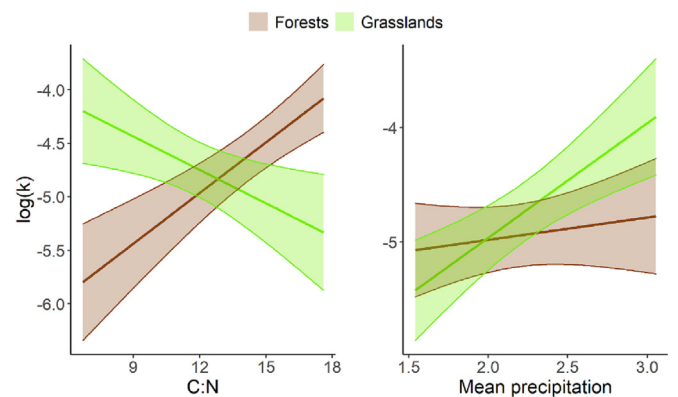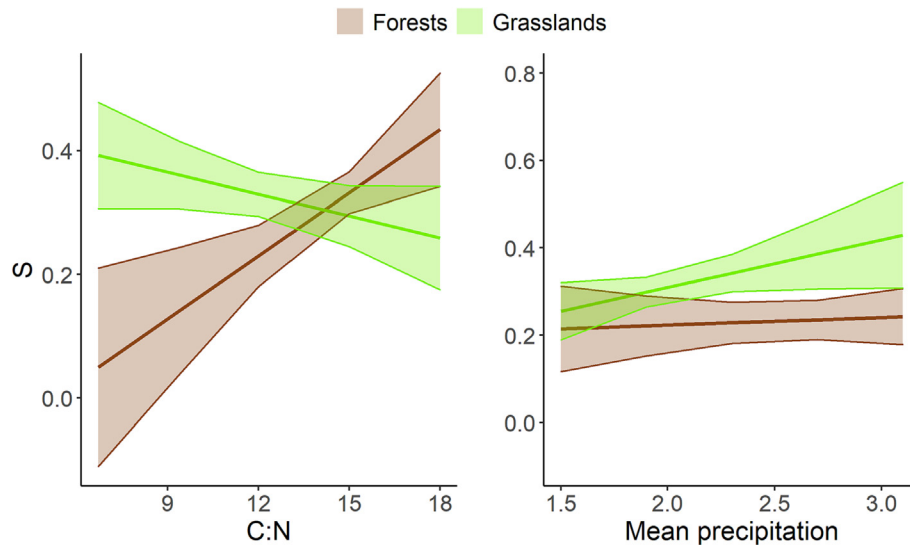
Fig. 6. Ecosystem-dependent partial effects of C/N ratios (left panel) and daily precipitation in spring 2017 (mm day^{-1}, right panel) on the stabilization factor *S*. Error bars indicate 95 % confidence interval.

4. Discussion

4.1. Modulating effects of ecosystem type on SOM decomposition

Estimated decomposition rates and stabilization factors for our sites showed a range similar to the ones reported by Keuskamp et al. (2013) in the initial description of the TBI methodology. Our results indicate that the two most important factors affecting decomposition rates in this region are precipitation during the incubation period and soil nitrogen through its relationship with soil carbon. Similar dual climate/soil influences have been routinely reported in other TBI studies (e.g. Althuizen et al., 2018; Bohner et al., 2019).

Our initial hypothesis has been confirmed by the strong interactions observed between ecosystem type and the two most influential variables in the models (precipitation and C/N ratio). In the case of grasslands, the decomposition rate *k* showed a decrease as the C/N ratio increases. Such result could be a reflection of the higher effort done by microbial decomposers to access N from the environment to support metabolization of carbon sources in the tea bags. However, the trend is the opposite in forests, with decomposition rates increasing as soil C/N ratio also increases. This pattern can appear as a result of soils with higher C/N being actively

