Long-Term Climate Regime Modulates the Impact of Short-Term Climate Variability on Decomposition in Alpine Grassland Soils

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ABSTRACT

Decomposition of plant litter is an important process in the terrestrial carbon cycle and makes up approximately 70% of the global carbon flux from soils to the atmosphere. Climate change is expected to have significant direct and indirect effects on the litter decomposition processes at various timescales. Using the TeaBag Index, we investigated the impact on decomposition of short-term direct effects of temperature and precipitation by comparing temporal variability over years, versus long-term climate impacts that incorporate indirect effects mediated through environmental changes by comparing sites along climatic gradients. We measured the initial decomposition rate (k) and the stabilization factor (S; amount of labile litter stabilizing) across a climate grid combining three levels of summer temperature (6.5–10.5°C) with four levels of annual precipitation (600–2700 mm) in three summers with varying temperature and precipitation. Several (a)biotic factors were measured to characterize environmental differences between sites. Increased temperatures enhanced k, whereas increased precipitation decreased k across years and climatic regimes. In contrast, S showed diverse responses to annual changes in temperature and precipitation between climate regimes. Stabilization of labile litter fractions increased with temperature only in boreal and sub-alpine sites, while it decreased with increasing precipitation only in sub-alpine and alpine sites. Environmental factors such as soil pH, soil C/N, litter C/N, and plant diversity that are associated with long-term climate variation modulate the response of k and S. This highlights the importance of long-term climate in shaping the environmental conditions that influences the response of decomposition processes to climate change.

Key words: decomposition; climate change; temperature; precipitation; litter bag; annual variability; grassland; tea bag index.

HIGHLIGHTS

- Decomposition increases with temperature and decreases with increased precipitation
- Stabilization of labile fraction of litter varies among long-term climate regimes
- Long-term climate modulates decomposition through environmental characteristics

INTRODUCTION

Litter decomposition contributes approximately 70% to the global CO_2 flux from soils and is estimated to be in the range of $68–77 \times 10^{15}$ gC y^{-1} (Raich and Schlesinger 1992; Raich and Potter 1995). On a global scale, litter decomposition is regulated by a combination of geographic, climatic, and litter quality variables (Zhang and others 2008). Climate affects decomposition processes directly and at short time-scales through temperature and water availability as biological processes are highly sensitive to these factors. On longer timescales, climate also affects decomposition indirectly, for example, by affecting the litter quality via plant community composition and structure, or by affecting decomposer and detritivore community composition (Aerts 2006). Climate change is expected to have substantial effects on both direct and indirect controls of decomposition processes, and the associated CO_2 release could have a positive feedback on global warming (Davidson and Janssens 2006; Crowther and others 2016). It is therefore important to determine both direct and indirect effects of climate change on soil carbon dynamics to quantify more accurately the role of soil under future projections of climate change (Classen and others 2015). In this study, we investigate the direct effects of climate change by studying the response of decomposition processes to short-term inter-annual climate variation (hereafter: short-term climate), whereas indirect effects are studied through the use of spatial climate gradients that represent long-term climate (hereafter: long-term climate) which is an important state factor shaping ecosystem structure and functioning (Chapin and others 2011). We use a climate grid in southern Norway, which combines three levels of summer temperature, that is, the mean of the four warmest months June–September, representing different biogeographic zones (alpine $\approx$ 6.5°C, sub-alpine $\approx$ 8.5°C, boreal 10.5°C) with four levels of mean annual precipitation (1 $\approx$ 600 mm, 2 $\approx$ 1200 mm, 3 $\approx$ 2000 mm, 4 $\approx$ 2700 mm) while avoiding correlation between climatic factors (Meineri and others 2013; Klanderud and others 2015). This study design allows us to disentangle the short-term (direct) and long-term (indirect) impacts of climate on litter decomposition in alpine grasslands in three consecutive growing seasons with contrasting climates.

Climate change scenarios predict greater increases in surface temperature and enhanced precipitation for northern high-latitudes (IPCC 2013). Because biological processes in these regions are generally temperature-limited, litter decomposition is expected to increase (Hobbie and others 2002; Robinson 2002). The effect of global warming on decomposition in these regions is often studied by artificial warming experiments. This type of intra-site experiment studies the short-term direct effects of warming on decomposition but disregards the long-term indirect effects of climate that shape the local environment through edaphic factors and plant and decomposer communities. In addition, these experiments can be troubled by artifacts such as soil drying, and they often ignore the role of and projected changes in precipitation (Aerts 2006). Field approaches that make use of extant climatic gradients—space-for-time approaches—can be performed at broad spatial scales, spanning entire or multiple continents (Berg and others 1993; Cornelissen and others 2007; Portillo-Estrada and others 2016), or at a local scale, spanning a single gradient (for example, Murphy and others 1998; Salinas and others 2011). Such gradient studies have the advantage of being able to incorporate indirect, long-term effects of climate. However, in most cases, covariation occurs between temperature and precipitation across the gradient, making it difficult to separate the effect of these climatic factors on decomposition. Temporal variability in climate between years is another way to study the direct effects of climate on decomposition (McCulley and others 2005). The combination of climatic gradients together with annual climate variability is an opportunity to study both the short-term direct and the long-term indirect effects of climate on decomposition.

The majority of litter decomposition studies use native leaf material, which makes them very realistic, but this approach also has a drawback. Litter can decompose faster when it is placed under the plants from which the litter originated (''home'') than at locations with different plant species

("foreign") (Ayres and others 2009; Veen and others 2015). This phenomenon is called "home-field advantage" (Gholz and others 2000) and can bias results in studies using one local litter across sites with different species. Recently, Keuskamp and others (2013) developed the TeaBag Index (TBI) as a standardized method to negate litter quality and litter trait effects from environmental drivers of decomposition processes by removing this litter bias. This method uses two types of tea with contrasting decomposability as standard litter substrate to characterize two parameters of the decomposition process. The decomposition rate constant (k) is a measure of the speed of initial litter decomposition and the stabilization factor (S) is a measure of the proportion of the labile fraction of litter that will finally stabilize and become recalcitrant and transform into soil organic matter (SOM). Decomposition of native litter and the standard tea litter shows similar responses to changes in temperature and precipitation, indicating that the TBI is a suitable approach for assessing the role of environmental variables on litter decomposition (Didion and others 2016).