accumulating organic carbon (Wojciech et al., 2019), as litter inputs are higher that decomposition losses. Such situation could happen in secondary forests stands developed on former pastures (either by natural tree regeneration or by active afforestation; Laganiere et al., 2010). Accordingly, high loads of high-quality litter from deciduous forests could support high metabolic rates in microbial decomposers, producing higher decomposition in tea bags, but at the same time producing soils with elevated C/N ratios, particularly in environments with relatively high precipitation such as the Pyrenean and Atlantic sites in our research. In fact, the observed patterns along our gradients could indicate an adaptation by microbial communities to their main C sources. Such adaptation could happen by means of two different process: 1) changes in microbial communities, as Blaser and Firestone (2005) reported microbial community composition as more influencing on some soil processes than climate; or 2) changes in the metabolism of the communities, as Throckmorton et al. (2012) reported that ecosystem-specific influences on decomposition rates can be more important than intrinsic differences among soil microbial communities. Throckmorton et al. (2012) argued that such results indicated that different soil microbial communities adapt to the prevailing C sources and environmental conditions in which they live. In our case, as we have not directly studied the soil microbial communities but their effects on standardized
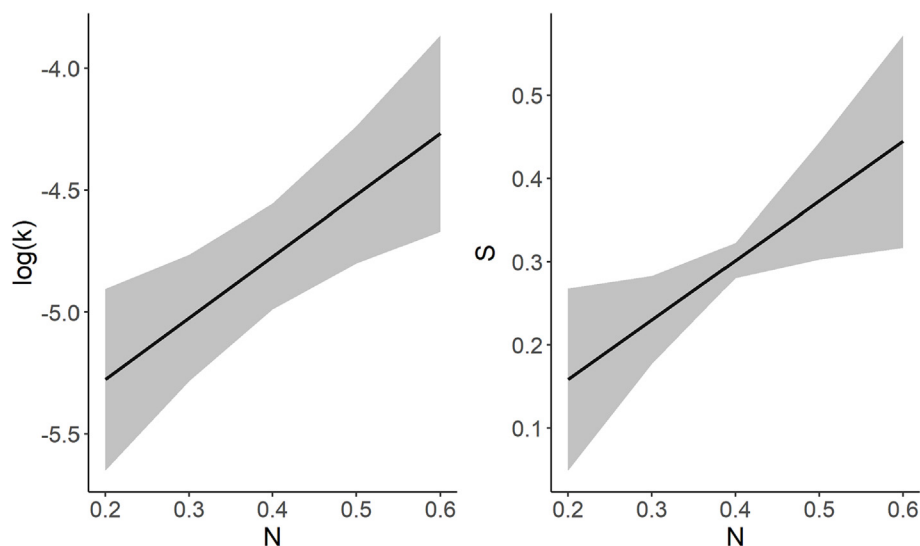

Fig. 7. Ecosystem-independent partial effects of N on the decomposition rate *k* (left panel) and stabilization factor *S* (right panel). Error bars indicate 95 % confidence interval.

litter, we cannot rule out the possibility that any of these process (or a combination of both) are in place. In fact, our observed differences in decomposing activity could be a reflection of both diverging microbial communities in grasslands and forests but also adaptation of such communities to diverging litter materials.

For the stabilization factor, a similar interaction between ecosystem type and C/N ratio was found. When the C/N ratio increased in grasslands, decomposition rates (k) decreased but a more complete decomposition took place since a lower stabilization factor (S) was reached. The results suggest a higher capacity for microbial decomposers to use the C sources in the tea litter. It should be noticed that the studied grasslands did not reach C/N ratios above 14, likely as C flows into the soil are limited by the consumption of aboveground plant biomass by large herbivores (Pausas and Bond, 2020), and by the grassland litter own nature, intrinsically less lignified than forest litter and hence with lower C/N ratios.

On the contrary, in forests the increase in C/N ratio was linked to an increase of decomposition rates (k), although a less complete decomposition of the labile fraction was reached (high stabilization factor, S). This fact reinforces the argument that soil organic matter is accumulating in forest ecosystems with higher litter inputs (Creamer et al., 2016), and lower herbivore consumption (Pausas and Bond, 2020). Therefore, microbial decomposers can cover their needs with the less recalcitrant fractions in the litter, causing a gradual increase in C soil, a clear difference in how C is recycled in grasslands.