In this study, we used the TBI to investigate the short-term (inter-annual variability) and long-term (environmental conditions shaped by differences in climate between sites) effects of climate on litter decomposition in mountain grasslands in southern Norway. We used a climate grid that consists of sites positioned along natural temperature and precipitation gradients explicitly selected to disentangle effects of temperature and precipitation. To investigate the response of decomposition processes to short-term climate variability we adopted the TBI approach in three consecutive summers that varied in temperature and precipitation. At each site, we measured a number of biotic and abiotic variables to determine how long-term differences in climate have shaped the local environment at the different sites. The combination of the climate grid and annual climate variability provided the opportunity to compare the impact of short-term variation in climate compared with long-term effects of regional climate gradients on decomposition processes.

Specifically, we aimed to (1) determine how decomposition processes are affected by short-term variation in temperature and precipitation, (2) evaluate whether these responses are consistent across regional climate gradients, and (3) assess to what degree short-term and long-term impacts of climate affect decomposition processes.

We expected decomposition to increase in sites and in growing seasons with higher temperatures and precipitation, because mountain ecosystems are known to be limited by temperature and productivity generally increases with precipitation. In addition, we expected the reaction to be strongest in sites where the other factor was not limiting, so that variation in precipitation (between sites and years) would cause larger differences in decomposition in the warmest locations than in cold locations where decomposition is limited by temperature. Vice versa, variation in temperature should cause larger differences in decomposition in the wettest location than in low-rainfall sites, where moisture limitation is more likely.

MATERIALS AND METHODS

Site Description

The study was conducted in a climate grid consisting of 12 calcareous grassland sites spread across natural temperature and precipitation gradients, spanning almost 1000 m in elevation and 175 km in geographical distance. The sites of this grid were selected based on interpolated climate data from the normal period 1961–1990 with a resolution of 100 m (Tveito and others 2005; Norwegian Meteorological Institute 2010). It combines three levels of summer temperature, that is, the mean of the four warmest months June–September, representing different biogeographic zones (alpine $\approx 6.5°C$, sub-alpine $\approx 8.5°C$, boreal $\approx 10.5°C$) with four levels of mean annual precipitation ($1 \approx 600$ mm, $2 \approx 1200$ mm, $3 \approx 2000$ mm, $4 \approx 2700$ mm; Figure 1). All sites are seminatural grasslands on shallow slopes (5–20°) associated with calcareous bedrock and plant communities within the plant sociological association *Potentillo-Festucetum ovinae* (Fremstad 1997), and other factors were kept as similar as possible, including aspect and land use (for more details see Meineri and others 2013; Klanderud and others 2015; and also see Table S1). The sites are fenced during the snow-free season to prevent ungulate grazers from damaging the experimental setup.

Climate Data

The Norwegian Meteorological Institute (NMI) provided mean daily interpolated data on air temperature and precipitation at a resolution of 100 m for each site for the whole study period 2014–2016 (Norwegian Meteorological Institute 2016). At each site, we measured soil temperature at 5 cm below ground with MT2-05 temperature sensors (Delta-T Devices, Cambridge, UK). Due to temperature sensor malfunction of climate stations at some sites,
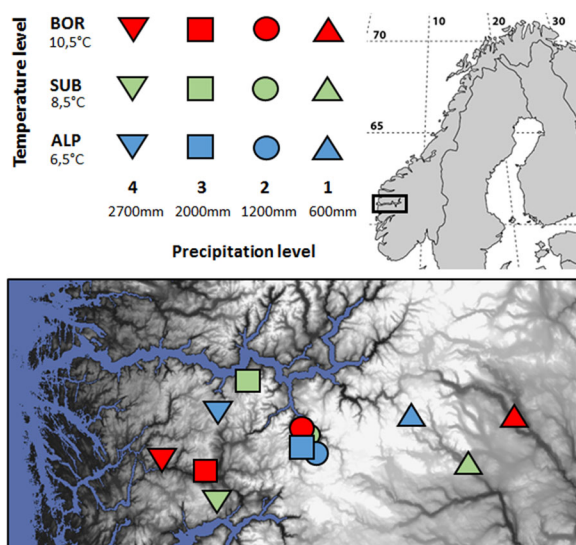
Figure 1. Location of the study sites along the temperature and precipitation gradients in southern Norway. The summer temperature range, alpine $\approx 6.5°$C, sub-alpine $8.5°$C, and boreal $\approx 10.5°$C, is indicated by the different colors. The levels of annual precipitation, $1 \approx 600$ mm, $2 \approx 1200$ mm, $3 \approx 2000$ mm, and $4 \approx 2700$ mm, are indicated with the different shapes, 1 (*Triangle*), 2 (*Circle*), 3 (*Square*), and 4 (*Inverted triangle*). Color coding and symbols presented in this figure will be consistent throughout the manuscript.

we do not have continuous soil temperature measurements at site level for each of the incubation periods. In 2014, iButton temperature loggers (DS1922L, Maxim Integrated, San Jose, CA, USA) were buried at five locations in each site at a depth of 8 cm. Temperature data from the different sources were compared to each other both visually and by pairwise Pearson correlation for the incubation periods of the tea bags ($\pm$ 3 months in summer, see method section on litter decomposition measurements) at the different sites (Figure S1). Because the temperature data of NMI for incubation periods corresponded well with both buried iButton and climate stations at all the sites, $R^2 > 0.93$ and $R^2 > 0.83$, respectively, we decided to use air temperature data from NMI in the analyses. Mean temperature and total precipitation were calculated based on data from NMI for the incubation periods of tea bags for each site and year (Table S2).

Environmental Variables Representing Long-Term Climate

Long-term climate plays an important role in shaping ecosystem structure by influencing the development

of soil and determining the types and diversity of plants and organisms that can occur (Chapin and others 2011). This climatically driven variability in the biotic and abiotic environment could affect decomposition (that is, indirect effect of climate). To characterize these differences, we measured a number of biotic and abiotic characteristics at each site.

Soil Properties

At each site, five composite soil samples were collected by combining three core rings (5 cm diameter). Soil pH of these samples was measured after mixing 30 g of sieved fresh soil with 30 ml deionized water. Additional soil samples from the surface layer (0–10 cm) were collected with a soil corer (25 mm diameter) from four locations at each site. These samples were oven-dried at $30°$C for 2–3 days, and roots were carefully removed. Dried samples were ground thoroughly and passed through a 1-mm sieve. Subsequently, 25 g subsample was milled at a frequency of 30 s^{-1} for 2–3 min with a mixer mill (MM200, Retsch Gmbh, Haan, Germany). Total soil C and N contents were measured using a Vario MI-CRO cube elemental analyzer (Elementar Analysensystem GmbH, Germany). Soil mineral N availability was determined in summer 2010 with ion-exchange resin bags (IERBs) (Fariñas 2011). At each site, 10 IERBs were buried at 5 cm depth at the beginning of the growing season and collected at the end of August. The NH_4^+ and NO_3^- were extracted from the resin bags with NaCl and measured colorimetrically with a SmartChem autoanalyzer. Total availability of N was calculated from the sum of NH_4^+ and NO_3^- concentrations standardized by the number of days the IERBs had been deployed in the different sites (Giblin and others 1994). Two extremely high values for available nitrogen (1059.67 and 487.78 mg g^{-1} N) at ALP3 were considered outliers and excluded from the analysis.

Vegetation Characteristics

Plant diversity was quantified for each site by recording all vascular plant species in five vegetation plots (25 $\times$ 25 cm) and estimating the percentage of cover by eye at peak growing season in 2015 and 2016. The Shannon diversity index of the individual plots was calculated for each individual plot in the separate years and averaged to get a mean diversity index per site.

Carbon (C) and nitrogen (N) concentrations of living aboveground vegetation were determined from pooled samples based on three circular plots of 5 cm diameter harvested from five locations within each site at peak growing season in 2011 (Fariña

2011). The samples were dried (24 h at 70°C), ground in a Wiley Mill (Thomas Scientific, Swedesboro, USA) and analyzed using a Costech ECS 4010 elemental analyzer (Costech Analytical, Valencia, CA).

Litter quality for each site was determined from dead leaves that easily detached from live graminoids representative of the vegetation community collected along a transect within the fenced experimental site. The litter was collected in August/September 2013 for alpine sites and after snowmelt in May/June 2014 for sub-alpine and boreal sites, as litter could not be collected in the autumn because snowfall started earlier than the die-off of graminoids.

As we assume that very little decomposition occurs during winter, we expect the litter collected after snowmelt to be representative of the litter quality entering the soil in summer at these sites. The litter was washed in deionized water to clean it of any soil particles, air-dried at room temperature for at least 7 days, and subsequently stored in a well-ventilated room until processing. For each site, two litter samples of 10 g were ground in a cyclone mill (TWISTER, Retsch GmbH, Haan, Germany). For each sample, two 5-mg subsamples were analyzed for C and N using a Vario MICRO cube elemental analyzer.

Litter Decomposition Measurements

Decomposition parameters were quantified for all 12 sites using the TeaBag Index (TBI) (Keuskamp and others 2013) for the summers of 2014, 2015, and 2016. For each site and year, air-dried, weighed Lipton green tea and Lipton rooibos tea bags with a nylon mesh were buried after snowmelt and collected after an in situ incubation period of 60–98 days, depending on the duration of the snow-free season (see Table S3). At each site, 10 replicates of each tea were buried pairwise, 8 cm below ground, and with at least 10 cm between the two tea types. For two sites, the number of replicate tea bag pairs was higher in 2015 (12 replicates in ALP3 and 16 replicates at ALP2). After collection, adhering soil particles and roots were removed and the tea bags were dried (48 h at 60°C) and weighed. Three additional tea bags of each type of tea were not buried but handled and dried the same way as the experimental tea bags to allow correction for weight loss during transport and drying.

The TBI uses two types—green tea and rooibos tea—with contrasting decomposability, that is, different labile and recalcitrant fractions, to determine two parameters of the decomposition process: decomposition rate k and stabilization factor S.

Some of the labile compounds of litter stabilize and become recalcitrant in late stages of the decomposition process depending on environmental factors. The retardation of decomposition may be so strong that decomposition reaches a limit value where total mass loss of litter virtually stops and at which point it becomes soil organic matter (SOM) (Berg and Meentemeyer 2002). Green tea decomposes quickly in comparison with rooibos tea and reaches its decomposition limit, while rooibos tea is still in its early stages of decomposition where labile material is still being decomposed. The difference between these litter types allows for an estimation of the decomposable fraction from green tea (a_g) and the decomposition rate constant k from rooibos tea at a single point in time.

The TBI assumes that during short-field incubations, the weight loss of the recalcitrant fraction is negligible. Consequently, the decomposition curve can be modeled using a standard decay curve:

$$W(t) = ae^{-kt} + (1 - a) \quad (1)$$

where W is the fraction of labile material remaining after time interval t, a is the labile fraction that decreases with decomposition rate k, and $1-a$ is the recalcitrant fraction of the litter for which we assume that the decomposition rate is negligible (that is, e^{-kt} is close to 1).

The TBI also assumes that incubation periods of about 90 days are long enough for green tea to reach the second phase of decomposition, where the remaining material will only decompose over very long timescales. This is represented by the deviation of the actual decomposed fraction a from the hydrolysable (that is, chemically labile) fraction H and can be interpreted as the inhibiting effect of environmental conditions on the decomposition of the labile fraction, that is, the stabilization factor (S). One can calculate the fraction of the labile component of green tea that did not decompose, but stabilized:

$$S = 1 - \frac{a_g}{H_g} \quad (2)$$

where a_g is the fraction of green tea remaining and $H_g = 0.842$ is the hydrolysable fraction of green tea (Keuskamp and others 2013). Assuming that for the labile fraction of rooibos tea, the same proportion will be stabilized, and one can predict how much material of rooibos tea (a_r) will remain in the second phase:

$$a_r = H_r(1 - S). \quad (3)$$

By substituting a_r in equation (1), and using the weight loss observed in rooibos tea, one can obtain the initial decomposition rate of the labile fraction of tea.