Both the decomposition rate and the stabilization factor were also significantly and positively affected by precipitation during the incubation period. On one hand, decomposition rates increased when more precipitation was present, likely due to increased metabolic activity during spring. On the other hand, high precipitation levels can cause longer periods of time in which soil pores are full of water and hence less oxygen is available for soil microbes (Davidson and Janssens, 2006). This in turn can cause a reduction on microbial decomposer activity that is translated into less complete litter decomposition and therefore a higher proportion of plant material being stabilized in soil (Althuizen et al., 2018). In the case of minimum temperature, it can cause a cessation of the microbial activity during the time that such temperatures last, which in turn is translated into less tea litter being consumed and hence a higher proportion of litter stabilized (Gavazov, 2010).

Precipitation also interacted with ecosystem type, as for both k and S in grasslands were much more sensitive to environmental changes than forests. This could have been caused by the influence of tree canopy in the soil microclimate, as canopy likely reduced maximum air temperature and increased air humidity (Von Arx et al., 2012), then preventing soil desiccation more effectively than grass cover. This in turn translates into more stable conditions for the microbial communities (Blaser and Firestone, 2005), and hence sustains higher decomposition rates in forests than in grasslands. However, as precipitation increases, part of it remains in the canopy and does not reach the soil (Llorens et al., 2011; Cardil et al., 2018), therefore causing a more stable and less noticeable change in soil moisture under tree canopy. As a consequence, forest soils are more shielded from precipitation changes, which translates into more horizontal linear models of decomposition rates than for grasslands (Figs. 4 and 5). The stabilization factor is under a similar influence, as higher precipitation causes higher stabilization in grasslands but has lower impact on decomposition of tea bags placed under the forest canopy. As explained before, the tree canopy in forests can cause on one hand a reduction of effective precipitation reaching the soil, and in the other hand an increase in water infiltration rates (Blanco, 2017). Both phenomena combined cause less anoxic conditions and hence less limiting conditions for microbial decomposers (Throckmorton et al., 2012).

4.2. Environmental factors independent of ecosystem type

Main climate variables were not the most important factors affecting decomposition variables, as our statistical models showed. For example, mean and maximum temperatures were not found to be an influencing factor on

decomposition rates, likely because incubation was done during spring, a time when all the sites have enough temperature to support microbial metabolic activity. Only low temperatures (T_{min}) affected the stabilization factor, likely by limiting microbial activity (Von Lützow and Kögel-Knabner, 2009; Conant et al., 2011). In addition, the relationship between precipitation and decomposition was modulated by ecosystem types, as explained above. These results are in line with Althuizen et al.'s (2018) observations that temperature is not relevant in the long term in sites of milder climates as other variables, such as C/N ratio, modulate microbial composition. However, we should point that our research was done in the spring-summer period, and fall-winter measures would probably have modified the results, particularly in the higher and colder sites, as also reported by Althuizen et al. (2018).

Some soil factors also had effects on SOM decomposition not related to ecosystem type. For example, significant direct influence of soil N on decomposition rates indicated a positive effect of nutrient availability on decomposer activity (Duddigan et al., 2020), as has already been reported for soils in this region (Blanco et al., 2011; Rodríguez et al., 2020). However, soil N also had an indirect effect through the C/N ratio, which was significantly influenced by the presence of different vegetation types (Rodríguez et al., 2021), as explained in the previous section. Both soil factors also indicate the limiting nature of soil available N on microbial decomposer activity (Averill and Waring, 2018). Our results also indicate that the stabilization factor S was more sensitive than decomposition rates to soil variables (i.e. pH and conductivity), in agreement with results by de Godoy Fernandes et al. (2021).

4.3. Land use change implications for soil C

As our results show, generalizing effects of grasslands transitioning to forests on SOM decomposition is hindered by the existence of important interactions between environmental factors and ecosystem types. On one hand, reduced precipitation under climate change (as predicted for this region; Amblar-Francés et al., 2020) will cause a deceleration of SOC decomposition (Alessandro and Nyman, 2017) and a reduction in stabilized litter (Becker and Kuzyakov, 2018), which based on our results will be particularly intense in grassland ecosystems. In addition, increased minimum temperatures will cause less litter being stabilized in the long term even if decomposition is slower (Fig. S1). On the other hand, the relatively quick replacement of many grasslands by forests observed in Europe as cattle ranching and sheep shepherding are being reduced by rural abandonment and socioeconomic changes, will translate into changes in microbial communities. Such changes would imply moving from communities specialized in dealing with more labile litter (dominated by grass leaves and particularly fine roots) to communities more specialized in using recalcitrant woody materials with higher C/N ratios (Creamer et al., 2016). Hence, it could be expected that woody encroachment of grasslands will translate into increases of soil C/N, increases in decomposition rates and a higher proportion of litter stabilized in soils. The beginning of this trend has already been observed by Bohner et al. (2019) in abandoned alpine grasslands, where these authors reported increases in both k and S as tall grasses and shrubs increasingly dominated the plant community.

5. Conclusions

Microbial decomposition of soil organic matter is influenced by a complex combination of site-dependent and site-independent environmental variables, in which vegetation cover plays an important role. Our research has shown that interactions of particular interest are those between vegetation cover, precipitation and C/N ratio. As those three elements are set to change due to anthropogenic activities (vegetation cover due to land use changes, precipitation due to climate change, and C/N ratio due to pollution and vegetation changes), it is expected that soil microbial decomposition processes will be altered in important extensions of European landscapes. This phenomenon in turn may have implications for the

capacity of soils in European forests and grasslands to act as C sources or sinks, and to the size of C stocks that can be permanently stored in them.

CRediT authorship contribution statement

Juan A. Blanco: Conceptualization, Methodology, Investigation, Writing – original draft, Supervision, Resources. **María Durán:** Investigation, Writing – original draft, Visualization. **Josu Luquin:** Methodology, Investigation, Data curation. **Leticia San Emeterio:** Investigation, Formal analysis, Writing – review & editing. **Antonio Yeste:** Formal analysis. **Rosa M. Canals:** Conceptualization, Methodology, Investigation, Writing – review & editing, Supervision, Resources.

Data availability

Data will be made available on request.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.164118.

References

Aerts, R., 1997. Climate, leaf litter chemistry and leaf litter decomposition in terrestrial ecosystems: a triangular relationship. Oikos 79, 439–449.

Åkesson, O., 2017. Tea Time for Soils: Decomposition Experiments in Swedish Long-term Field Trials. Second cycle, A2ESLU, Dept. of Ecology, Uppsala.

Alessandro, O., Nyman, P., 2017. Aridity indices predict organic matter decomposition and comminution processes at landscape scale. Ecol. Indic. 78, 531–540.

Althuizen, I.H.J., Lee, H., Sarneel, J.M., Vandvik, V., 2018. Long-term climate regime modulates de impact of short-term climate variability on decomposition in alpine grassland soils. Ecosystems 21, 1580–1592. https://doi.org/10.1007/s10021-018-0241-5.

Amblar-Francés, M.P., Ramos-Cañizado, P., Sanchís lladó J., et al., 2020. High resolution climate change projections for the Pyrenees region. Adv. Sci. Res. 17, 191–208. https://doi.org/10.5194/asr-17-191-2020.

Averill, C., Waring, B., 2018. Nitrogen limitation of decomposition and decay: how can it occur? Clim. Change Biol. 24, 1417–1427. https://doi.org/10.1111/gcb.13980.

Barton, K., 2022. Multi-model inference. Accessed on September 27th 2022. Available at: https://cran.r-project.org/package=MuMIn.

Becker, J.N., Kuzyakov, Y., 2018. Teatime on Mount Kilimanjaro: assessing climate and land-use effects on litter decomposition and stabilization using the Tea Bag Index. Land Degrad. Dev. 29 (8), 2321–2329.

Blanco, J.A., 2017. Bosques, suelo y agua: explorando sus interacciones. Ecosistemas 26, 1–9. https://doi.org/10.7818/ECOS.2017.26-2.01.

Blanco, J.A., Imbert, J.B., Castillo, F.J., 2011. Thinning affects Pinus sylvestris needle decomposition rates and chemistry differently depending on site conditions. Biogeochemistry 106, 397–414.