From the 736 tea bags buried, 25 were not retrieved (12 green tea, 13 rooibos tea) and 24 were badly damaged (10 green tea, 14 rooibos tea) and were therefore excluded from analysis. To reduce the number of data points lost because of damaged tea bags, we calculated the mean S per site for each year and used these mean values to predict a_r for the calculation of k. After this, we had 21 missing values for S and 23 for k.

Data Analysis

To determine the effect of short-term annual climate variability on the decomposition parameters k and S, we constructed a mixed effects model, where we included year as a fixed factor and site as a random factor, using lmer in the R package lme4 (Bates and others 2011). To assess the effect of temporal and spatial climate variability on k and S, we used variance decomposition to quantify how much of the total variation in k and S is explained by year and site, respectively. To determine the effect of temperature and precipitation on k and S, we used linear regression on the complete dataset and for the separate temperature and precipitation levels. Environmental variables (pH, available nitrogen, soil C/N, plant C/N, litter C/N, and plant diversity) were analyzed for differences between temperature and precipitation levels using two-way ANOVA, and for significant results ($P < 0.05$), pairwise T tests were performed to compare the different temperature or precipitation levels to each other ($P < 0.05$). Collinearity between environmental variables was evaluated using Pearson's correlations. We used multiple linear regression models to assess the relationship between k and S and the climatic and environmental predictor variables (temperature, precipitation, temperature level, precipitation level, pH, available nitrogen, plant C/N, soil C/N, litter C:N, plant diversity). Average values for k and S were calculated for each site in each year prior to model construction. Models selection followed a backward selection procedure using the R package: drop1 (Chambers and Trevor Hastie 1992). Based on Akaike's information criterion (AIC) scores, we only selected models of greater complexity when inclusion of an additional model parameter reduced AIC by more than 2 (Burnham and Anderson 2002). Variance decomposition was used to determine how much of the variation in k and S was explained by each variable in the various models. All data analyses were performed in R version 3.4.0 (R Core Team 2017).

RESULTS

Annual Climate Variability

Summer climate during the incubation period of the tea bags, which commenced shortly after snowmelt at each site, varied between the three growing seasons of this study (Figure 2A–F, Table S2). The year 2014 was relatively warm and dry, 2015 was a relatively cold, and 2016 had more precipitation. In 2014, temperature was on average 3.11 and 1.84°C warmer across the grid compared to 2015 and 2016, respectively. Temperature decreased across biogeographic zones, boreal > subalpine > alpine, and this was consistent across the various years (Figure 2A–C,Table S2). Total amount of precipitation was on average 39 and 33% higher in 2016 compared to 2014 and 2015, respectively, although not all sites received more precipitation and the magnitude of the precipitation difference varied between sites (Figure 2B, Table S2). Observed precipitation showed some inconsistencies relative to the original setup of climatic levels within the climate grid (based on climate data of NMI over 30-year normal period).

Environmental Characteristics of Sites

Soil pH was higher in sites with a colder alpine climate and sites at the high end of the precipitation gradient ($F_{2,48} = 61.29$, $P < 0.001$ and $F_{3,48} = 5.25$, $P < 0.01$, respectively) and showed a positive correlation with both soil available N and soil C:N ratio (Pearson's $\rho = 0.56$, $P < 0.001$ and $\rho = 0.64$, $P < 0.001$, respectively). Soil C/N also increased along the temperature and precipitation gradients of the grid, being significantly higher in alpine sites and sites with high precipitation ($F_{2,48} = 6.47$, $P < 0.05$ and $F_{3,48} = 18.45$, $P < 0.001$, respectively). Plant C/N and litter C/N were higher at sites on the high end of the precipitation gradient ($F_{3,48} = 15.45$, $P < 0.001$ and $F_{3,24} = 137.66$, $P < 0.001$, respectively). Plant diversity increased toward colder sites, with plant diversity being significantly higher in alpine sites than in boreal and sub-alpine sites ($F_{2,48} = 21.87$, $P < 0.001$; Table 1).

Short-Term and Long-Term Climate Controls on Litter Decomposition Processes

There was a clear difference between decomposition of the two tea types after incubation time, as relative mass remaining ranged from 0.19 to 0.59 (g g^{-1}) for green tea and 0.62–0.88 (g g^{-1}) for rooibos tea. The highest values for relative mass
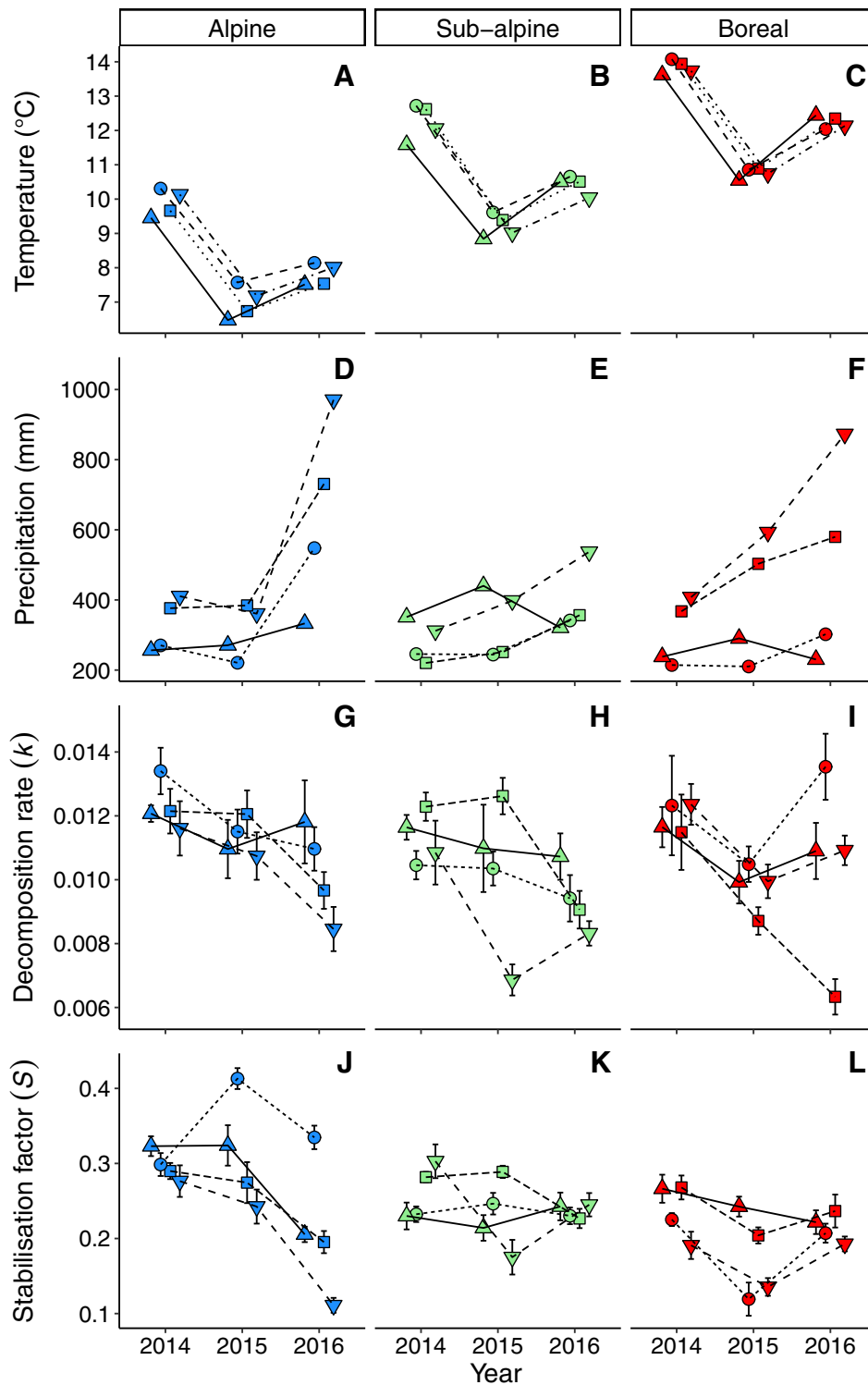
Figure 2. Mean temperature (**A–C**), total precipitation (**D–F**), mean $k \pm$ S.E. (**G–H**), and mean $S \pm$ S.E (**J–K**) for the different precipitation levels, $1 \approx 600$ mm (*Triangle*), $2 \approx 1200$ mm (*Circle*), $3 \approx 2000$ mm (*Square*), and $4 \approx 2700$ mm (*Inverted triangle*), within each temperature level, alpine ≈ 6.5°C (*blue*), sub-alpine ≈ 8.5°C (*green*), and boreal ≈ 10.5°C (*red*).

Table 1. Environmental Characteristics of Sites

Site	Soil pH	Available N (mg g^{-1} m^{-2} day^{-1})	Soil C/N	Plant C/N	Litter C/N	Plant diversity (Shannon's H)
Boreal						
BOR1	5.2 ± 0.1	114.5 ± 44.4	11.6 ± 0.5	23.9 ± 2.2	37.7 ± 1.4	2.39 ± 0.16
BOR2	5.2 ± 0.1	108.8 ± 23.1	11.8 ± 0.7	27.6 ± 1.6	30.3 ± 1.0	2.00 ± 0.36
BOR3	5.2 ± 0.1	68.1 ± 20.0	13.0 ± 1.4	27.6 ± 3.7	42.4 ± 1.1	2.17 ± 0.30
BOR4	5.3 ± 0.1	58.7 ± 19.3	13.4 ± 0.3	28.5 ± 4.3	41.8 ± 2.0	1.50 ± 0.28
Sub-alpine						
SUB1	5.5 ± 0.1	116.3 ± 57.0	13.1 ± 1.6	19.4 ± 0.8	36.7 ± 0.8	2.01 ± 0.1
SUB2	5.1 ± 0.1	77.7 ± 15.6	11.6 ± 0.3	28.5 ± 3.3	34.6 ± 1.0	2.21 ± 0.22
SUB3	5.6 ± 0.3	150.1 ± 92.8	14.6 ± 1.1	31.2 ± 5.4	47.9 ± 1.4	2.20 ± 0.38
SUB4	5.7 ± 0.2	162.1 ± 95.3	14.0 ± 0.9	27.8 ± 2.2	53.9 ± 2.2	2.86 ± 0.15
Alpine						
ALP1	5.7 ± 0.2	181.6 ± 161.0	12.8 ± 0.8	24.6 ± 3.3	25.2 ± 0.4	2.57 ± 0.30
ALP2	6.0 ± 0.1	175.7 ± 84.8	12.9 ± 0.6	21.9 ± 2.4	42.1 ± 1.5	2.53 ± 0.43
ALP3	6.1 ± 0.2	140.1 ± 94.3	13.5 ± 1.3	31.7 ± 3.9	49.3 ± 2.2	2.50 ± 0.33
ALP4	6.0 ± 0.4	70.7 ± 16.2	14.8 ± 1.6	27.5 ± 1.3	31.8 ± 0.2	2.51 ± 0.19

Mean ± S.D. of environmental variables for the alpine, sub-alpine and boreal sites along precipitation gradients (low [1] to high [4]) in southern Norway. For information on the significances of differences in environmental variables between temperature and precipitation levels, see text.

remaining of green tea corresponded with the shortest incubation times (60 days, $n = 8$). This shows that our data are generally within the range of Keuskamp and others (2013) and that the assumptions made by the TBI can be applied to our dataset, as rooibos tea remained in the first phase of decomposition and green tea has generally entered the second phase of decomposition.