Blaser, T.C., Firestone, M.K., 2005. Linking microbial community composition and soil processes in a California annual grassland and mixed-conifer forest. Biogeochemistry 73, 395–415. https://doi.org/10.1007/s10533-004-0372-y.

Blume-Werry, G., 2021. The belowground growing season. Nat. Clim. Chang. 12, 11–12.

Bohner, A., Karrer, J., Walcher, R., Brandl, D., Michel, K., Arnberger, A., Frank, T., Zaller, J.G., 2019. Ecological responses of semi-natural grasslands to abandonment: a case studies in three mountain regions in the Eastern Alps. Folia Geobotanica 54, 211–225. https://doi.org/10.1007/s12224-019-09355-2.

Burnham, K.P., Anderson, D.R., 2002. Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach. 2nd edn. Springer-Verlag, New York, NY, USA.

Bussotti, F., Pollastrini, M., Holland, V., Bruggemann, W., 2015. Functional traits and adaptive capacity of European forests to climate change. Environ. Exp. Bot. 111, 91–113.

Cardil, A., Imbert, J.B., Camarero, J.J., Primicia, I., Castillo, F.J., 2018. Temporal interactions among throughfall, type of canopy and thinning drive radial growth in an Iberian mixed pine-beech forest. Agric. For. Meteorol. 252, 62–74. https://doi.org/10.1016/j.agrformet.2018.01.004.

Conant, R.T., Ryan, M.G., Agren, G.I., et al., 2011. Temperature and soil organic matter decomposition rates – synthesis of current knowledge and a way forward. Glob. Change Biol. 17, 3392–3404. https://doi.org/10.1111/j.1365-2486.2011.02496.x.

Creamer, C.A., Filley, T.R., Boutton, T.W., Rowe, H.I., 2016. Grassland to woodland transition: dynamic response of microbial community structure and carbon use patterns. J. Geophys. Res. Biogeosci. 121, 1675–1688. https://doi.org/10.1002/2016JG003347.

Davidson, E.A., Janssens, I.A., 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 440, 165–173.

de Godoy Fernandes, P.H., de Souza, A.L.T., Tanaka, M.O., Sebastiani, R., 2021. Forest Ecosystems. 8, p. 13. https://doi.org/10.1186/s40663-021-00293-0.

De Vries, F.T., Manning, P., Tallowin, J.R.B., Mortimer, S.R., Pilgrim, E.S., Harrison, K.A., Hobbs, P.J., Quirk, H., Shipley, B., Cornelissen, J.H.C., Kattge, J., Bardgett, R.D., 2012. Abiotic drivers and plant traits explain landscape-scale patterns in soil microbial communities. Ecol. Lett. 15, 1230–1239.

Duddigan, S., Shaw, L.J., Alexander, P.D., Collins, C.D., 2020. Chemical underpinning of the tea bag index: an examination of the decomposition of tea leaves. Appl. Environ. Soil Sci. 2020, 6085180. https://doi.org/10.1155/2020/6085180.

EEA, 2017. Climate Change, Impacts and Vulnerability in Europe 2016 – An indicator Based Report. EEA Report No 1/2017European Environment Agency.

Gabriel, C.E., Kellman, L., 2014. Investigating the role of moisture as an environmental constraint in the decomposition of shallow and deep mineral soil organic matter of a temperate coniferous soil. Soil Biol. Biochem. 68, 373–384.

Gavazov, K.S., 2010. Dynamics of alpine plant litter decomposition in a changing climate. Plant Soil 337, 19–32. https://doi.org/10.1007/s11104-010-0477-0.

Herrera-Alvarez, X., Blanco, J.A., Imbert, J.B., Alvarez, W., Rivadeneira-Barba, G., 2020. Coarse Woody Debris invertebrate community is affected directly by canopy type and indirectly by thinned in mixed Scots pine – European beech forests. Forests 11, 975.

Huxman, T.E., Wilcox, B.P., Breshears, D.D., Scott, R.L., Snyder, K.A., Small, E.E., Hultine, K., Pockman, W.T., Jackson, R.B., 2005. Ecohydrological implications of woody plant encroachment. Ecology 86, 308–319.

Kaiser, K., Wemheuer, B., Korolkow, V., Wemheuer, F., Nacke, H., Schöning, I., Schrumpf, M., Daniel, R., 2016. Driving forces of soil bacterial community structure, diversity, and function in temperate grasslands and forests. Sci. Rep. 6, 33696.

Kauppi, P.E., Sandstrom, V., Lipponen, A., 2018. Forest resources of nations in relation to human well-being. PLoS One 13. https://doi.org/10.1371/journal.pone.0196248.

Keuskamp, J.A., Dingemans, B.J.J., Lehtinen, T., Sarneel, J.M., Hefting, M.M., Muller-Landau, H., 2013. Tea Bag Index: a novel approach to collect uniform decomposition data across ecosystems. Methods Ecol. Evol. 4, 1070–1075. https://doi.org/10.1111/2041-210X.12097.

Laganiere, J., Angers, D.A., Pare, D., 2010. Carbon accumulation in agricultural soils after afforestation: a meta-analysis. Glob. Chang. Biol. 16 (1), 439–453.

Llorens, P., Latron, J., Álvarez-Cobelas, M., Martinez-Viilta, J., Moreno, G., 2011. Hydrology and biogeochemistry of Mediterranean forests. In: Levia, D.F., et al. (Eds.), Forest Hydrology and Biogeochemistry: Synthesis of Past Research and Future Directions, Ecological Studies. 216, pp. 301–319. https://doi.org/10.1007/978-94-007-1363-5_14.

Lo, Y.H., Blanco, J.A., Seely, B., Welham, C., Kimmins, J.P., 2011. Generating reliable meteorological data in mountainous areas with scarce presence of weather records: the performance of MTCLIM in interior British Columbia, Canada. Environ. Model Softw. 26, 644–657. https://doi.org/10.1016/j.envsoft.2010.11.005.

Lo, Y.H., Blanco, J.A., Canals, R.M., de Andrés, E., San Emeterio, L., Imbert, B., Castillo, F.J., 2015. Land use change effects on carbon and nitrogen stocks in the Pyrenees during the last 150 years: a modeling approach. Ecol. Model. 312, 322–334.

Meteonavarra, 2022. Meteorología y climatología de Navarra.Available at http://meteo.navarra.es/ (Accessed on January 28th 2022).

Mori, T., 2022. Validation of the Tea Bag Index as a standard approach for assessing organic matter decomposition: a laboratory incubation experiment. Ecol. Indic. 141, 109077. https://doi.org/10.1016/j.ecolind.2022.109077.

Nakagawa, S., Johnson, P.C.D., Schielzeth, H., 2017. The coefficient of determination R2 and intra-class correlation coefficient from generalized linear mixed-effects models revisited and expanded. J. R. Soc. Interface 14, 20170213.

Palmero-Iniesta, M., Pino, J., Pesquer, L., Espelta, J.M., 2021. Recent forest area increase in Europe: expanding and regenerating forests differ in their regional patterns, drivers and productivity trends. Eur. J. For. Res. 140, 793–805.

Pansu, M., Gautheyrou, J., 2007. Handbook of Soil Analysis: Mineralogical, Organic and Inorganic Methods. Springer, Berlin.

Papadakis, J., 1970. Climates of the World, their Classification, Similitudes, Differences, and Geographic Distribution Self-edited. (Buenos Aires, Argentina).

Pausas, J.G., Bond, W.J., 2020. On the three major recycling pathways in terrestrial ecosystems. Trends Ecol. Evol. 35 (9), 767–775.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., R Core Team, 2020. nlme: linear and nonlinear mixed effects models. https://CRAN.R-project.org/package=nlme.

Prescott, C.E., 2002. The influence of the forest canopy on nutrient cycling. Tree Physiol. 22, 1193–1200.

Quinn, G., Keough, M., 2002. Experimental Design and Data Analysis for Biologists. Cambridge University Press, Cambridge, UK.

R Core Team, 2021. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria URL https://www.R-project.org/ URL.

Rahman, M., Castagneyrol, B., Verheyen, K., Jactel, H., Carnol, M., 2018. Can tree species richness attenuate the effect of drought on organic matter decomposition and stabilization in young plantation forests? Acta Oecol. 93, 30–40.