There was no overall relationship between k and temperature when data from all sites across the three incubation periods were combined into one regression model (Figure 3a). However, short-term annual variation in climate had a significant effect on k ($\chi^2(2) = 33.47$, $P < 0.001$). On average, k was 12.1 and 15.6% higher for 2014, a warm and relatively dry year, than for 2015 and 2016, respectively (Figure S2a). This temporal pattern is illustrated within each of the temperature levels within the grid, where k consistently increased with temperature, although not significantly in the alpine (alpine: $R^2 = 0.003$, $P = 0.07$, sub-alpine: $R^2 = 0.05$, $P < 0.05$, boreal: $R^2 = 0.08$, $P < 0.01$; Figure 3A, Table S4). Furthermore, k decreased with increasing precipitation (Figure 3B, Table S4), both for the complete dataset ($R^2 = 0.07$, $P < 0.001$) and within each temperature level of the climate grid (alpine: $R^2 = 0.12$, $P < 0.001$, sub-alpine: $R^2 = 0.13$, $P < 0.001$, boreal: $R^2 = 0.07$, $P < 0.01$). The precipitation gradients within the grid had no distinguishable effect on decomposition rate either in relation to annual temperature vari-

ability or annual precipitation variability (Table S4).

Short-term annual climate variability only explained 22% of the variance in mean k, whereas long-term climate variability (temperature and precipitation gradients) explained 44% (Table 2). A model combining both long-term and short-term annual climate variability improved the proportion of variance explained to 72%. The variance explained by long-term climate variability can be mediated by a number of local environmental characteristics of the sites, namely: pH, soil C/N, and plant diversity. Although none of the environmental factors showed any significant relation with k across the grid or along climatic gradients except for a decrease in k with increasing plant diversity within the sub-alpine sites ($R^2 = 0.32$, $P < 0.05$), the selected model contained a number of environmental variables. This model explained 66% of the variation in k and included the predictors temperature (1.3% explained variation), precipitation (20.3%), plant diversity (22.3%), pH (14.3%), and soil C/N (7.7%) and showed no bias toward any climatic level (Figure S3a).

Short-term annual variation in climate had a significant effect on S ($\chi^2(2) = 28.62$, $P < 0.001$). On average, S was 8.3 and 17.1% higher for 2014, a warm and relatively dry year, compared to 2015 and 2016, respectively (Figure S2b). Stabilization factor S was negatively related to temperature for the complete dataset ($R^2 = 0.03$, $P < 0.001$) as it decreased
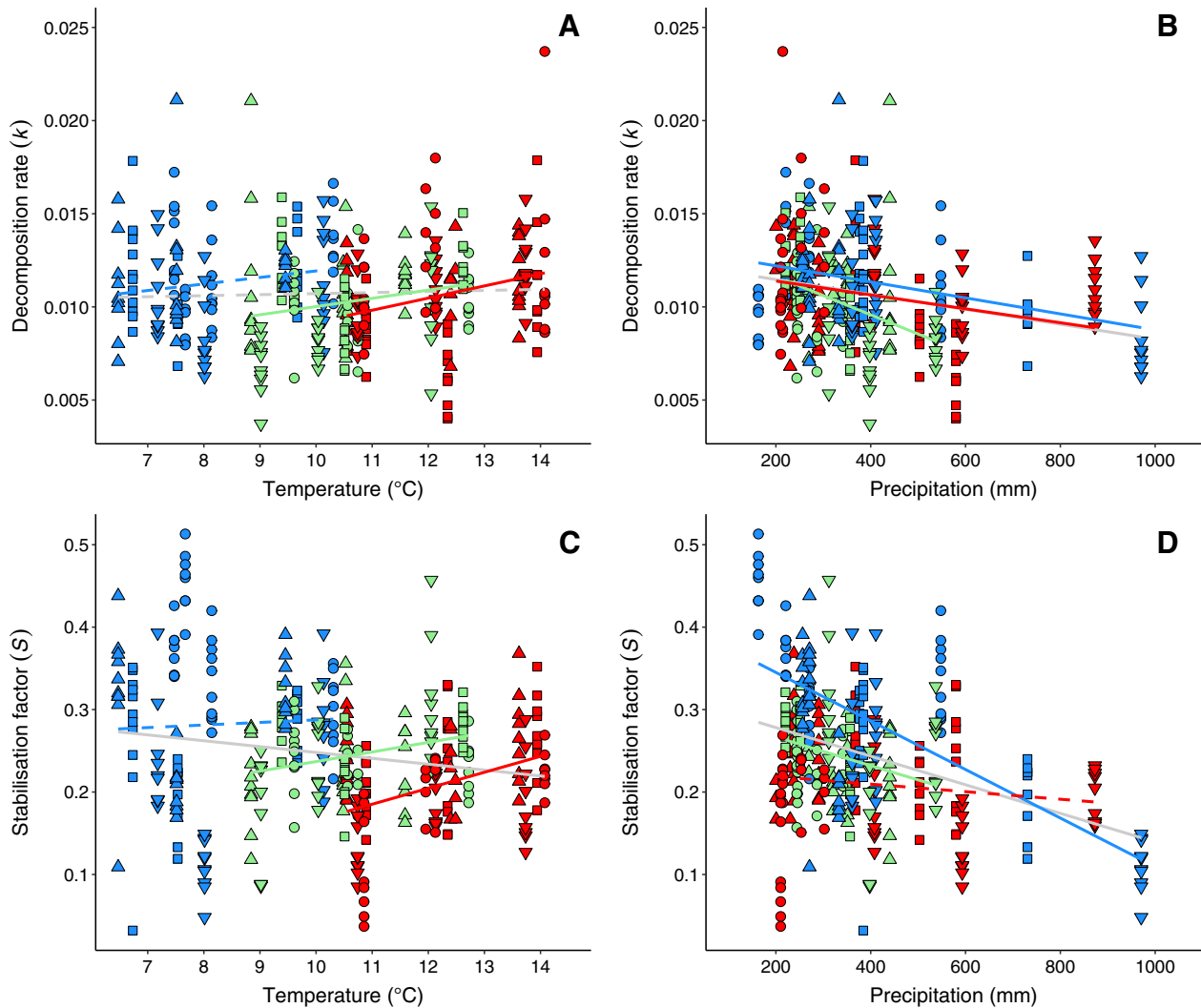
Figure 3. Decomposition rate k in relation to temperature (**A**) and precipitation (**B**), and stabilization factor S in relation to temperature (**C**) and precipitation (**D**). Colors correspond to temperature level; alpine ≈ 6.5°C (*blue*), sub-alpine ≈ 8.5°C (*green*) and boreal ≈ 10.5°C (*red*). Different symbols correspond to precipitation level 1 ≈ 600 mm (*Triangle*), 2 ≈ 1200 mm (*Circle*), 3 ≈ 2000 mm (*Square*), and 4 ≈ 2700 mm (*Inverted triangle*). *Colored lines* indicate relation between k or S and the climatic variable for the particular temperature levels, while *gray lines* show the relationship between k or S and the climatic variable across the entire grid. *Solid lines* indicate significant relationships while *dashed lines* indicate nonsignificant relationships.

Table 2. Regression Models for Decomposition Rate k and Stabilization Factor S

Decomposition proxy	Model	Adj R^2	AIC	Var. expl. (%)
Decomposition rate k	$t + p$	0.17*	− 465.82	22
	TL × PL	0.18	− 459.67	44
	$t + p$ + TL × PL	0.55**	− 480.59	72
	$t + p$ + pH + Pdiv + Soil C:N	0.60***	− 489.83	66
Stabilization factor S	$t + p$	0.27**	− 209.35	33
	TL × PL	0.33*	− 206.75	54
	$t × p$ + TL × PL	0.63***	− 226.45	78
	$t × p$ + TL + Litter C:N	0.58***	− 226.69	65

Multiple regression models relating site averaged decomposition rate k (n = 36) and stabilization factor S (n = 36) to climatic and environmental variables. Climatic and environmental variables included in the models represent: temperature level (TL), precipitation level (PL), mean air temperature (t), total precipitation (p), pH, soil C/N ratio (soil C/N), litter C/N ratio (litter C/N), and plant diversity (Pdiv).
Significance of models are shown, with */**/*** indicating P < 0.05, P < 0.01 and P < 0.001, respectively.

along the temperature gradient within the grid (Figure 3C). However, within the different temperature levels of the grid S increased in warmer years (Figure 3C and Table S4), although not significantly for alpine sites (alpine: $R^2 = 0.001$, $P = 0.6$, sub-alpine: $R^2 = 0.07$, $P < 0.01$, boreal: $R^2 = 0.15$, $P < 0.001$). Increased precipitation had a negative effect on S for the complete dataset ($R^2 = 0.15$, $P < 0.001$), which was consistent within the different temperature levels of the grid except for boreal sites (alpine: $R^2 = 0.44$, $P < 0.001$, sub-alpine: $R^2 = 0.08$, $P < 0.01$, boreal: $R^2 = 0.02$, $P = 0.13$; Figure 3D). Increased temperature had a stronger effect on S in sites at higher temperature levels of the grid, whereas the effect of increased precipitation weakened toward warmer sites (Figure 3C, D, Table S4). Within the precipitation levels of the grid, temperature did not have a significant effect on S, whereas increased summer precipitation significantly affected S across all precipitation levels, except for precipitation level 2 (Table S4). The precipitation gradients within the grid had no distinguishable effect on the stabilization factor in relation to annual temperature variability, but showed a consistent pattern with increased summer precipitation, except for precipitation level 2 (Table S4).

Short-term annual climate variability only explained 33% of the variation in S, while long-term climate (temperature and precipitation gradients) explained more than half (54%) of the variation (Table 2). Combining climate gradients and short-term annual climate variability in one model improved the percentage of variance explained to 78%. Litter C/N was the only significant environmental variable and improved the model by reducing complexity as it substituted the precipitation gradient (Table 2). Stabilization tends to increase with higher litter C/N across the grid, however, not significantly. Although litter C/N ratio does not have a significant relationship with S, the selected model that incorporated litter C/N as a predictor explained the variation in S quite well and shows no bias toward any climatic levels (Figure S3b). The best model for S explained 65% of the variation and included temperature (7%), precipitation (20.2%), and their interaction (10.1%), temperature levels (19.6%), and litter C/N (8.3%).

DISCUSSION

The selected models explained the variation in decomposition rate and stabilization factor relatively well—72 and 65% of the variation, respectively, and included factors representing short-term annual variability in climate as well as factors representing long-term effects of climate. Long-term climate, represented by the climatic gradients within the grid or environmental factors reflecting long-term climate regimes, explained a large part of the variation in both litter decomposition rate and in litter stabilization: 44 and 28%, respectively. Short-term annual variation in summer precipitation was another major driver and explained about 20% of the variation for both litter decomposition and litter stabilization, whereas short-term annual variation in summer temperature only had a minor influence on decomposition processes.

We observed an increase in decomposition rate of the labile fraction with increased summer temperature within each temperature level in the climate grid. The enhanced decomposition with increased temperatures is in accordance with our expectations, and is in line with results from experimental warming studies in cold biomes (Aerts 2006) and a study using the TBI along an elevational gradient (Didion and others 2016). However, factors related to long-term climate differences between sites appear to be such strong modulators that this temperature effect was not visible across the grid (Figure 3A, Table S4). The selected model for k supports the proposition that long-term climate shapes environmental conditions that modulate k, most likely through differences in soil pH, soil C/N, and plant diversity that together explained almost 45% of the variation in decomposition rate. Soil pH and plant diversity varied significantly along the temperature gradient, and soil C/N varied significantly along both temperature and precipitation gradients. Soil pH, soil C/N, and plant diversity have been shown to influence soil microbial community composition both directly and indirectly (Zak and others 2003; Rousk and others 2010; Wan and others 2015). We speculate that differences in decomposition rate across the grid could be partly traced back to differences in microbial community composition between sites. In our study sites, soil pH is a strong determinant of microbial community composition between alpine and sub-alpine sites (Guittar and others unpublished results), which matches the differences observed between climatic regimes.