Raich, J.W., Potter, C., 1995. Global patterns of carbon dioxide emissions from soils. Glob. Biogeochem. Cycles 9, 23–36. https://doi.org/10.1029/94GB02723.

Reynolds, B.C., Hunter, M.D., 2001. Responses of soil respiration, soil nutrients, and litter decomposition to inputs from canopy herbivores. Soil Biol. Biochem. 33, 1641–1652. https://doi.org/10.1016/S0038-0717(01)00085-2.

Rodríguez, A., Canals, R.M., Plaixats, J., Albanell, E., Debouk, H., García-Pausas, J., San, Emeterio L., Ribas, A., Jimenez, J.J., Sebastià, M.T., 2020. Interactions between biogeochemical and management factors explain soil organic carbon in Pyrenean grasslands. Biogeosciences 17, 6033–6050.

Rodríguez, A., Canals, R.M., Sebastià, M.T., 2021. Positive effects of legumes on soil organic carbon stocks disappear at high-legume proportions across natural grasslands in the Pyrenees. Ecosystems. https://doi.org/10.1007/s10021-021-00695-9.

Running, S.W., Nemani, R.R., Hungerford, R.D., 1987. Extrapolation of synoptic meteorological data in mountainous terrain and its use for simulating forest evapotranspiration and photosynthesis. Can. J. For. Res. 17, 472–483.

Saura-Más, S., Estiarte, M., Peñuelas, J., Lloret, F., 2012. Effects of climate change on leaf litter decomposition across post-fire plant regenerative groups. Environ. Exp. Bot. 77, 274–282.

Schimel, J.P., Hättenschwiler, S., 2007. Nitrogen transfer between decomposing leaves of different N status. Soil Biol. Biochem. 39, 1428–1436.

Seely, B., Welham, C., Blanco, J.A., 2010. Towards the application of soil organic matter as an indicator of ecosystem productivity: deriving thresholds, developing monitoring systems, and evaluating practices. Ecol. Indic. 10, 999–1008.

Sylvain, Z.A., Wall, D.H., Cherwin, K.L., Peters, D.P.C., Reichmann, L.G., Sala, O.E., 2014. Soil animal responses to moisture availability are largely scale, not ecosystem dependent: insight from a cross-site study. Glob. Chang. Biol. 20, 2631–2643.

Throckmorton, H.M., Bird, J.A., Dane, L., Firestone, M.K., Horwarth, W.R., 2012. The source of microbial C has little impact on soil organic matter stabilisation in forest ecosystems. Ecol. Lett. 15, 1257–1265. https://doi.org/10.1111/j.1461-0248.2012.01848.x.

Von Arx, G., Dobbertin, M., Rebetez, M., 2012. Spatio-temporal effects of forest canopy on understory microclimate in a long-term experiment in Switzerland. Agric. For. Meteorol. 166-167, 144–155. https://doi.org/10.1016/j.agrformet.2012.07.018.

Von Lützow, M., Kögel-Knabner, I., 2009. Temperature sensitivity of soil organic matter decomposition - what do we know? Biol. Fertil. Soils 46, 1–15.

Wang, L.X., Otgonsuren, B., Godbold, D.L., 2017. Mycorrhizas and soil ecosystem function of co-existing woody vegetation islands at the alpine tree line. Plant Soil 411, 467–481.

Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setälä, H., van der Putten, W.H., Wall, D.H., 2004. Ecological linkages between aboveground and belowground biota. Science 304, 1629–1633.

Wieser, G., Holtmeier, F.K., Smith, W.K., 2014. Trees in a changing environment: ecophysiology, adaptation, and future survival. Treelines in a Changing Global Environment. 9, pp. 221–263.

Wojciech, P., Ewa, B., Jaroslaw, L., 2019. Soil biochemical properties and stabilisation of soil organic matter in relation to deadwood of different species. FEMS Microbiol. Ecol. 95, fiz011. https://doi.org/10.1093/femsec/fiz011.

Zhang, D.Q., Hui, D.F., Luo, Y.Q., Zhou, G.Y., 2008. Rates of litter decomposition in terrestrial ecosystems: global patterns and controlling factors. J. Plant Ecol. 1, 85–93.