In a year of increased precipitation, initial decomposition rates of the labile fraction slowed down across the whole grid and were consistent within each temperature level (Figure 3B). Although the direction of the change is in contrast with our expectations, this shows that short-term variation in precipitation affects decomposition in the same way across sites in different climatic re-

gimes. This implies that temperature-limited sites have the same sensitivity to short-term variation in precipitation as warmer sites. In other regional and global cross-biome studies (using a one-phase model), decomposition rate is usually positively correlated with mean annual precipitation (Epstein and others 2002; Zhang and others 2008; Portillo-Estrada and others 2016), although a few studies that use a two phased TBI decomposition model show a decrease in decomposition with increased mean annual precipitation or increasing soil moisture (Didion and others 2016; Sarneel and Veen 2017). Besides differences in model assumptions, a possible explanation for the negative effect of precipitation on decomposition rate could be that, in our relatively moist study region, high amounts of precipitation induce oxygen limitation to microbial communities and therefore limit decomposition rates (Schuur 2001), but not the degree to which material is broken down. Soil respiration, which reflects decomposition, has also been found to be very low under conditions with very high soil moisture content (Suh and others 2009).

We found that stabilization of labile material is also modulated by long-term climate, as S decreases along the temperature gradient within the grid (Figure 3C, Table S4). This is in accordance with another study that found the size of the recalcitrant fraction of standard plant material to increase with elevation (Coûteaux and others 2002). However, within each temperature level of the grid, and in particular in the boreal and sub-alpine sites, we found that short-term temperature variation has the opposite effect on stabilization as S increased with higher summer temperatures (Figure 3C, Table S4). This indicates that long-term climate is an important modulator for stabilization of labile material through shaping environmental conditions, but that short-term increases in temperature can have a significant and opposite effect on this stabilization. Increased temperatures could lead to smaller amounts of labile litter being stabilized and turned into SOM in the long-term, even if short-term results indicate the opposite.

Higher amounts of precipitation showed varied effects on litter stabilization along the temperature gradient within the grid, but were relatively consistent along the precipitation gradient (Table S4). In contrast to our expectations, the colder sites were more sensitive to short-term variation in precipitation compared to the warmer (boreal) sites. In alpine and sub-alpine sites, increased precipitation decreased stabilization and SOM production, while in boreal sites, it had no effect (Figure 3D, Table S4). This again shows that the long-term climate regime played an important role in mod-ulating stabilization. The temperature gradient explained about 20% of the variation in S, but this variation could not be attributed to any of the measured environmental characteristics. The only environmental variable that was included in the model that best explained S was litter C:N, but this explained only a relatively minor proportion of the variation (8.3%). Organic matter can be stabilized in various ways: by physical stabilization through micro-aggregation, chemical stabilization through intimate association with silt and clay particles, and biochemically through formation of recalcitrant SOM compounds (Six and others 2002). It is therefore possible that other environmental factors, such as soil structure or soil clay and silt content, could explain the difference in stabilization to changes in summer precipitation along the temperature gradient.

Overall, there was a very clear difference between the effects of short-term variation in climate (that is, between growing season) and long-term effects of the different climate regimes characterizing the different sites (that is, climatic gradients). We expected decomposition processes in cold locations to be most sensitive to temperature, and decomposition processes in warm locations to be more sensitive to precipitation. Instead, we see that k and S showed a stronger response to short-term variation in temperature in warmer sites and that short-term variation in precipitation had a greater effect on S in colder sites. The decomposition rate of the labile fraction was enhanced by increased summer temperatures within each temperature level, though across the grid this relationship with temperature was not found. On the other hand, high precipitation had a consistent inhibiting effect on decomposition as it lowered the decomposition rate across the grid and within each temperature level. More stabilization of labile material occurred with increased annual temperatures within most temperature levels, whereas a decrease in stabilization was found across the different long-term climatic regimes. Further, stabilization of labile material was more sensitive to short-term variation in precipitation in colder sites compared to warmer (boreal) sites. Increased temperatures would thus stimulate decomposition, but at the same time, would also lead to more stabilization and transformation to SOM, whereas increased precipitation limits decomposition but also decreases stabilization of litter and transformation to SOM. These results imply that, over the short-term, increases in temperature and precipitation could offset one another in terms of effects on decomposition processes, as climate change is expected to both increase temperature and precipitation in northern

ecosystems. However, short-term effects within a particular climatic regime are not necessarily predictive of the long-term outcome after the ecosystem has adapted to a new climate.

CONCLUSIONS

We studied the short-term, direct (that is, annual variation in temperature and precipitation) and long-term, indirect (that is, climatic regime of different sites) effects of climate on two phases of the decomposition process: decomposition rate (k) and stabilization factor (S) using TeaBag Index (TBI) in three consecutive growing seasons in a climate grid combining both temperature and precipitation gradients. We found that the response of the initial decomposition rate of labile litter to annual increases in temperature is rather consistent within climate regimes but not across different climate regimes, whereas annual increases in precipitation had a consistent negative effect both within and across climatic regimes. Stabilization of the labile litter fraction increased with higher annual temperatures within climatic regimes, whereas stabilization decreased from sites experiencing colder climate regimes to warmer climate regimes. In addition, stabilization was more sensitive to short-term variation in precipitation in sites with colder climatic regimes compared to warmer sites. Short-term effects of temperature and precipitation within a particular climatic regime showed discrepancy with long-term climate and will therefore not necessarily reflect changes due to climate change on the long-term. Environmental characteristics of the sites related to long-term climate (for example, soil properties and plant diversity) played a significant role in regulating decomposition processes. This supports the statement that multiple factors regulate litter decomposition but that they change in predominance as the values of regulatory factors also change (Bradford and others 2016). Our findings highlight the importance of long-term climate in shaping environmental conditions that influence the response of decomposition processes to climate change.

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